

**Breeding for resilience:  
a strategy for organic  
and low-input farming systems?**

**EUCARPIA 2nd Conference  
of the "Organic and Low-Input Agriculture" Section**

**1-3 December 2010, Paris**

**Le Comptoir général  
80 quai de Jemmapes  
75000 Paris - FRANCE**

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## **Breeding for resilience: a strategy for organic and low-input farming systems?**

Isabelle Goldringer, Julie Dawson, Alice Vettoretti, Frédéric Rey

Global change is increasingly affecting agricultural production and threatening food security. Organic and low-input farming systems are less demanding in fossil energy and might thus contribute to moderating global carbon emissions. Moreover, under increased uncertainty and variability in environmental conditions, these systems offer solutions for buffering against climatic extremes, disease epidemics, changing nutrient availability, and other stresses that will add to already heterogeneous environmental conditions.

2010 has been designated the Biodiversity Target year by Parties to the Convention on Biological Diversity. Yet, it is clear that biological diversity in agroecosystems, measured as the number and abundance of species as well as genetic diversity within cultivated plants, is still decreasing, largely due to the negative impacts of intensive industrial agriculture. Overall, ecosystem services delivered by biodiversity such as plant disease control, soil fertility and pollination are jeopardized by its decline. These threats present an opportunity for the organic sector to develop original and innovative strategies for biodiversity preservation and increased resilience in the field.

The second EUCARPIA meeting of the Section Organic plant breeding and low-input agriculture organised in Paris, France, from the 1<sup>st</sup> to the 3<sup>rd</sup> of December 2010, by INRA – UMR Génétique Végétale Le Moulon and ITAB, wishes to take inspiration from the ecological sciences to highlight the use of biodiversity in agriculture while taking advantage of the new tools coming from genomics. Therefore, the symposium will deal with breeding strategies for organic and low-input farming systems with a special emphasis on approaches that allow for more resilience in response to global change. Some 130 participants representing 20 countries will attend the symposium, including students, researchers and other professionals from universities, institutes, breeding companies, governmental institutions, Non Governmental Organizations and farmers.

The programme features 30 oral and 37 poster presentations, covering the following areas:

- Improving resilience of agro-ecosystems
- Utilizing and conserving agrobiodiversity in agricultural landscape
- Global change and adaptability
- New insights into the mechanisms of adaptation to local conditions and organic farming
- Breeding for diverse environments and products
- Regional participatory plant breeding

The scientific committee has selected oral presentations based both on their scientific quality and with the aim to cover a diverse range of crops and species, different approaches, methods and viewpoints. Because the place and the conditions in which thoughts develop are also important, the organising committee chose Le Comptoir Général – a new space with strong environmental endowment dedicated to large audience events (80 Quai de Jemmapes, Paris 10<sup>ème</sup>, France) – as the location for the event, and an organic caterer (Grain de vie). We hope that all this will allow for fruitful discussions and the emergence of new ideas and collaborations for a future more resilient low-input and organic agriculture.

## Programme

### Wednesday, December 1st

#### 13:00 – Arrival – registration

#### 13:30 – Welcome & introduction

Welcome by Isabelle Goldringer, chair of the organising committee.

Welcome by Zoltan Bedo, chair of the Eucarpia association.

13:20	Introduction by Edith Lammerts van Bueren, chair of EUCARPIA Section Organic & Low-input Agriculture	The challenges and the framework for future breeding for organic and low-input agriculture, and the goals of the conference
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#### 14:00 – 14:50 – Improving resilience of agro-ecosystems

14:00	Keynote speaker: Dr Jerry Glover, Science and Technology Policy Fellow, American Association for the Advancement of Science, Washington DC	Improving Agroecosystem Resilience and Sustainability
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#### 15:10 – 17:20 – Session 1 : Utilizing and conserving agrobiodiversity in agricultural landscapes

15:10	Invited speakers: Dr Devra Jarvis, Senior Scientist, Bioversity International, Rome, Italy Dr Bhuwon Sthapit, Senior Scientist, Biodiversity International, New Delhi, India	Participatory plant breeding as a strategy for supporting the assessment, access, use and benefit of traditional crop genetic diversity in the farmer's production system: Overview and the case of the Mansara rice ( <i>Oryza sativa</i> L.) landrace in Nepal
16:00	Géza Kovacs	Evolutionary breeding of cereals under organic conditions
16:20	Emmanuelle Porcher	Crop genetic diversity benefits farmland biodiversity in cultivated fields
16:40	Maria-José Suso	Potential power of the plant-pollinator relationship as a tool to enhance both environmental and production services of grain legumes in the context of low-input agriculture: what do we do?
17:00	Anders Borgen	Quality traits in conservation varieties

#### 17:30 – 19:00 – Posters – 1st session

Utilizing and conserving agrobiodiversity in agricultural landscapes / Global change and adaptability / New insights into the mechanisms of adaptation to local conditions and organic farming

#### 20:00 – 23:00 – Dinner followed by a debate, with “Fondation Sciences Citoyennes”

“Organic agriculture: so few research, so many questions - what are the lock-in, how to release?”

<http://sciencescitoyennes.org> (in French)

### Thursday, December 2nd

#### 8:30 – 11:00 – Session 2 : Global change and adaptability

8:30	Invited speaker: Dr Martin Wolfe, Principal Scientific Advisor, ORC, UK	Steps towards an ecological future
9:15	Invited speaker: Dr R. Ford Denison, Adjunct Professor, University of Minnesota, USA	Economic, ecological, and evolutionary tradeoffs as past constraints and future opportunities
10:00	Thomas Döring	Breeding for resilience in wheat - Nature's choice

10:20	Maria Finckh	Population developments from the F5 to the F9 of three wheat composite crosses under organic and conventional conditions
10:40	Sébastien Barot	Breeding new cultivars to enhance positive feedbacks between soil and crops?

### 11:30 – 12:30 – Posters – 2nd session

Breeding for diverse environments and products / Regional participatory plant breeding

### 12:30 – 13:30 – Lunch

### 13:30 – 16:00 - Session 3: New insights into the mechanisms of adaptation to local conditions and organic farming

13:30	Invited speaker: Dr John M. Warren, Senior Lecturer in Agro-ecology, IBERS, UK	Building resilience in organic and low-input farming systems: an ecological geneticists view
14:20	Olga Scholten	Breeding onions for low-input and organic agriculture: Genetic analysis of the interaction between Allium species and arbuscular mycorrhizal fungi
14:40	Monika Messmer	Genetic variation for nutrient use efficiency in maize under different tillage and fertilization regimes with special emphasis to plant microbe interaction
15:00	Roeland. E. Voorrips	Validation of associations between plant traits and Thrips damage in cabbage
15:20	Bernard Rolland	Wheat varieties in competition with weeds for sustainable agriculture, in particular organic farming
15:40	Abdul Rehman Khan	DNA methylation patterns in VRN1 gene in vernalized and non-vernalized wheat

### 16:30 – 19:00 - Session 4: Breeding for diverse environments and products

16:30	Invited speaker: Dr Kevin Murphy, Assistant Research Professor, Washington State University, USA	Breeding for Diversity: Examples from quinoa, buckwheat, hops and spelt
17:20	Paolo Annicchiarico	Breeding forage and grain legumes for adaptation to specific agroclimatic regions and cropping systems: opportunities and limitations,
17:40	Pedro Revilla	Grain quality in traditional maize varieties for bakery under organic conditions
18:00	Mickael Fleck	Chinese cabbage variety 'Atsuko' as an example of biodynamic vegetable breeding with Kultursaat association, Germany
18:20	Ilze Skrabule	Evaluation of potato breeding clones in organic and conventional growing conditions
18:40	François Warlop	Urgent need for new fruit breeding methods better adapted to low-input agro ecosystems

20:00 – 23:00 – Dinner and concert performance : Balval <http://www.myspace.com/balval>

### Friday, December 3rd

### 8:30 – 11:00 - Session 5 : Regional participatory plant breeding

8:30	Invited speaker: Dr Lorenz Bachmann, MASIPAG, Germany	Farmer-led participatory plant breeding. Methods and impacts. The MASIPAG farmers Network in the Philippines
9:20	Julie Dawson	On-farm conservation and farmer selection as a strategy for varietal development in organic agricultural systems

9:40	Erica N.C. Renaud	Will regional selection provide better adapted and performing broccoli varieties combining agronomic, quality (phytochemical) traits and farmers' preferences in the USA?
10:00	P. Mendes-Moreira	On-farm conservation and participatory maize breeding in Portugal; lessons learnt and future perspectives
10:20	Jingsong Li	Participatory breeding in China in maize and its institutional challenges
10:40	Bernd Horneburg	Participation, utilization and development of genetic resources in the Organic Outdoor Tomato Breeding Program

**11:00 – 12:00 – Closing session**

**13:00 – 19:00 – Visit** (optional) to the Bergerie de Villarceaux (95) : an organic farm with an experimental platform for research in organic farming

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Follow the tomato! This picture indicates posters that are eligible for the poster content (posters made by PhD, post-doctoral positions, engineers, master students...)

## Introduction

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### Future breeding for organic and low-input agriculture: integrating values and modern breeding tools for improving robustness

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*Keywords:* robustness, resilience, plant breeding, organic and low input agriculture, adaptation model

#### **Introduction**

Organic production and also the attention for plant breeding for organic agriculture is still increasing in Europe. The question often raised is how much does plant breeding for the organic sector differ from modern plant breeding and does a ban on GMO also include refraining from molecular marker assisted selection (MAS)? In this paper I will first elaborate on the values in organic agriculture and its related systems approach as a central focus in organic agriculture and will then discuss in which way molecular marker assisted selection can be of use for plant breeding for organic and low-input agriculture.

#### **Values and systems approach**

The central values of organic agriculture are summarized in IFOAM (International Federation of Organic Agricultural Movements) four basic principles: health, ecology, fairness and care (IFOAM, 2005). The original, long term driving force behind organic farming systems was and is to support a stable crop growth by avoiding soil degradation through a management system that restores and maintains soil health as the basis for healthy crops, animals and humans. Soil fertility includes a good soil structure to allow roots to exploit a large soil volume, a good water holding capacity through improved level of soil organic matter. The organic fertilisers together with a wide crop rotation contribute to a diversity of soil organisms. In this sense sustainable soil management is considered the basis of a robust farming system. However a farming system should be designed in such a way that at all levels it can contribute to sustainable crop production. Napel et al. (2006) plea for a adaptation model rather than a control model if the focus is to support yield stability rather than yield as such, see figure 1. In the mainstream agriculture the 'control' model is the prevailing approach. The main focus is to protect crops from unwanted fluctuations influencing the production as much as possible by keeping away disturbances depending on continuous monitoring and direct intervention with chemical-synthetic crop protectants and to look for single solutions.

In the 'adaptation model' not optimising the financial income but stabilising crop production and income is the main focus. In this approach in which we can recognise the aims of organic farming approach, the goal is designing a site-specific farm system such that at all levels (plant, field, farm) can minimise the impact of sources of variation or disturbances, rather than ruling out the sources of variation.



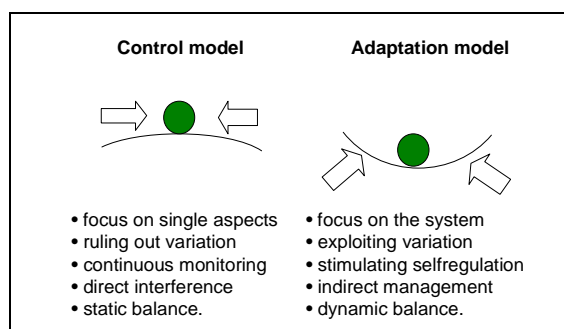


Figure 1. The control model versus adaptation model, adapted after Napel et al. (2006)

Besides increasing soil fertility and improving crop management to enhance disease suppression, also the choice of varieties can contribute to achieving yield stability. Many authors have discussed important crop traits to make crop robust and less vulnerable for unfavourable conditions. Not only disease resistances and field tolerance, but also other kind of traits is of importance such as a deeper rooting system to exploit larger soil volumes for resources, different plant features that contribute to early and late weed suppression. Another category of variety characteristics to enhance yield stability are focussed on exploiting genetic diversity in breeding approaches such as evolutionary breeding, or breeding for combinability in variety mixtures or crop mixtures in an intercropping system.

### Breeding for resilience

Breeding for organic and low-input farming systems with a special emphasis on strategies that allow for more 'robustness' or 'resilience' in response to global change is a challenge. While organic and low-input agricultural systems are more exposed than conventional ones to heterogeneous environments, low nutrient availability and biotic as well as abiotic stress, global change might increase uncertainty in environmental conditions by producing drastic variation in climate, epidemic pressures, nutrient availability, etc. These changes could be considered an opportunity for the organic sector to develop original and innovative strategies for high level resilience or robustness. We will have to realize that certain plant traits such as deeper root system or nutrient efficiency can contribute to crop robustness, but that there are more mechanisms that support robustness in the broad sense which we have not yet fully explored to be able to optimise breeding programs for organic and low-input agriculture. We can learn from the ecological sciences to reconsider e.g. the use of biodiversity without ignoring the new tools coming from genomics.

### Molecular marker assisted selection (MAS)

Breeding for resilience or robustness includes quantitative traits such as nutrient-efficiency, root architecture or polygenetic disease resistances. Such complex traits depend on more genes and are very much influenced by the environment. As low-input management of organic agriculture results in a larger influence of varying environmental conditions (in time and geographically) on crop performance, also the QTLs (Quantitative Trait Loci ) may interact with environmental conditions and QTLs found in one environment will not always be the ones found in another environment. However, plant geneticists consider molecular marker assisted selection for such complex traits a useful additional tool in plant breeding programs to make selection more efficient.

Standards for organic agriculture do not exclude the use of molecular markers, but still there is uncertainty whether molecular tools are in line with the values of organic agriculture. Use of genomics, e.g. molecular tools as easily associated with genetic modification which is banned in organic agriculture. Modern breeding techniques, including in-vitro techniques, have been discussed for many years in the organic sector not only for ecological but also for ethical reasons not wanting to violate the integrity of life (Lammerts van Bueren et al., 2003). The outcome so far is that breeding techniques applied on whole plant level are applicable, and techniques on cell (tissue) level as the lowest level of self organised life, are still under debate. More clarity is on the ban of techniques beyond cell level and interfering directly at DNA level, e.g. genetic modification, cell or protoplast fusion etc.

Organic and low-input farming conditions require breeding for robust varieties that contribute to resilience at all levels of the farming systems, which may be hampered by too much focus on the molecular level. The question therefore is how information and selection on molecular level can be integrated in an approach that takes all

levels of the crop production into account. Pros and cons for use of molecular markers in breeding for organic and low-input agriculture were the topic of a Eucarpia-Bioexploit plant breeding workshop in 2009. Through a SWOT analysis produced during the workshop the strengths, weaknesses, opportunities, and threats of the use of molecular markers were explored (Lammerts van Bueren et al., 2010). Clear strengths were identified, e.g. better knowledge about gene pool of breeding material, more efficient introgression of new resistance genes from wild relatives and testing pyramided genes. There were also common concerns among breeders aiming at breeding for organic and/or conventional agriculture, such as the increasing competition and investments to get access to marker technology, and the need for bridging the gap between phenotyping and genotyping especially with complex and quantitative inherited traits such as nutrient-efficiency. Integrating more disciplines such as agronomy and crop physiology in breeding research will provide ways to bridge that gap and to deal with the interaction between genotype, environment and management (Struik and Yin, 2009). Their approach, including QTL-based ecophysiological modelling, could provide the tools to breed for complex trait, such as nutrient-use efficiency, in a more efficient way making use of markers.

The extent to which MAS can be used efficiently in breeding for organic farming to complement or to replace part of the phenotypic selection will be highly dependent on the specific trait and the availability of markers that reflect the genetic basis of the special needs for organic farming. With respect to the organic sector there is a need to show and discuss with molecular scientists examples of ‘good practices’ of breeding and research projects with MAS approaches as well as to be updated on how new protocols may be adapted. This last aspect reflects the concern of the organic sector that the development and use of molecular markers often include the use of harmful chemicals and enzymes produced from genetically modified organisms. One of the opportunities is that the general development within molecular techniques is moving towards replacing harmful chemicals by alternatives that cause less damage to the lab-workers and the environment.

### Discussion and conclusions

The question is whether we fully understand the plant characteristics or interactions with soil organisms that contribute to intrinsic robustness for achieving sustainable crop production. Napel et al. (2006) indicates that the adaptation model is closely linked with concepts such as a) robustness in the narrow sense, which can be defined as the ability to switch between underlying processes to maintain the balance, b) resilience, as the ability to regain the balance after a disturbance, and c) resistance, as insensitivity to disturbance. Napel et al. argue that none of the three concepts alone describes fully the adaptation model, but that robustness in the broad sense is minimal variation in a target feature following a disturbance, regardless of whether it is due to switching between underlying processes, insensitivity or quickly regaining balance. These authors also state that there are many unanswered questions regarding optimal utilisation of biological robustness mechanism and further research is needed. This will also apply to breeding research to support the development of varieties that enhance the intrinsic robustness in the cropping system.

### Acknowledgement

The author gratefully acknowledge funding from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the EU Integrated Projects Bioexploit (Exploitation of natural plant biodiversity for the pesticide-free production of food) FOOD-CT-2005-513959 and EU SOL (High Quality Solanaceous Crops for Consumers, Processors and Producers by exploring of natural biodiversity) FOOD-CT-2006-016214, and the Eucarpia Section Organic and Low-input Agriculture.

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## Keynote paper: Improving resilience of agro-ecosystems

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### Improving Agroecosystem Resilience and Sustainability

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Of human activities, agriculture has been identified as the most disruptive to the functioning of ecosystems and the greatest threat to biodiversity (Cassman and Wood, 2005). While supplying most of our food calories, a large portion of our fibre needs and an increasing amount of our energy demands, farmed landscapes generally exhibit degraded soil, air, water and wildlife habitat conditions compared to those maintained by natural ecosystems (Cassman and Wood, 2005; Foley et al, 2005). Compounding these negative impacts, the demand for food, especially meat, is increasing; much of the land area most suitable for annual crops is already in use; and production of nonfood goods (e.g., biofuels) increasingly competes with food production for land (Godfray et al., 2010). And despite doubling of yields of major grain crops since the 1950s, more than one in seven people currently suffer from malnutrition (Food and Agriculture Organization, 2009). Climate change, resource scarcity, and continued land degradation, which forces farmers onto even more marginal lands, exacerbates the problem of developing sustainable agricultural systems in the 21<sup>st</sup> century. To ensure food and ecosystem security, farmers need more options to produce farm products under different, generally less favorable circumstances than those under which increases in crop yields were achieved this past century.

As Foley et al. (2005) have illustrated, though, tradeoffs between ecosystem services and agricultural productivity may be typical but not absolute. An ideal agricultural system would produce abundant yields over long time periods and support ecosystem services at similarly high levels as natural ecosystems, but would not require energy intensive inputs that pose environmental risks. It would also be resilient to the many challenges facing farmers around the world, including environmental, social, political, and economic disturbances that frequently affect agricultural production. In extensive regions of the world where high quality lands, resources, and infrastructure are limiting, the ability of an agricultural system to function well in marginal conditions will be an increasingly important attribute.

Central to solving many agriculture related problems is addressing the production of the annual grain crops—cereals, oilseeds, and legumes—planted on almost 70% of croplands, which combined supply a similar portion of human calories (Monfreda et al., 2008; Pimm, 2001). During the last half of the 20th century, farmers, overall, kept pace with or exceeded global food demands by expanding production onto previously unexploited landscapes and by utilizing new crop varieties, agricultural technologies, and management practices. Annual crop production, though, often compromises essential ecosystem services, pushing some beyond sustainable boundaries, particularly in marginal conditions (Pimm, 2001).

Now, however, much of the global lands best suited for farming are already in use. More marginal lands, which are at greater risk of degradation when producing our most widely grown crops, are increasingly being exploited. The best lands have soils at low or moderate risk of degradation under annual grain production but make up only 12.6% of global land area (16.5 million km<sup>2</sup>) (Eswaran et al., 1999). Supporting more than 50% of world population is another 43.7 million km<sup>2</sup> of marginal lands (33.5% of global land area), at high risk of degradation

under annual grain production but otherwise capable of producing crops (Eswaran et al., 1999). Agricultural innovations developed during the Green Revolution were most successful in raising yields on the better agricultural lands where industrial inputs provided the highest payoffs. The successful reproduction of the yield increases of the Green Revolution in areas inhabited by people most in need of agricultural advancement will require a shift in focus to less ideal landscapes and conditions.

The expanded use of currently existing perennial crops and the development of new ones provide an increasingly viable pathway for developing resilient and sustainable farming systems even in more marginal conditions. Perennial crops can provide multiple ecosystem services essential for sustainable production more effectively than annual production systems (Boody et al., 2005; Tilman et al., 2006; Glover et al., 2007; Jordan et al., 2007). Previous studies of perennial grasslands from which aboveground biomass has been removed for long periods of time indicate their potential to serve as a model for highly resilient and sustainable agricultural systems. For example, levels of total soil nitrogen (TSN) in unfertilized perennial grass plots in the Continuous Hay Experiment at Rothamsted did not decline after twice-annual harvests over a 120-yr period (Jenkinson et al., 2004); nor did biomass yields decline (Jenkinson et al., 1994). Studies of unfertilized harvested grasslands in the Russian Chernozem revealed that TSN and soil organic carbon (SOC) stocks had not been reduced after more than 50 yrs of annual harvesting when compared to unharvested grasslands (Mikhailova et al., 2000; Mikhailova and Post, 2006). In another study of unfertilized harvested perennial grasslands in Kansas, USA (Fig. 1), the grasslands, annually harvested for roughly 75 years, yielded similar amounts of nitrogen per hectare in annual harvests of biomass as adjacent high-input wheat fields yielded in grain (Glover et al., 2010a). The grasslands, despite being harvested each year, supported a range of ecosystem functions at higher levels than the annual croplands, including higher levels of soil fertility and structure and more complex biological communities (Culman et al., 2010).

These unfertilized, harvested grasslands do not represent the full potential of perennial cropping systems but likely represent their lowest potential. Through plant breeding and improved fertility management these studies indicate that perennial cropping systems could greatly outperform annual systems in terms of production and environmental performance. Historically, though, herbaceous perennial crops have offered less valuable farm products than their annual counterparts and have been primarily used to feed animals. Until recently making the transition from extensive reliance on annual crops to perennial crops for a wide range of basic agricultural products was impractical or technically impossible. Achieving this transition is now more feasible because of advances in plant breeding, which provide plant breeders greater potential to develop perennial crops that produce a wider range of valuable products, such as grains (Glover et al., 2007), and innovations in post-harvest processing. There is also growing potential to use a wider range of plants to produce non-traditional bio-products including fuels, solvents, and durable and bio-degradable materials (Regauskas et al., 2006; Nash, 2007).

Important plant breeding innovations include the use of molecular markers associated with desirable traits that accelerate perennial breeding programs by allowing plant breeders to characterize and exploit plant genetic variation more effectively (Xu and Crouch, 2008). Marker assisted selection coupled with high throughput genotyping by which genotypes of large numbers of plants can be rapidly and inexpensively determined facilitates the combining of desirable genes without the need for field evaluation over many years and in every selection cycle. Naturally occurring genes that permit exchange of DNA between chromosomes of different species or genera can be used to obtain offspring with desirable traits from both parents (Qi et al., 2007). Plant breeders can use genetic modification to introduce new genes, to modify existing genes, or to interfere with gene expression in specific cases. Without classically trained plant scientists, however, opportunities offered by these innovations will not be fully realized.

The development of perennial versions of the major grain crops is one example of the possibilities for realizing highly resilient production systems offered by recent plant breeding innovations. Past efforts to develop perennial grain crops were hindered by the lack of modern plant breeding technologies. Efforts in the former Soviet Union and in the United States to develop perennial wheat in the 1960s were abandoned in part because of plant sterility and undesirable agronomic characteristics difficult to overcome with the technologies of the time (Cox et al., 2006). More recently, agronomists and plant breeders in Argentina, Australia, China, India, Nepal, Sweden, and the United States have initiated programs to identify and improve, for eventual use as grain crops, a range of perennial species and hybrid plant populations derived from annual and perennial parents.

These programs include the development of perennial versions of rice, wheat (Fig. 2), maize, sorghum, pigeon peas, and oilseed crops from the sunflower, flax, and mustard families (Sacks et al., 2003; Snapp et al., 2003; Ploschuk et al., 2005; Cox et al., 2006; Bell et al., 2008; Eriksson, 2009;). Additional plant taxa have potential to be developed as perennial grains (Cox et al., 2006).

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Breeding for resilience: a strategy for organic and low-input farming systems?  
 Improving resilience of agro-ecosystems

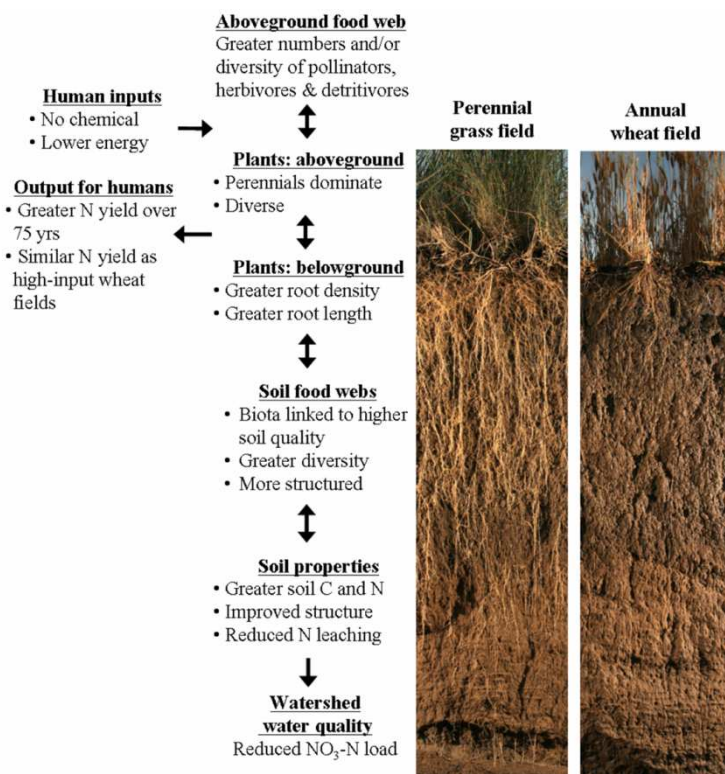


Figure 1. Ecosystem attributes of harvested perennial grass fields (on left) compared to annual wheat fields (Glover et al., 2010a).



Figure 2. Seasonal development of annual winter wheat (left in each panel) and its wild perennial relative, intermediate wheatgrass (right in each panel).

**Session 1**  
**Utilizing and conserving agrobiodiversity in agricultural landscapes**  
**Wednesday, December 1st - 3:10 pm**

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Participatory plant breeding as a strategy for supporting the assessment, access, use and benefit of traditional crop genetic diversity in the farmer's production system: Overview and the case of the Mansara rice (*Oryza sativa* L.) landrace in Nepal.

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Genetic information held in farmer's traditional varieties is crucial to the development of heat, drought, salinity, pests and diseases-resistant, fast-growing, high-yielding new varieties, necessary to combat food insecurity in the face of climate change. Agricultural biodiversity assets that are important for the poor are being lost or not optimally used because neither the rural poor themselves nor research and development teams understand their value or manage them sufficiently well. The purpose of this paper is to illustrate how simple participatory method of assessing diversity, identifying locally adapted farmers' varieties under rainfed and low input agriculture. In addition to methods of diversity assessment and decentralized breeding, we discuss the importance and present methods within a PPB strategy for improving both access to local genetic materials and information on performance of these materials to the farming community. We note that the decision to implement a PPB programme, and therefore its success, will depend on the farmer and the farming community having the knowledge, institutions and leadership capacity to evaluate the benefits that this action will have for them. We therefore emphasize within the PPB strategy the importance of strengthening local institutions to enable local communities to take a greater role in the management of their resources.

As a case study, we present a rice variety maintained by poor farmers in poor soil as a recurrent parent to improve the productivity and quality of the variety as a test case. The breeding goal is set by focused discussion with farmers during participatory diversity assessment. Mansara is a landrace adapted to poor soil, drought conditions, and low-input production system of hill environments of Nepal. The productivity of the landrace is poor, straw quality and cooking qualities are inferior and fetches poor market price. The landrace is currently grown by resource poor rice farmers who do not have better varietal options to replace Mansara in their low potential land. Farmers interviewed discussed the need to improve both the quality and yield of the local Mansara rice variety by crossing with a quality modern variety. As an alternative to a full replacement of the

local Mansara rice with an exotic variety, the breeding team worked together with the farming community to transfer the quality and the high yield potential to the farmer's Mansara variety without losing adaptive trait of this traditional variety. Selections were carried out in the target environment of Mansara growing areas using participatory plant breeding and participatory variety selection method during 1998 to 2010 in Begnas village (1000-1300m) as a strategy of promoting on-farm conservation of local rice diversity. Modified bulk breeding strategy was used and segregated bulk of 3 types were selected by farmers in their Mansara growing rice plots and selected own seed and exchange the materials with neighboring farmers. A PPB farmer group is formed to coordinate local level farmers, selection process and plant breeders from NGO and the Nepalese Agricultural Research Council (NARC). Farmer selected Mansara is now superior in terms of productivity but also in cooking quality. The variety is being proposed for registration in the name of community. The paper concludes that use of local germplasm as the female parent from the marginal production environment and selection by local farmers' under target environment is a viable on-farm management strategy to increase the acceptability and adaptation of new varieties by creating additional incentive for farmers from the extant diversity.



## Evolutionary breeding of cereals under organic conditions

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*Keywords:* organic farming, genetic resources, cereals, synthetic hexaploids, breeding

### Introduction

Success of organic farming is highly depends on the biodiversity used in its complex management system. Although it is relatively easy to guarantee the necessary diversity at farm level, it is difficult to find appropriate varieties for a (large scale) market oriented production of individual crop species, where only a few varieties that were specifically bred for organic farming are available. It is estimated that more than 95% of the varieties used in recent organic farming were bred for the conventional high-input sector, and these varieties lack important genetic diversity and traits required under changing environmental conditions (see Lammerts van Bueren et al. 2010 for review). To cover the complex needs of organic farming, development of a new type of crop varieties is needed, which is probably based on the genetic complexity of the varieties. During the decades several concepts were developed to increase functional diversity of crop varieties. One of the easiest ways is to develop multiline varieties or variety mixtures, but its application is constrained by the concern of farmers and processors about the anticipated negative effect on the homogeneity of the quality. An alternative possibility for maintaining genetic diversity and evolutionary fitness within crop varieties is to create composite cross populations. Unfortunately the application of these methods are limited to within species crosses, and do not give the possibility for increasing the genetic diversity of a given species by using the advantage of natural selection. The main aim of our recent research is to combine the advantage of composite crosses with the possibility of interspecific crosses to introduce new adaptive traits into the cultivated populations.

Based on the previous results obtained from within variety composite crosses of wheat new evolutionary breeding concepts were developed by using interspecific crosses to develop evolutionary adaptive composite cross populations. The simplest way to create such composite populations is the crossing of relative species having the same ploidy level. Another possibility is the creation of new synthetic hexaploids. The results of such breeding strategies will be presented.

### Materials and methods

#### *Creation of interspecific composite cross population*

- Studying the behaviour of the interspecific composite crosses a new composite cross population was developed by crossing 2 selected wild emmer (*Triticum dicoccoides*), 3 selected emmer (*Triticum turgidum* ssp. *Dicoccon*) and 2 modern durum wheat (*Triticum turgidum* ssp. *Durum*) varieties according to the half-diallel crossing scheme. The F1 generation of each cross were grown under greenhouse conditions and the seeds obtained were mixed to create a population. Starting from F2 generation the population were grown under organic farming conditions in each subsequent generation.

#### *Development of new composite cross population from new synthetic hexaploids Triticum species*

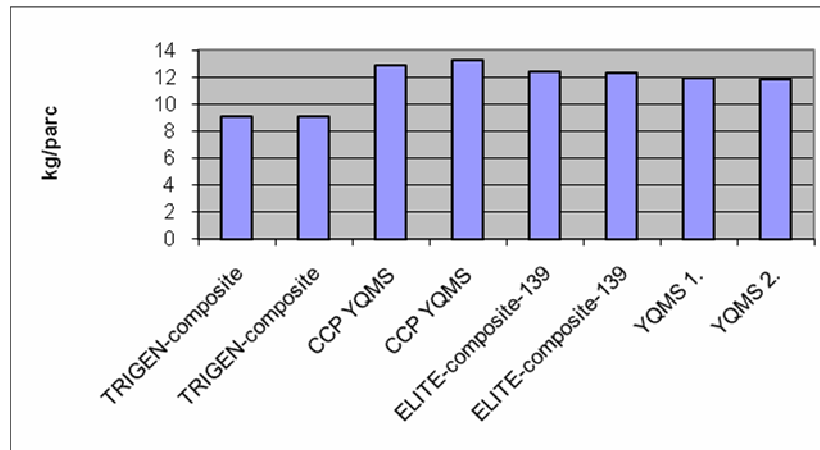
- In the first step of the research new synthetic hexaploids were developed by crossing different tetraploid *Triticum* species having AB genome structure (*Triticum turgidum* ssp. *durum* *Triticum turgidum* ssp. *dicoccon*, selected lines from the population originated from the composite cross population mentioned above), and one another tetraploid *Triticum* carrying AG genome, namely *Triticum timopheevii*. In each case, individual lines were selected on the basis of their agronomic performance, and the best two lines were

crossed with two highly crossable semidwarf einkorn breeding line showing excellent crossability in interspecific crosses. The hybrid seeds were screened for their chromosome number, and the true triploid seeds were selected. After genome doubling, the seeds were grown in greenhouse, and the obtained seeds were sown under organic field in the next autumn. The populations were multiplied for two subsequent generations before further crossing and testing. Some of the selected lines were used to establish interspecific composite crosses, meanwhile the remaining part of the population were used in direct breeding of a new species or were used in bread wheat crosses to increase the gene pool of the common wheat.

## Results

### *Tetraploid interspecific composite crosses*

According to the results of the composite crosses originated from the different tetraploid species their yield is relatively low in comparison of composite crosses developed from common wheat. Under our organic growing conditions the CCP YQMS are showing the highest yield followed by the Elite composite developed from modern Hungarian wheat varieties, and the yield of tetraploid composite is the lowest. On the contrary its yield stability is better than most of the common wheat populations. The diversity of the interspecific composite, understandingly, is the highest one, and several different types can be identified.



Recently this population is in F8 generation, and it is still segregating, and is producing several new types with sometimes very strange phenotype. Starting from F4 generation a number of breeding lines were isolated, and the best lines are over yielding even the modern common wheat control, and shows excellent adaptability and resistance against both biotic and abiotic stresses. Based on the promising results obtained during the development of interspecific composite crosses at tetraploid level, similar work had been initiated using hexaploids Triticum species, such as *T. aestivum*, *T. spelta*, *T. macha* and *T. sphaerococcum* to be able to increase the gene pool of the common wheat.

### *Development of synthetic hexaploids and interspecific composite crosses (Neodomestication)*

In the first step of the experiment different hexaploids amphiploids had been developed via interspecific crosses of durum and einkorn, emmer and einkorn, having ABA genome constitution. After successful genome doubling, the new synthetic hexas were multiplied via and characterised for their agronomic performance using individual spike rows under field conditions. Stable and segregating lines were then separated from each other. The phenotypically stable lines were multiplied and the best lines were selected for further crosses. During the same time the segregating lines were planted to an extreme environmental condition to be able to select materials for marginal environmental conditions, and to maximise the genome rearrangement of the given materials. The selected lines were used as parents of composite cross population, and were crossed with bread wheat to be able to introduce new genes into the gene pool of the bread wheat.

According to the results field experiment of the ABA synthetic composite population, we can conclude, that the yield of the population is still not competitive with the bread wheat composite crosses (the yield is lower with about 10%), but the disease resistance of the population is excellent, no infection were found at all. The lower

yield is practically based on the relatively high percent of open flowering plants, which were mostly male sterile. The rate of outcrossing was also very high, and several new combinations were identified in the next generation. Using pedigree breeding methods, several high yielding and high quality lines (even with very strange quality parameters) were developed, and they are recently used for breeding to be able to develop new variety from this new species.

Based on the above mentioned results, a new neodomestication protocol had been established. If we are able to produce agronomically useful synthetic hexaploids with different genome construction, we would be able to recreate the situation which is similar that of the Fertile Crescent some thousands years ago. Namely, if the synthetics are able to grow together and there is the possibility of the (natural) cross between them, new agronomically useful species should be born in a relatively short period, and (local farmers) breeders can pick up the useful plants, a new domestication should be carried out. In the same time, the genetic diversity of common wheat should be increased by introducing novel genes and gene combination by crossing the new hexaploids with the bread wheat. Finally, interspecific composite cross population can be established via crossing the different interspecific hybrids with each other. According to our preliminary result, the cross between ABA genome structure synthetic hexaploids with ABD bread wheat varieties, gives a high fertility and results in a high recombination frequency between the wheat D genome and the einkorn A genome (as the other has their natural pair). To broaden the possibilities, synthetic hexaploids with AGA genome structure is under development (*T. timopheevii*, AG, crossing with *T. monococcum* Am), and the first synthetic plants are under multiplication in field conditions. The final aim of such project is to set up interspecific composite cross population in which the parents are carrying different genome structure (ABD, ABA, AGA) at hexaploids level to be able to maximise the evolutionary potential of the hexaploids wheat.

Acknowledgements. The recent research was partly financed by the ALKOBEEER project financed by the Hungarian Science and Technology Office, and partly by the European Commission FP7 SOLIBAM project.

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## Crop genetic diversity benefits farmland biodiversity in cultivated fields

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**Keywords:** varietal mixture, biodiversity, predatory arthropods, ecosystem services, biological control

### Introduction

During the second half of the 20<sup>th</sup> century, homogenization has been the dominant model of agriculture in industrialized countries, both at the local (field) scale and at the landscape scale (via e.g. land consolidation). The current intensive farming systems are based on high consumption of fossil fuels, pesticides and synthetic fertilizers, which homogenize environmental conditions both within and among fields. This practice has tended to eliminate genetically diverse local varieties in favour of a limited number of genetically homogeneous varieties (inbred lines or hybrids) that were selected to grow under stable, nutrient-rich conditions (e.g. FAO, 1997). Conservation programs were launched to halt the loss of crop genetic diversity, but mainly in the form of seed banks. The variability of crop varieties was thus merely considered a "genetic resource", i.e. a static gene pool which could be used by conventional breeding programs designed to obtain distinct, homogeneous, and stable varieties. This model is now questioned, especially with increasing awareness of global change, and agrobiodiversity is now viewed as a key component of sustainable farming systems.

In this context of renewed interest for diversity in farming system, the genetic diversity of cultivated varieties should play a central role, not only as a genetic resource isolated from cultivated land, but also as a necessary condition for the existence of agroecosystems that are resilient to environmental change. The presence of genetic diversity within field is indeed likely to provide ecological services that go far beyond a mere reservoir for breeding programs. On the one hand, genetic diversity in the field can stabilize production (Kiaer et al., 2009) and provide greater resistance to pests and diseases (Zhu et al., 2000; Altizer et al., 2003) much more efficiently than monocultures. On the other hand, crops represent a large proportion of biomass in agroecosystems (i.e. they are foundation species sensu Whitham et al., 2006). Previous studies in community genetics demonstrated that the genetic diversity of dominant plant species in a natural ecosystem could benefit specific diversity in the whole associated community (Bailey et al., 2009; Whitham et al., 2006): the loss of crop genetic diversity in agroecosystems may similarly impact wild farmland species.

Farmland biodiversity provides numerous ecosystem services such as pollination (e.g. Klein et al., 2007), biocontrol, mycorrhizal symbioses or nutrient cycling, and is therefore essential to agroecosystem functioning. However many species associated with rural landscapes are threatened by agricultural intensification (Robinson & Sutherland, 2002; Tschamntke et al., 2005), among which crop homogenization may play a significant role. If this were the case, increasing the genetic diversity of crops could thereby constitute an easy-to-implement solution to preserve farmland wild species and associated ecosystem services. Here, we tested the effect of wheat genetic diversity on wild plants and invertebrates using an experimental approach in the field.

### Materials and methods

The experiment was run in La Bergerie de Villarceaux, an organic farm located 70 km north-west of Paris, France. In winter 2007-2008 ten contiguous square plots (60 m wide) were sown with either a "pure line" bread wheat variety (*Triticum aestivum* "Renan", INRA, five plots) or a genetically diverse seed mix including 30

landraces and several pure line varieties. These two diversity treatments were distributed in a checkerboard pattern (Figure 1).

In each plot, we sampled (1) springtails in May 2008, using five systematically located soil cores per plot; (2) ground-dwelling arthropods (spiders and carabids, which are generalist predators) in May and June 2008, using five systematically located pitfall traps per plot and (3) vascular plants in May and June 2008, using 25 systematically located 1m<sup>2</sup> quadrats per plot. Individuals from all taxonomic groups were identified to species level as much as possible, and species abundance was measured as the number of individuals for animal taxa, and as the frequency of occurrence within 1m<sup>2</sup> quadrats for plants.

In each plot, we describe the diversity of wild communities with two different metrics: species richness  $S$ , i.e. the total number of species, and Shannon diversity index  $H' = -\sum_{i=1}^S p_i \ln p_i$ , where  $p_i$  is the abundance of species  $i$ . We also measured a number of wheat phenotypic and phenological traits (tiller number, flowering date, total height at maturity, length, width and position along the stalk of the first leaf, and spike number) on ca. 200 individuals per plot. This allowed us to compare the actual phenotypic diversity between the pure line variety and the variety mix, and to examine the relationship between phenotypic diversity and the diversity of wild species within plots.



Figure 1: Aerial photography of the field

## Results

Overall, we observed a positive impact of wheat genetic diversity on the specific diversity of arthropod taxa: the average number of species per plot and the Shannon diversity index were always higher in the mix of varieties than in the pure line variety, and the difference was significant in many instances (Table 1). In addition, there was a significant correlation at the plot level between wheat phenotypic diversity and the species richness of Linyphiids spiders ( $F_{1,97} = 7.80$ ,  $P = 0.0063$ ) and predatory carabids ( $F_{1,97} = 5.37$ ,  $P = 0.0226$ ). In contrast, we observed no significant difference in the species richness of plant communities between the pure line variety and the variety mix (Table 1).

*Table 1: Mean (standard error) species richness and Shannon diversity index in the mix of varieties and in the pure line varieties, for the six taxonomic groups studied. Significant differences are in bold.*

Taxonomic group	Mean (SE) species richness		Mean (SE) Shannon diversity index	
	Mix of varieties	Pure line variety	Mix of varieties	Pure line variety
Springtails	<b>5,16 (0.33)</b>	<b>4,28 (0.37)</b>	1,27 (0.07)	1,10 (0.08)
Linyphiids	<b>4,26 (0.32)</b>	<b>3,5 (0.30)</b>	0,82 (0.05)	0,61 (0.05)
Other spiders	4,94 (0.21)	4,68 (0.20)	<b>1,27 (0.05)</b>	<b>1,05 (0.05)</b>
Predatory carabids	3,58 (0.17)	3,26 (0.18)	0,86 (0.04)	0,74 (0.05)
Herbivorous carabids	1,14 (0.10)	1,1 (0.11)	0,18 (0.05)	0,18 (0.05)
Plants	6,27 (0.18)	6,54 (0.17)	1,38 (0.03)	1,42 (0.03)

## Discussion

Using an experimental approach in the field, we demonstrated a positive effect of wheat genetic diversity on species diversity of associated invertebrate species.

Our results however demand further validation. First, we did not specifically demonstrate that genetic diversity per se was responsible for the differences in community diversity between the two treatments, because a single pure line variety was used and we may have simply observed a mere genotype effect. Future experiments should therefore compare several pure line varieties with a range of variety mix of contrasting genetic and, most importantly, phenotypic diversity (Hughes et al., 2008). Second, the observed effect remained modest in size: the maximum difference in average species richness between the two treatments was around one species, which raises the issue of the effective role of crop genetic diversity in agroecosystems, which are submitted to severe environmental disturbance causing much larger effects on biodiversity (Tschardt et al., 2005). However, we nonetheless detected significant differences despite the limited temporal and spatial scope of our experiment (a few hectares over six months), and we are confident that the use of genetically diverse crops over broader spatial and temporal scales can have appreciable effects on farmland communities.

If the results of the present study are confirmed, the mechanisms underlying the relationship between crop genetic diversity and community diversity remain to be elucidated. In ecological studies of the impact of genetic diversity on communities and ecosystems, higher species diversity is generally thought to result from a larger number of ecological niches available. Two alternative mechanisms may explain why genetic diversity of dominant species provides more numerous niches: an increase in the diversity of food resources, or an increase in habitat resources, via a larger structural heterogeneity of plants. The ‘food resource’ hypothesis may partly explain the higher diversity of springtail species, some of which feed on roots and on their associated microbial communities, but overall our observation that mostly predatory species are affected by wheat genetic diversity suggests that the structural heterogeneity hypothesis is the most likely. Wheat phenotypic diversity may for example provide a wider range of microhabitats for invertebrate species, which are known to be sensitive to vegetation architecture (e.g. Langellotto & Denno, 2004).

The positive effect of crop diversity on farmland biodiversity, if confirmed over broader scales and for other crop species, may have potential effects on ecosystem services such as biological control and soil regeneration. These effects will have to be tested by measuring ecosystem services directly. Overall, these results strongly suggest that crop genetic diversity, through the use of mixtures of varieties, could therefore benefit both biodiversity and crop production, especially in organic farming and should be promoted as a tool toward sustainable farming.

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## Potential power of the plant-pollinator relationship as a tool to enhance both environmental and production services of grain legumes in the context of low-input agriculture: what do we know?

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*Keywords:* crop improvement- faba bean- floral traits- pollination- soybean

### General Introduction

There is mounting evidence of pollinator decline all over the world. Low-input agriculture is predicted to enhance diversity in agroecosystems. In this contribution, we address the question of how the observed positive effect of organic farming on pollinator diversity and density could be used in grain legume improvement. Plant breeders have the opportunity to develop improved cultivars and practices consistent with the multifunctional character of agriculture by maximizing the services provided by pollinators. Production and environmental services, yield and yield stability heterosis-mediated and preservation of bee fauna by providing foraging places and nesting sites for bees, can only be achieved in tandem, because these two realities are closely intertwined by plant-pollinator relationships. In legumes, bees, as agents of cross-pollination, help seed set and increase the level of heterozygosity and heterosis potential of cultivars, which in turn improve resistance to biotic and abiotic stresses.

Our viewpoint is that the exploration of plant-pollinator interaction will allow the development of environmental and production services of grain legumes by designing crops with appropriate flowers for specific pollinators, providing a win-win scenario and at the same time creating new opportunities for maintenance of crop diversity. We advocate the need to recover and improve functional floral traits that may have been lost through extended breeding for conventional systems. These traits are related to the ability of crops to benefit from associative relationships with beneficial species such as insect pollinators. Fully understanding the patterns of plant-pollinator interactions could answer a wide range of basic and applied questions in breeding. This contribution is an attempt to examine the conceptual framework for plant-pollinator relationship approaches. We discuss advantages of this approach by using our research on soybean and faba bean as exemplar crops.

We first analyse our preliminary work on soybean relevant for the choice of appropriate procedures to develop a hybrid seed production technology that uses insect-aided natural crossing mechanisms of the crop to increase seed set on male-sterile lines, i.e. female parent. Next, we examine studies aiming to analyse the effectiveness of early directional selection for outcrossing in populations. Finally, we discuss experiments related to seed multiplication methodologies that examine the ways in which knowledge of plant-pollinator relationships could contribute to define efficient plot isolation strategies for cultivar maintenance. These studies would be clearly of interest for breeders, farmers, and stakeholders. The effective implementation of the approach proposed as a tool to better associate ecological concerns and the needs of crop production can procure indirect benefits to the farmer as a supplementary source of income (green payments).

### Soybean exemplar case



*Background and Aims.* An efficient pollen transfer mechanism is crucial for the successful development of soybean hybrid seed technology. In the production of soybean hybrid seed it is a standard practice to plant parental lines and leave pollination to bees. In soybean, the proportion of seed set varies dramatically among closely lines (Ortiz-Perez et al. 2006 a,b) and low seed set might be caused by reduced bee visitation rates. To increase seed set, breeders need to identify floral traits that are positively associated with visitation rates. Floral size and shape, is well known to influence pollinator visitation and thus potentially should be an important determinant of seed set. Our research is focused on studying the variation of floral traits as a possible cause of high or low seed set in certain genotypes, with the goal of being used as a management strategy to enhance hybrid seed technology. We present the results of an experiment designed to test whether floral traits exert sufficient pressure on bees to account for the seed set shifts seen among soybean genotypes.

*Material and Methods.* A segregating population from a cross between a male-sterile, female-fertile genotype (*ms2 ms2*) and a male parent (fertile version of T359) allowed the identification of three main categories of seed set lines: high (average number of seeds per plant from 51.75 to 12.5), intermediate (average number of seeds per plant from 8.45 to 7.35) and low (average number of seeds per plant from 4.87 to 1.53). A total of 15 lines in  $F_5$ , 7 lines with high seed-set, 3 lines with intermediate seed-set and 5 lines with low seed-set resulted from insect-mediated cross-pollination were chosen for the study.

We used digital image analysis to measure four standard traits: length, width, area and angle between the two symmetrical sides of the standard. We calculated the ratio of length and width and the perimeter as the sum of length and width. We compared floral traits between high, intermediate and low seed-set level categories in order to evaluate the hypothesis that the differences in seed set may have arisen as a by-product of differences in floral shape and size. The comparison was made using discriminate analysis. The basis idea underlying the discriminant analysis is to determine whether seed set groups differ with regard to floral shape and size and then to use floral traits to help in the development of hybrid technology. If floral traits are different between the seed set groups, we can use this information to identify specific traits to facilitate pollen transfer, thus providing appropriate guidance in the selection of lines to improve seed set.

*Results and Discussion.* The first discriminant function contained a highly significant amount of discriminatory power and accounted for 44% of the variation, indicating that changes in the floral traits result in significant shifts in seed set. This implies that floral traits capture a significant amount of differences between seed set categories to be used in the development of hybrid seed technology. This discriminant function significantly distinguishes between the high and low seed set categories.

Discriminant analysis quantifies the importance of different floral traits in the separation of seed set categories. Examining trait weights, we found that the trait that contributed mainly to the discrimination among the levels of seed set is standard angle. Another variable which also greatly contribute to the discrimination is the standard length. The power of the discriminant traits is determined by the proportion of correct classifications. The accuracy of the assignment varies among the levels of seed set. As expected the large number of misclassification occurs among the intermediate seed-set group, only 20%. The global picture emerging is that high and low seed set lines significantly differ in flower size and shape and that, significant increases in the standard length and in the standard lobe (less flattened standards) are associated with increasing seeds set. This is not unexpected and fit very well to the fact that pollinators often visit flowers on plants with large floral displays. Floral size and shape is of primary importance to pollinators in guiding foraging decisions. The success of hybrid seed production depends on floral traits that manipulate pollinators by promoting the receipt of pollen. Seed set in soybean relied primarily on leafcutter bees because of their ease of management and transportation. In many male-sterile lines, the number of bee visitations can be the limiting step in obtaining optimal seed set. Increasing the attractiveness and facilitating the manipulation of the flowers, by less flattened standards, to leafcutter could, therefore, provide a useful means of improving seed set in these lines.

## **Faba bean exemplar case**

### **1) Effectiveness of early directional selection for outcrossing in populations**

*Background and Aims.* The basic philosophy that has been held by faba bean breeders is to make use of yield heterosis using synthetic varieties. The exploitation of heterosis in faba bean could be improved by increasing the level of heterozygosity which could be achieved through selection for outcrossing. We briefly examine our

research (Suso et al. 2010) addressing the question of open pollinated population response to directional selection for outcrossing. Artificial selection for high and low level of outcrossing was performed in two different faba bean populations to investigate the possibility of modifying the level of allogamy. Under field conditions, the level of allogamy is affected by the availability of pollinators and by the floral traits that either facilitates selfing or outcrossing. Selection for allogamy could affect faba bean floral attractiveness to pollinators. The study of the responses of floral traits to outcrossing selection provides the opportunity to advance in the required search for ways to develop the environmental services of faba beans.

*Materials and Methods.* Two-way selection for high and low level of outcrossing was performed in two experimental populations. Floral traits were classified into three classes: phenology, design, and display. Outcrossing rates were estimated in the populations using isozyme loci and the multilocus estimation program MLTR. To understand the selection process discriminant analysis was used.

*Results and Discussion.* The pattern of responses to outcrossing selection showed a trend opposite to that expected. We did achieve a significant increase on autogamy but when selecting for allogamy and in the process of selection for selfing the progeny returned to the intermediate values of allogamy. Discriminant Analysis revealed how the patterns of outcrossing in the selected populations were due to significant and simultaneous change in floral traits and provided evidence showing that floral traits evolve in response to changes in mating patterns, limiting or contra-balancing the effects of selection.

Selection for outcrossing changed the floral display schedule. Floral display advertises rewards to potential pollinators, and therefore, floral displays have an important influence on mating patterns. Accordingly, to develop improved techniques for managing pollinators as effective tools for crossing, future studies on the constraints to increase allogamy, it should be focused on the analysis of the relationship between floral traits and pollinator behaviour. This approach, in turns, will allow the development of both pollinator-friendly varieties and the enhancement of faba bean environmental services.

## **2) Maintenance breeding methodologies: isolation among breeding materials**

*Background and Aims.* Ensuring adequate isolation between genetically heterogeneous breeding materials is an important step in maintenance breeding. To use the presence of local pollinators to maintain the level of heterozygosity and heterogeneity of landraces seed multiplication under open pollination conditions is required. Generally, physical distance is used to prevent pollen flow. Pollen flow can be further interrupted by planting barriers of the same or another species. In insect-pollinated plants, floral traits are expected to play an important role in determining pollen mediated gene flow variation. We examine the results of a study previously reported by Suso et al. (2008) carried out to illustrate effectiveness of different isolation zones on diverse genotypes for the control of pollen mediated gene flow.

*Material and Methods.* The study analysed the pollen flow in experimental plots that included four homozygous marker genotypes. The strategies tested for preventing gene flow were (1) an isolation zone devoid of vegetation, (2) the same size isolation zone sown with *Vicia narbonensis*, and (3) the same size isolation zone sown with two trap crops: a male sterile line and a tetraploid genotype of *V. faba*. Paternity analysis in conjunction with a multiple regression approach allowed the determination of floral traits that accounted for the largest proportion of variation in pollen mediated gene flow across each of the four isolation zones.

*Results and Discussion.* There were large variations in the patterns of gene flow among the genotypes and isolation zones. Gene flow was not at random but depending upon the genotype and isolation zone. Specific associations between genotype and barriers can bring about gene flow, just as high, or even higher, than those produced by a barren zone. The effect of isolation zone on the gene flow might be explained in terms of their influence on pollinator behaviour. Multivariate regression models showed that differences in floral traits accounted for more than 70% of the variation in inter-plot gene flow. The relative importance of various floral traits depends on the isolation zone. Given that pollen mediated gene flow frequency is influenced by the triple interaction between pollinator behaviour, barriers and crop floral traits this interaction should be considered for decision-making of appropriate diverse population maintenance.

### **General Conclusion**

The use of plant-pollinator related traits in low-input agriculture offers great potential benefits to breeders and farmers: 1) new levels of crop yield based in the exploitation of heterosis and diversity, 2) better design maintenance methodologies for diverse populations and 3) new uses for the crop, environmental services like supporting bee pollinator conservation. However, the effectiveness of this insect-aided technology based breeding strategy is nowadays hampered by non biological constraints, related to the assumed time consuming evaluation and little relevance to breeders.

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## Yield performance in heritage spring wheat and barley varieties in organic farming

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### Introduction

The grain yield in cereals has increased considerably since the beginning of industrialisation of agriculture, including introduction of modern plant breeding 100-150 years ago. Since beginning of this period, plant breeding has selected and bred varieties suitable to the changing agricultural conditions, and hence to increasing extent focused on conventional high input agriculture as this type of agriculture developed. Most pronounced is probably the plant height, and old high plants are likely to lodge at the nitrogen level normally seen in modern industrial agriculture. Modern varieties are characterised by a high degree of specific resistance genes against selected pathogens on the expense on a broad durable polygenic resistance (Robinson 1995). Organic farming is a low input agricultural system, with some similarities with former agriculture compared with modern industrial agriculture. Therefore, heritage varieties selected for, and adapted to the low input agriculture in former days could have some advantages compared with modern varieties, and/or the advantage of using modern varieties could be less pronounced in the low input organic system. On this background, a project in Denmark has multiplied and studied agronomic and quality traits of mainly Danish heritage varieties compared with modern varieties under organic cropping conditions.

### Methods

In spring wheat, 54 varieties no longer in the European Common Catalogue of Varieties of Agricultural Plant Species were compared with 22 high quality varieties on the current Common Catalogue. Trials were carried out over three years at the organically managed farm Mørdrupgård at Zealand, and in one year at Gl.Estrup, Jutland, Denmark. Landraces from Nordic Genebank (NordGen) were extracted by Hans Larsson at Swedish University of Agriculture. Landraces were multiplied, and within the landraces, selection lines were developed. True varieties developed by plant breeders in the period 1882 to 1988 were extracted from Nordic Genebank and multiplied prior to the experiment. This group represent the majority of spring wheat varieties in The Nordic Genebank. As spring wheat has always been a minor crop in Denmark, most of the varieties including the landraces are of Swedish origin, and bred for this area. The modern varieties were delivered by plant breeders in Sweden, Germany, Switzerland and Austria based on their recommendations for good performing varieties with high baking quality under organic conditions in Denmark. The varieties did therefore not necessarily represent the mean of modern varieties.

Protein content was estimated by NIT analysis. Baking test assessing loaf volume was done at Darzau Getreidezüchtung on 20g seed samples in two replicates. Data for loaf volume is only available from the trial at Mørdrupgård 2008.

Two field trial in barley was sown in one year on each of the farms. Varieties included three modern approved malting barley varieties, three 2-rowed landraces, three 6-rowed landraces and one 6-rowed and seven 2-rowed varieties from the period 1868 to 1965. The modern varieties were selected as the recommended malting varieties by the dominating seed companies in Denmark. The older varieties and landraces were selected as the varieties giving the best tasting beer in an experiment among beers brewed on malt from 46 old Danish barley varieties prior to the start of the experiment.

Experiments were sown in 12 m<sup>2</sup> plots in 4 replicates, except spring wheat at Gl.Estrup which had only 3 replicates. Three spring wheat varieties, Øland (landrace), Dacke (released in 1990) and Fiorina (released in 2001) were included in all blocks and experiments in spring wheat, and the mean of these varieties were used to

calculate a relative yield and relative protein content within each block. The widely grown modern malting variety Power was used as standard variety for comparison between the two barley experiments. Statistical p-values were developed by F-test in GLM.

## Results and discussion

### Spring wheat

As can be seen in Figure 1, there seem to be a tendency of improved yield during the period of modern plant breeding. However, this tendency is weak ( $R^2=0.17$ ). When looking exclusively within the group of heritage varieties 1882-1988, the tendency of improved yield during the period seem to disappear ( $R^2=0.02$ ). The protein content seems to be constant over the period ( $R^2=0.02$ ).

When the spring wheat varieties were grouped in three groups, landraces, old varieties and modern varieties, a significant effect of group could be seen on grain yield ( $p < 0.001$ ). The modern varieties had a significant higher yield than the old varieties and landraces. The modern varieties had a relative mean grain yield of 107.9 (105.6 – 110.2), the old varieties had a relative mean grain yield of 88.7 (86.8 – 90.9) and the land races had a relative mean grain yield of 88.1 (85.2 – 90.9).

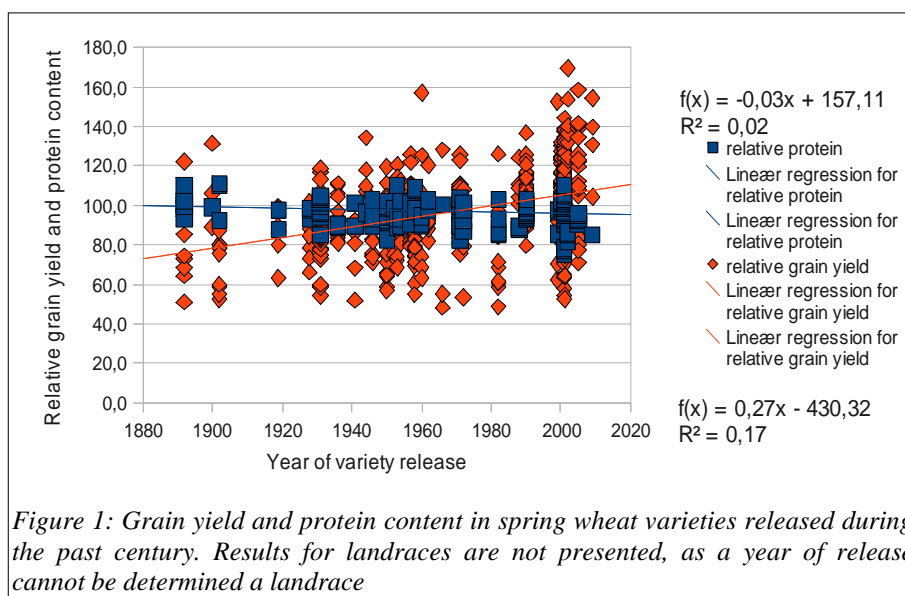


Figure 1: Grain yield and protein content in spring wheat varieties released during the past century. Results for landraces are not presented, as a year of release cannot be determined a landrace

This indicates that the increased yield in the current varieties compared with the heritage varieties may not be an effect of plant breeding, but an effect of the origin of the seed or the selection of varieties to be included in the trial. Plant breeding has developed gradually through the century, and if the increased yield had been an effect of plant breeding, an increased yield would most likely also have been seen also during the period 1882 and 1988. The modern varieties were delivered from seed companies and selected based on their recommendations for organic production, whereas the heritage varieties were multiplied from genebank material and gives a broader diversity of the varieties from this period. This may explain the difference between the varieties on the European Common Catalogue of Varieties of Agricultural Plant Species compared the varieties no longer on this list. Hence, the result does not support the finding of other authors, who concluded a gradual increase in yield though the past and former centuries (Murphy et al 2008, Perry and D'Antuono 1989). In variety field trials, a negative correlation between grain yield and protein content is often observed (Schlehuber and Tucker 1959).

In three out of the four experiments, a weak tendency of negative correlation between yield and protein content could be observed for all groups, landraces, heritage varieties and modern varieties (except the group of modern varieties at Mørdrupgård in 2006), but this tendency was not significant (Figure 2 a-c). In the field trial at Gl.Estrup 2007, no correlation between yield and protein content was seen (Figure 2d). The soil at Gl.Estrup is a eutrophic peat soil, whereas the soil type at the tree experiments at Mørdrupgård is a more normal middle loam soil with less nitrogen available in the soil.

There were no correlation between protein or gluten content and loaf volume in these trials. Most likely, the gluten content were in all varieties so high that other factors were limiting for loaf volume. Under these conditions, heritage varieties had 9% higher loaf volume than modern varieties ( $p > 0.99$ ). This result is surprising, since modern varieties had a higher gluten index, and a high gluten index is considered as a quality criteria for wheat breeding.

#### Spring barley

As can be seen in Figure 3, the modern varieties were the highest yielding with a mean relative yield of 104.2% of Power, and the heritage varieties had an average lower yield of 75.4%. Landraces had the lowest yielding with a mean yield of 46.3% of Power (data not presented in Figure 3). There was a weak but still significant difference between the groups in yield ( $p = 0.09$ ). However, only few varieties were included in each group and therefore individual varieties had huge impact of mean yield of each group. In the group of heritage varieties, several varieties performed a yield not statistically different from the variety Power, which was the most widely grown modern malting barley in Northern Europe at the time of the experiments.

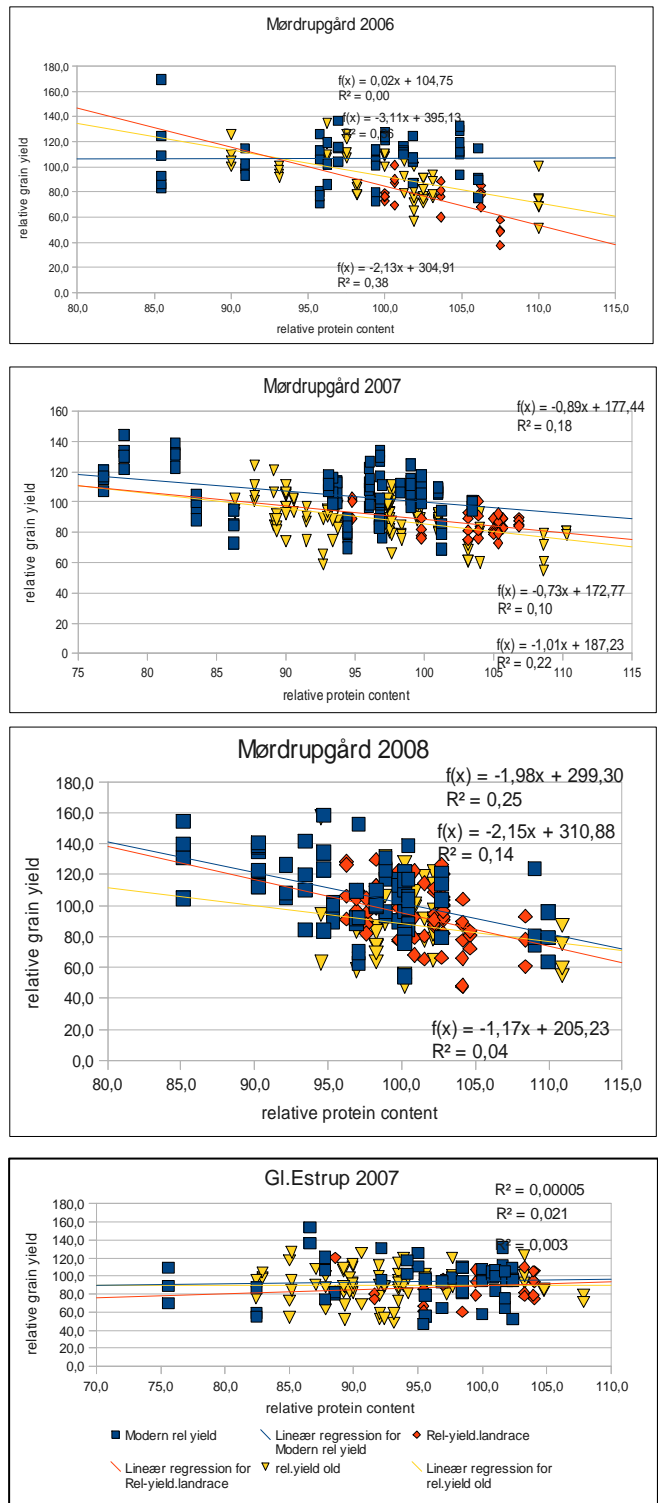


Figure 2a-2d: correlation between yield and protein content in 4 spring wheat field trials grouped in landraces, old true varieties, and modern varieties.

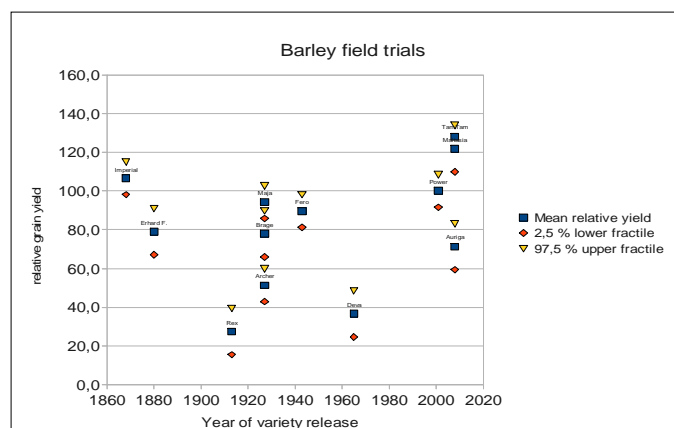


Figure 3: Barley grain yield in two years field trials. grain yield in landraces not presented as landraces has no specific year of release

These results indicate that the age of the variety is less important for a high yield than the yield potential of the specific variety. In general modern varieties perform well under organic conditions, but so do some of the heritage varieties.

Mineral content of the spring wheat varieties in this study was included in a study of grain mineral content published by Hussain et al 2010. The study indicates an improved mineral content in older varieties and landraces compared with modern varieties, which confirms conclusions of Murphy et al 2008. Our study point on improved baking quality in terms of loaf volume in older varieties compared with modern wheat varieties.

The study contradict the general agreement that modern varieties are better than older varieties. This seem not to be the case for the heritage varieties when grown under the organic conditions in this study. Both high yielding and low yielding varieties are found both among heritage and modern varieties.

Modern varieties are bred for high input condition, and under these conditions, the conclusion of improved yield as a consequence of plant breeding may be correct, but our study shows that under Danish organic condition, there may be a slightly higher average grain yield in the modern varieties, but the difference is surprisingly small, if existing at all. It indicates that organic farming in Denmark has achieved little, if at all, from modern conventional plant breeding, as several very old varieties are fully compatible in terms a yield with the normally grown modern varieties.

The results calls for targeted breeding for low input conditions, as breeding for high input condition contribute little to the benefit of organic agriculture and other low input conditions. Research is needed to quantify the effect of the different yield components of breeding on grain and straw yield and quality at low input conditions.

#### Acknowledgement

The experiment was supported by the Ministry of Food and Agriculture, program for plant genetic resources

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## Session 1 - Posters

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### 1. Opportunities for genetic base broadening of crops

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*Keywords:* genetic base broadening, sustainability, climate change, barley

Plant breeding often comes with trade-offs; inbreeding over thousands of generations has contributed to the creation of a stable high quality end product but has also led to the loss of variation in many other valuable traits such as pathogen resistance and nutrient uptake abilities. In part this dilemma is created because of conflicting needs: homogeneity in crops to obtain a stable product quality and heterogeneity in crops to deal with the wider pressures from the environment. Biotechnological solutions, such as introducing a few specific genes in food crops to satisfy these needs may be a temporarily solution, but it might be more sustainable to look at other ways of dealing with environmental factors that determine crop success. A sustainable way of dealing with environmental crop pressures like diseases, nutrient deficiencies and water availability, may be to increase the variation within the crops so that these 'varietal populations' are able to deal with these pressures as in natural populations. By crossing several varieties with each other to increase the variation within a crop species it is possible to create a composite cross population. Even for highly inbreeding species, like most cereals, it is possible to create more diverse crops also referred to as populations.

A pioneer of composite cross populations was Harlan in Davis CA in the 1920s, who crossed a dozen barley lines with each other to construct a population that was to be used merely to study local adaptation. After that, composite cross (CC) populations have been researched extensively for yield, heading time and pathogen resistance (Allard, 1988; Goldringer et al, 2006; Harlan, 1921; Ibrahim & Barrett, 1991; Paillard et al, 2000). In terms of yield, Suneson (1956) found that the first generations of the CC populations were underperforming compared with cultivar varieties (under low input), but after 29 generations these populations outperformed modern cultivars available at that time. Heading time studied by Goldringer et al (2006), in North-South distributed populations demonstrated that there was still a lot of maintained variation reflecting the complex underlying genetics of this character. In terms of pathogen resistance, extensive work has been carried out on the evolutionary dynamics of rare alleles involved with resistance against different pathogens (Ibrahim & Barrett, 1991; Paillard et al, 2000). Both studies found that the loss of rare alleles due to genetic drift and local adaptation influenced resistance against powdery mildew. In certain locations where there was pathogen pressure, alleles were maintained whereas they were lost in places with lower pathogen pressures.

We are creating populations from barley cultivars and landraces to study whether these populations can be used for agricultural purposes and as a model for adaptation of populations to dynamic environments in relation to climate change. In this study we have been looking at genotypic and phenotypic changes of single genotype cultivars and landraces of barley which were subsequently hybridized over three generations in the green house. Morphological traits under study include length of ears, total plant height, leaf surface area and number of seeds. Microsatellite analysis will also be used to determine variation within and between populations. Studies will examine variance for morphological traits between intercrossed populations and single line blends.

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## 2. SOLIBAM, Strategies for Organic and Low Input Breeding and Management, a European programme (2010-2014)

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*Key-words:* breeding, crop management, organic agriculture, diversity

SOLIBAM (Strategies for Organic and Low Input Breeding and Management) is based on “diversity” : genetic diversity within crops, diversity in cropping systems and in management practices, and wild biodiversity present in agro-ecosystems. Through the history of agriculture, agronomy, crop management and plant breeding have always been linked. Our research hypothesis is that developing diversity at all levels is the best strategy for improving crop adaptation to fluctuating environmental conditions and for increasing yields and yield stability in organic and low-input systems.

The project is a consortium of 23 organisations from 12 countries in Europe and Africa, which began in March 2010 for a duration of 4.5 years. We aim to increase our understanding of the vital role of diversity in agricultural activities, through plant breeding and crop management, and their impacts from the soil to the market.

The programme is developed around three groups of species: cereals (wheat, maize, barley...), legumes (faba beans, niébé, beans) and vegetable (broccoli, tomato).

Plant breeding for resilience begins with the identification of traits important for adaptation to low-input/organic conditions over a wide range of agro-climatic conditions in Europe. The development of efficient phenotyping and genotyping tools aims to monitor both heritable variation and genetic diversity during the selection processes. Molecular analysis of functional polymorphisms will increase accuracy in breeding methodologies and the understanding of adaptive phenomena.

Examples will be achieved through the creation of novel diversity within the range of species involved in the project, enhancing existing diversity within-crop to cope with current and increasing variation in environmental conditions faced in organic and low-input agriculture and to provide greater buffering potential. The diversity explored includes landraces adapted to specific pedo-climatic regions, physical mixtures of varieties, new crosses between existing farmers' varieties, new synthetic varieties and composite cross populations.

Crop management integrated approaches will help to exploit crop diversity for resilience to enhance crop performance in the different contexts taking into account the perspectives of local stakeholders. Specifically, it is expected that inclusion of legumes in association with arable and vegetable crops and that diversifying cropping systems will increase diversity-driven agroecological services that have consequences for agronomically important outcomes such as increased crop yield, yield stability, weed suppression and soil fertility.

A large part of the methodological development will involve several kinds of stakeholders and especially farmers in the framework of participatory research in relation to the diversified management practices, needs, expectations and traditions of farmers, end-users and consumers. Participatory Plant Breeding and Management (PPBM) strategies are perhaps best suited to meet the specific and original demands of organic and low-input agriculture, particularly related to small-scale farms or farms in marginal areas. Particular attention will be paid to quantifying the effects and interactions of breeding and management innovations on crop nutritional, organoleptic and end-use quality. Elsewhere, with the collaboration of small regional seed companies, we aim to compare the effectiveness of different breeding strategies under conventional, low input and certified organic farming conditions and to set up optimal breeding strategies for the production of varieties suitable for organic and low-input farming.

Environmental, economic and social sustainability will be assessed at three levels: i) the cropping system, ii) the farm and iii) the food supply system, from breeder to farmer to consumer (including technical and legal aspects of non-conventional varieties developed on-farm).

### 3. Suitability of the emmer wheat land races for the organic farming

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**Keywords:** emmer, genetics resources, agronomic traits, quality, organic farming

The paper aims to refer to a possibility of selection of suitable genetic resources of emmer wheat [*Triticum dicoccum* (SCHRANK) SCHUEBL] for the organic farming system (the varieties being suitable for growing and having a high nutritive value). Morphological characteristics of the emmer wheat cultivars indicate their high competitiveness to weeds. The length of plant is thought to be a positive aspect, however it does not contribute to a reduction of the resistance to lodging. This one is assured by a combination of width and length of stalk in the case of the tested cultivars. The flag leaf is narrow but long enough to provide a sufficient assimilation surface. The emmer wheat is characterised by a fast growth of phytomass in the initial growing stages. It contributes to an enhance of the resistance to drought, which seriously damages the crop stands in the grain filling stages. It also inclines to the resistance to drought, as it has lower  $\delta^{13}\text{C}$  discrimination values, compared to the control varieties. The plants are, therefore, highly resistant to usual wheat diseases under a natural pressure of pathogenes. The emmer wheat provides lower, but more stable yield level. Particular emmer wheat cultivars grown in an absence of nitrogen achieve the similar hectare yield as the wheat control varieties. Low harvest index is a factor reducing the efficiency of the emmer wheat growing. They have short very dense spikes and their grain weight per spike is reduced. The correlation between the number of grains in spike and weight of spike may serve as a selective criterion of the above-mentioned characteristics. The emmer wheat cultivars are more stable from the point of view of the establishment and following reduction of the number of grains in spikelet, compared to the soft wheat, grown in the conditions of absence of nitrogen. The emmer wheat cultivars also have a high protein content in grain and they almost do not swell (the crucial factors of the final use of the production). Therefore, their grains are not suitable for yeasty products. The quality of proteins, represented by the proportion of the essential amino acids, is similar to the one of the soft wheat cultivars. Lysine is the first limiting amino acid. The emmer wheat grains are suitable for the production of wholemeal flour. Traditional technologies are used there - they assure the added value of the product made of the emmer wheat flour. Acknowledgement: This work was supported by the research project No. MSM 6007665806 of Ministry of Education, Youth and Sports of the Czech Republic and research project No. NAZV QH82272 of the National Agency for the Agricultural Research of the Ministry of Agriculture of the Czech Republic.

### 4. Pluridisciplinary points of view on Resilience: Lessons for breeding?

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Physicists consider resilience as synonymous with material resistance to shock. Computer scientists describe resilience as a system functioning correctly despite some shortcomings. For ecologists, it represents the regeneration ability of an organism or a population, and also, the capacity for an ecosystem to return to equilibrium after a disruption. For instance, it could be seen as the natural resistance of some fishes based on their fecundity level.

Economists distinguish resilience that ensures the integral conservation of an economical state (stationary socio-economy) from those that contributes to a reform (dynamic socio-economy). Psychologists compare resilience to invulnerability i.e the ability to take act of a traumatic event to overcome it.

More and more expressions like *resilient system*, *resilient business* and *resilient community* appear into scientific (or not) publications related to very diverse disciplines. They characterize a type of homeostasis allowing systems to recover their initial conditions or to maintain their initial functions into dynamic and changing environments.

Changing environments are increasingly frequent into agriculture and may be controlled or not. The authors of this paper propose to consider this concept of Resilience and its associated notions "Complexity", "Equilibrium", "Tolerance threshold",

"Resources", ... from several multidisciplinary point of view. The objective is to enlarge the understanding of this term and give new ideas for plant breeding oriented to low input cropping systems.

## 5. Evolution of Maize landraces throughout its conservation

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Genetic resources of Maize, conserved in genebank, include populations and inbred lines. Those landrace populations were cultivated until the appearance of F1 hybrid varieties after the fifties. Afterwards, they have been collected either by scientists either by breeders. Around 1980, under the authority of the French BRG (Bureau des Ressources Génétiques = French Genetic Board) the organization of French genetic resources focused around networks of conservation. For Maize, this network involves French institutions (INRA, GEVS, CTPS...) and private breeders (Pro-Maïs).

The management of those populations is shared in-between partners of the network. INRA is in charge of the animation of the network and conservation of those populations in the genebank. The multiplication is carried out by all partners under the same rules. A minimum of 400 plants are used to practice cross controlled pollinations (200 plants as male and 200 as female). 200 ears are harvested; 12 equilibrated mix with 3 kernels of each ear are specially dried out to 7% humidity and conserved in sealed bags. The rest constitute the bulk. Until 1993, all the populations were multiplied every 15 years. Due to improvement in the conservation conditions, the gap between 2 multiplications should be increased.

Elsewhere, landraces have also been conserved and multiplied in farms, under different conditions and different ways of multiplication. Non Governmental Organisations (Bio d'Aquitaine...) are also involved in collection and conservation of those landraces under different conditions. We would like to compare the different ways the conservation.

The objective of this project is to understand the genetic evolution of the diversity involved in different adaptive response, depending on the way of conservation. Is there a genetic drift in one or the other way of conservation and multiplication?

Field trials will compare landraces populations with same names (for example: "Grand Roux Basque"). These populations will be collected in different genebanks, farms, organisations of conservation and programs on diversity (FarmSeedOpportunities, RESGEN088...). A set of 5 original landraces throughout 3 generations of multiplication will be the start of this trial. Phenotypic characters as well as molecular markers will be used for this purpose. We hope to develop a method to understand the evolution of the diversity depending on the way of conservation.

This project is funded by the European Union in the SOLIBAM project (Strategies for Organic and Low-input Integrated Breeding and Management: FP7-KBBE 245058, from 2010 to 2014).

## 6. Breeding for resistance to common bunt (*Tilletia caries*)

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**Keywords:** resistance, population, plant pathology

Common bunt is one of the most devastating plant diseases, and a permanent threat to organic wheat production. The diseases can be controlled by the use of resistant varieties, but only few varieties with known resistance are available in the list of approved varieties in EU. Like any other disease resistance, virulence in the pathogen can develop if resistance is based on a single gene in a pure line variety. Breeding for a resilient resistance in organic farming therefore should be based on multiple gene resistance and/or based on different genes within a diverse wheat population. The breeding program therefore focuses on screening for resistance in a broad basis of varieties including modern and heritage varieties of bread wheat, spelt and other closely related wheat species. New resistant lines have been identified within species of species *Triticum aestivum*, *T.spelta*, *T.macha*, *T.dicoccon*, and *T.timopheevii*. In the BIOBREED project, DNA markers will be developed for the resistance found in the screenings. A composite cross population of bread wheat has been established based on 30 varieties with resistance or low susceptibility to bunt, and selection under field disease pressure and marker assisted breeding will be used to improve resistance in the population for long term stability of the resistance.



## 7. Growing together - evolution of plant height in wheat composite cross populations

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**Keywords:** Competition, composite cross populations

Conventional breeding relies on active selection of the best performing plants from multiple crosses, while discarding the remainder of the offspring. In evolutionary breeding (Suneson, 1956, Wolfe 2008), on the other hand, the entire offspring population is sown in bulk, without active selection. Instead, by using a random sample of the harvested grain as seed for the next generation, year after year, evolutionary breeding lets natural selection act on these composite cross populations (CCPs). Since the more productive plants will contribute more seed to the next generation than poor performers, this process can lead to yield improvement of the populations. However, in comparing the better and poorer performing genotypes, it is unclear which plant traits will determine the general evolutionary paths taken by the CCPs. One obvious general factor is the competitiveness of the individual plants when grown together. Our question was therefore how competitiveness of wheat plants evolves within the CCPs over generations. Specifically, we investigated the evolution of plant height within the CCPs as one indicator of competitiveness.

Three composite cross populations of winter wheat were generated from various crosses of 20 parent varieties. These were trialled in randomized field trials with 3 replicates in 4 locations (2 organic sites and 2 non-organic sites) in the UK over 6 years. Straw height was measured from the ground to the base of the ear each year for 10 plants per population. We found a general trend for the average plant height within the populations to increase over time. At the same time, variability of height tended to decrease over time. These effects were clearer on the conventional than on the organic sites, which could be related partly to the use of growth regulators in the conventional systems.

As plants compete against each other for light within the populations, taller plants will tend to outcompete short-strawed plants. Taller plants may therefore produce more offspring and contribute more to the next year's seed, explaining the increase of average crop height of the populations over years. At the same time, however, the trade-off in resource allocation within the plant may cap this development, because, at some point, longer straw will mean fewer resources available for grain formation. It is important to observe the populations further to test this idea of a saturation effect in plant height.

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## 8. Conservation and use of local cider-apple cultivars in Asturias (NW Spain)

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**Key-words:** Biodiversity, disease resistance, evaluation, fruit quality, germplasm

The traditional search of fruits with favourable traits has created a tremendous diversity of apple cultivars. In Asturias (NW Spain) cider has been made for centuries, and linked to this tradition there is a high diversity of apple cultivars. These ancient resources should be conserved because of the potential loss of agronomic and adaptive traits that could be used in future breeding programs and because of they represent a biodiversity and historical link. As most of these local cultivars were poorly represented just in one or a few high-stem old orchards, there were problems with in situ preservation and therefore serious risk of extinction of many of them. The aim of this work was to explore, to conserve, to evaluate and to make the most interesting cultivars available for apple growers. An intensive exploration was conducted in Asturian old apple orchards during 1995-1998. Cultivar selection was made with the following criteria: good level of disease resistance, high and regular yield and/or high level of polyphenols (bitter cultivars are demanded by cider makers). A total of 425 accessions were collected and added to the Apple Germplasm Bank of this region, which currently has more than 800 apple accessions, 550 of them being local cultivars. The 425 collected accessions were planted, conserved, evaluated and characterized in three different orchards. As a result, 12 cultivars have been selected for their agronomic and technological

traits and will be available for apple growers soon. Therefore, this work has contributed to the conservation and the reuse of underutilized apple genetic resources.



## 9. Dry season sorghum: diversity agrarian and diversity cultural

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*Key words:* Sorghum, crop diversity, local nomenclature, Northern Cameroon

Dry season sorghum has now become a staple food for most of the Northern Cameroon populations. The crop is cultivated during the dry season on clay soils characterized by a very high water retention capacity (vertisols) that are unfit for rainy season agriculture.

Providing a second harvest in the middle of the dry season, it represents an important food surplus, thus enabling a large number of families to go through the lean season more easily. Moreover, such an off-season activity lightens the very dense yearly work schedule of the northern Cameroon farmers. Over the past fifty years its culture has increased exponentially.

Our research program - conducted by a team of geneticists, geographers and anthropologists - ambitions to understand how cultural factors and biological ones interact and take part in the shaping of dry season sorghum's genetic diversity.

Dry season sorghum can be subdivided into two main groups: 1/ Muskuwaari (in Fulani) is transplanted on untilled vertisol fields after the last rains of the wet season; 2/ Baburi (in Fulani) is transplanted during the end of the rainy season on hoed soils - whose water retention capacity is weaker than that of the vertisols. For this study we will focus on two neighboring ethnic communities who, despite dwelling in a relatively restricted area, grow dry season sorghum in very different ways (different techniques, tools, and agricultural calendars). The Tupuri mostly grow the Baburi form, and have been doing so for a long time. It is supposed that these Baburi have been diffused from the southerner Bénoué valley. As for their Wina neighbors they have adopted the Muskuwaari forms at the turn of the last century. Our study endeavors to understand to what extent these different ways of cultivating dry season sorghum would be induced by the cultural and ethnic specificities of the studied communities, or the environmental constraints or again the genetic properties of these two groups of dry season sorghum.

The terminology and categorization of the different types of sorghum have been established through thorough ethnobotanical surveys (free- listing, pile-sorting, interviews). The various agricultural techniques (field preparation, transplantation) and uses (transformation, consumption and trading) have been described through direct observation and repeated interviews.

The genetic analyses of samples from the different named categories of sorghum by microsatellite molecular markers will allow us to characterize the genetic diversity at work in both communities.

The first results indicate a very high cultural value of dry season sorghum for the Wina from the village Djongdong. Farmers named over 15 different types under the Muskuwaari category, and most of the farmer (63%) claim to have acquired their seeds from their fathers. The Tupuri from the village of Bouzar grow two main types of Baburi, while also growing some types of Muskuwaari whose cultural value seems much less important than for the Wina: the Tupuri prefer growing wet season sorghum and groundnuts instead. The relations between the actual genetic diversity of the cultivars and the diversity of the types as named by the farmers will be discussed.



## 10. Is it possible to associate the resilience of non intensive agro-ecosystems with the high yield of modern varieties?

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Over the last thirty years, volumes of global agricultural production have increased significantly thanks to the specialization of farming systems and the search for high yielding varieties. The scientific and technological development related to globalization process has resulted in agro-ecosystems mainly based on the use of a low number of species and genetically uniform varieties with high performance. Nevertheless this strong increase in world food production has today many failures, due to the loss of genetic diversity and the low resilience in agro-ecosystems. In particular, the resilience of non intensive but low productive agro-ecosystems is usually ensured by farmers' strategies for the management of environmental risks which is usually based on a large panel of interspecific and infraspecific taxons. One may wonder if it is possible to associate the resilience of non intensive agro-ecosystems with the high yield of modern varieties. This question was recently addressed by several authors.

We achieved a literature review to put evidence on strategies that combine both productivity of agricultural systems and conservation of natural resources. To reach this goal we carried out a critical analysis of measures used by farmers in intensive and non-intensive agro-ecosystems, such as the cultivation of different varieties in the same field, or the combination of plant breeding systems and meta-population management.

Conservation approaches have been implemented, including in situ, on farm or dynamic conservation, in order to preserve biodiversity and evolution process. Several studies on different species showed that the regular introgression of local varieties by genes from improved material associated with mass selection is an efficient mean to cope with environmental uncertainty and ensure resilience. It has also been demonstrated that it is possible to increase yield while maintaining resilience in harsh environments. For example VomBrocke et al. (2003) investigated the consequences of farmer's strategies pertaining to seed management and the choice of pearl millet varieties that are cultivated on the production of pearl millet fields in Rajasthan, a very dry region of India. Nevertheless, further experiments are needed in order to evaluate whether maintenance of genetic diversity is compatible in the long term with high production levels.

## Session 2

### Global change and adaptability

Thursday, December 2 nd - 8:30 am

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#### Steps towards an ecological future

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#### Introduction

Two of the current public discussions are first, how to feed the burgeoning and aspirational world population as it grows towards its 2050 peak, and second, how to slow down, or better, halt the continuing loss of biological diversity and weakening of the associated ecosystem services. Curiously, and depressingly, these two discussions are usually held separately even though they are two sides of the same problem. Concerning the natural world, the most obvious impact is spatial because of the spread of both agricultural land and of urbanisation, eliminating natural habitats. But there are also major negative interactions caused by industrial agriculture, principally large-scale monoculture, that result in, for example, pollution of air, soil and water.

However, because of current and future food shortages, some argue that we need to carry on expanding agricultural production using industrial methods, whatever the cost to the natural environment – except, perhaps, for a little tinkering around the edges. Others argue for the 'parks and prairies' divisions – complete separation of agricultural and natural areas. However, in our view, the problems of food production and biodiversity need to be treated as one: by doing so, we may achieve sustainability in its most profound sense, in that our existence as well as that of other species can be sustained in the future. In this paper we show how plant breeding can be used to integrate food production and biodiversity in a mutually beneficial approach.

#### The monoculture problem

Large-scale monocultures are convenient in terms of labour, input applications and harvesting. Breeding for this approach has led to spectacular increases in grain yield per hectare although the biological and financial costs of obtaining and maintaining such yields are high, and increasing. Moreover, the artificiality of the monoculture approach creates problems that are difficult to resolve. For example, yellow rust (stripe rust) disease of wheat is caused by a fungal pathogen, *Puccinia striiformis*, which co-evolved with wheat and its relatives over a long period. The pathogen survived by evolving into a spore-producing 'factory'. Originally, this was because of the difficulty of dissemination of such a small organism among scattered host plants, and of ensuring that it would be able to 'find' a host plant with which it was compatible (matching virulence).

Disastrously, the introduction of bread wheat as a major crop followed by the massive increase in area and in monocultural production, has meant that the success rate of the spores produced by the pathogen increased by several orders of magnitude, leading to large-scale epidemics. These have been delayed to some extent by breeding resistant varieties and developing fungicides, but this has not prevented further evolution of the pathogen in its new global environment. As man helps to distribute host and pathogen germplasm worldwide, Milus et al. (2009) and others have shown that pathogen lines have now emerged that are more aggressive than

previously known. Moreover, the pathogen is able to express this aggression over a wider temperature range than ever before.

Unfortunately, similar developments are occurring with other plant pathogens – and also with pests and weeds. For instance, the massive use of glyphosate and genetically modified plants carrying herbicide tolerance has resulted in glyphosate-resistant weeds (target and non-target) becoming a major problem (Gaines et al., 2010). These are all examples of the effects of loss of biodiversity in the host crop: the new straitjacket of controlled biological and physical environments attempts to deny the evolution, on the one hand, of the pathogen or weed, and on the other, of the complex community of micro- and macro-organisms that are often involved positively in the nurture and protection of the crop.

In this context, it is interesting to note the observations of Jarvis et al. (2008) who found extraordinary reserves of crop genetic variation maintained by small farmers for use either as insurance or for cropping in mixtures. This shows the continuous link in the tradition of farmers relying on diversity as crop breeders for the past 10,000 years or so.

### **Polyculture, functional biodiversity and population breeding**

#### *Background to population breeding*

The idea and practice of using composite cross populations (CCPs) as an alternative to monoculture breeding was first developed in California for barley and later formulated by Suneson (1956) as evolutionary breeding. Winter wheat CCPs were generated and first trialled in the UK, starting with a DEFRA project (AR 0914: 2001-2008; Generating and evaluating a novel genetic resource in wheat in diverse environments) and continuing in a DEFRA-LINK project (LK 0999: 2008-2012; Adaptive winter wheat populations: development, genetic characterisation and application). The initiative is also being continued and expanded in a new EU Seventh Framework programme, Strategies for Organic and Low-input Integrated Breeding and Management (SOLIBAM), which runs from 2010 to 2015. These initiatives have benefited from other recent population projects, particularly in France (Goldringer et al., 2006), and have also led to a range of other related projects in Europe. The population trials in the first project are analysed by Döring et al. in this conference and their parents by Jones et al. (2010).

Developments through these projects will allow a major expansion of the principle of evolutionary breeding to include more crops, a greater geographical exposure and perhaps most important, a much wider consideration of how they might be used to develop more productive, robust and biodiverse farming systems to overcome the long-term weaknesses of monoculture.

#### *Performance of the populations*

On average, the UK CCPs show only a small yield advantage over the means of their parents. However, Döring et al. (this conference) have shown emphatically how the CCPs are better able to cope with environmental variation temporally both among and within sites relative to their parents (the variety mixtures were similar but less so). Such buffering against all variables among sites and years must be due to the immense diversity among genotypes in the populations and mixtures.

Because of the numbers of parents of notably different origins and ages used, it is likely that, by simple calculation, the potential number of genotypes generated in the CCPs would have exceeded the numbers of seeds produced by some orders of magnitude. The mixture diversity, on the other hand, was limited to the original numbers of parents physically mixed into each, unless there was out-crossing in subsequent generations (mixture seed was harvested as a bulk each year and re-sown, to mimic the CCPs). Despite the initial burst of diversity in the segregating populations, they were expected to return to homozygosity within, roughly, ten generations, assuming no out-crossing. However, even a small degree of out-crossing, which is common in wheat, would maintain some heterozygosity and segregation permanently in the populations and mixtures.

The field trials with the Y (yield), Q (quality) and YQ (yield and quality parents intercrossed) populations under both organic and non-organic conditions showed consistent yield ranking in the expected order of Y (highest yield) and Q (lowest), which was reversed for protein content. However, there was a tendency for the YQ population to be closer to Y than to Q for yield and closer to Q than to Y for protein production. This is a potentially valuable difference from the generally accepted observation among breeders of a negative correlation between yield and quality among homozygous inbred selections; it raises the possibility of a dual purpose crop.

For most of the characters assessed during the life-cycle of the populations, the performance was at least equal to that of the means of the parents, the main exception being Hagberg Falling Number. The poor performance in



this aspect was due, presumably, to the variation in maturity of the many different genotypes in the populations. Despite this apparent deficiency, quality tests with the populations for both industrial and artisanal bread-making have been generally positive.

There was also a concern that plant height would increase to unacceptable levels over the years, with plants in the populations evolving towards higher competitiveness for light. However, an analysis of plant height over several plant generations could not confirm this since height was highly variable and environment-dependent.

One of the proposed advantages for the CCPs was the potential ability to adapt to different sites. This has been proved at least at a large scale in that the YQ seed sent to Hungary in the early stages of the first project (AR 0914) adapted rapidly to cold winter survival and quickly came to yield as much as local varieties in that country (Kovács, pers. comm.). However, we do not yet know whether more localised selection is possible or whether such effects might be hidden in the overall environmental variability; this will be investigated further in the coming year.

#### *Improving the population approach*

Although the YQ CCP is proving effective and adaptable at the European level, this does not mean that it is the best varietal combination for all purposes. Nevertheless, provided large seed stocks are maintained from year-to-year, this single, 20-parent population might be adaptable to a wide range of uses and environments. For example, we are in the process of mass selection of dark grains for improved baking quality because grain colour is associated with protein level. In another trial we are selecting the population for performance as a spring wheat. If this is effective, we may then be able to select the spring population for dark grain.

Populations are relatively easy, inexpensive and quick to generate so that different populations can be produced for different needs, either in terms of end-use or environmental adaptation. Indeed, the greater the range of populations generated and used, the greater the overall diversity to help constrain the increase and successful evolution of pests, pathogens and weeds.

For some purposes it may not be necessary to generate new populations – the required objective may be better achieved simply by adding a specific variety, variety mixture or even population to the original population. This approach, using population-variety mixtures will be investigated for yield performance in SOLIBAM.

On the other hand, there is also an interest in developing populations that may go beyond the scope of current varieties and populations, for example, in dealing with climate change. This approach, which may require pre-breeding of the required population components, is also being tested in SOLIBAM through generation of synthetic hexaploid and tetraploid wheats which may lie outside the evolutionary history of the crop. Populations based on such novel parents may, for example, extend the tolerance of the crop to dry/wet/cold/warm environments and to the variability within each. Buffered populations should be more successful than individual genotypes or ideotypes.

And we should go further. Different species under evolutionary breeding should be brought together in different forms of inter-cropping to ensure selection for mutual inter-crop performance. This may provide a rapid and effective means of dealing with some of the competition problems that have been encountered in earlier attempts at inter-cropping different species. The ultimate objective in our view is to use such approaches to improve the potential and performance of eco-agroforestry systems as a way of maximising diversity within and among crops so as to integrate food production and support for biodiversity.

These methods of breeding within the crop at the population or community level rather than at the level of the single genotype would also provide a more rapid and practical way of exploiting the wide range of genotypes available in gene-banks and farmer collections. As an approach to ecological farming, this should help further to reduce or eliminate the need for external inputs while encouraging the maintenance of a wide range of biodiversity.

#### *What are the possibilities for such a radical approach?*

An initial hurdle lies in the current breeding and legal framework. The development of pure line breeding in the context of the industrial agriculture revolution over the last hundred years has led to a system which, although it may be regarded as highly productive, is also highly restrictive and expensive in terms of the use of oil and other resources. One can argue that in its original intention the system was set up to protect the farmer from unscrupulous breeders and seed merchants. Currently, however, it can be regarded as a system to protect breeders and seed merchants from the farmer and change. But, as always with the law, when the needs change, changes are needed.

As the variety laws stand at present, populations of wheat and other self-pollinated crops cannot be legally traded. Ironically, the use and trading of variety mixtures is permitted in the EU – the seed lot simply requires a label declaring the varieties used in the mixture. Unfortunately, a seed lot of a population, even though it could be labelled in a similar way, is not permitted.

Ideally, we should encourage a change in the 50 year old law. Indeed, the officials involved in administering the law have agreed that a possible alternative would be a system of transparency and traceability in which any farmer buying population seed would be able to know the parents used in the population and its production history. The seed would be subjected to seed health testing as in the current system. The only problem with this proposal is that, if accepted, it might take ten years or more to become law.

Other alternatives would be to use populations only in closed contract deals involving an end-user, or to create new species from the relatives of wheat used as population parents. Both of these approaches would be outside the existing law.

A more radical approach would be to follow the Open Access system currently used in software development (Kloppenborg, 2005). This would eliminate the charging involved in the system. Anyone involved in population production would have to accept that the material that they produced would be freely available (other than seed production costs) to anyone who wanted to exploit it further.

If the population approach and its potential applications do prove to be biologically sound and desirable in a time of climate change and resource scarcity, then a lack of action over the law could lead rather quickly to an anarchical approach, which may not be altogether satisfactory.

### Conclusion

As resources become more scarce and climates less predictable, large-scale monoculture will become less able to cope and there will be a need for more biodiversity at all levels. We believe that one effective, cheap and readily applied solution will be the use of high-yielding composite cross populations that provide rapid and dynamic responses to change while limiting the potential for evolution in pathogens, pests and weeds. However, this approach will need to be integrated into farming systems that make greater use generally of plant diversity and which thus enhance the importance and maintenance of biodiversity as a whole. This in turn will improve ecosystem services and so help to ensure a sustainable future for a wide range of organisms, including humans.

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## Economic, ecological, and evolutionary tradeoffs as past constraints and future opportunities

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By definition, tradeoffs constrain our ability to achieve two or more goals simultaneously. How, then, can a tradeoff represent an opportunity? Conditions change, shifting the balance among conflicting goals, and thereby creating new opportunities. This point will be illustrated using three examples of tradeoffs that constrained past options, but which may today represent new avenues for crop improvement. These examples are: 1) tradeoffs in the deployment of limited crop diversity in time and space, 2) tradeoffs from conservation of matter, applied to symbiotic nitrogen fixation, and 3) tradeoffs between individual-plant fitness (favored by natural selection) and the collective performance of crop communities.

The first set of tradeoffs is directly relevant to the use of crop diversity in agriculture. Crop diversity over time, i.e., crop rotation, can help control weeds and disease. But there can be tradeoffs between these benefits and the desire to grow the most-profitable crop(s) more often. There can also be tradeoffs between crop diversity over time and crop diversity in space. Intercropping, in particular, can quickly use up diversity that could otherwise be used for longer crop rotations (Denison *et al.*, 2003). For example, if there are only two crops that can be grown profitably in some region, then our choices are: 1) a two-year rotation or 2) growing the same two-crop intercrop year after year. I know of no published comparisons between these two different ways of deploying crop diversity, over enough years to see which is more sustainable, but would bet on crop rotation.

What opportunities are implied by these tradeoffs? Suppose that plant breeders and agronomists develop or identify four additional crops that can be grown profitably in the region just discussed. Then options expand to include a six-year sole-crop rotation, or a three-year rotation among two-crop intercrops, among other options.

Domesticating, improving, or even identifying alternative crops is an expensive undertaking, but some global trends may help direct more resources towards this goal. One such trend is increasing public interest in local food and in dietary diversity. This may be reinforced by global movement of pests, which should increase farmer interest in the risk-reduction benefits of crop diversity. The combination of longer growing seasons (Menzel & Fabian, 1999) and increased atmospheric CO<sub>2</sub> may create new crop niches, although other climatic changes, like increased drought, may impose new constraints.

A second set of tradeoffs has constrained the use of legume crops, forages, and green manures, in ways that may also be posed to change. So long as the natural gas used to make nitrogen fertilizer remained inexpensive, economics tended to favor nitrogen fertilizer over symbiotic nitrogen fixation, despite environmental tradeoffs. These environmental tradeoffs include increasing nitrate levels in well water in some areas, even as some pesticide levels decreased (Rupert, 2008), and "dead zones" in coastal waters. Increasing prices for natural gas should stimulate interest in symbiotic nitrogen fixation, as a potentially more-profitable alternative to nitrogen fertilizer.

Some less-understood tradeoffs may have equally important implications for the increased use of symbiotic nitrogen fixation. The Law of Conservation of Matter, applied to individual elements, generates a variety of tradeoffs in the use of resources by crops. Up to 25% of the carbon that legumes acquire via photosynthesis can be transferred to their symbiotic rhizobia (Minchin *et al.*, 1981). A given carbon atom may be respired to CO<sub>2</sub> to power N<sub>2</sub> fixation, but it may also be used for current or future rhizobial reproduction. For example, rhizobial cells can hoard enough polyhydroxybutyrate to reproduce without external carbon resources or to survive months of starvation (Ratcliff *et al.*, 2008). Differences among rhizobial strains in diversion of plant carbon from N<sub>2</sub> fixation to rhizobial reproduction can partly explain the tenfold differences in plant growth with different rhizobial strains from the same soil (Burdon *et al.*, 1999). For the rhizobia, conservation of matter leads to tradeoffs among alternative uses for plant carbon. But this could also be seen as a tradeoff between plant growth and rhizobial reproduction.

What future opportunities are implied by these tradeoffs? Some carbon allocation to rhizobial reproduction is needed, of course, but greater allocation to N<sub>2</sub> fixation would increase the efficiency of N<sub>2</sub> fixation, raising the value of legume crops and forages. In regions where legumes play only minor roles today, increased use of legumes with enhanced N<sub>2</sub> fixation would simultaneously reduce the need for external nitrogen inputs (synthetic fertilizer, animal manure, compost, etc.) and increase crop diversity, with all of its pest-control and risk-reduction benefits.

How can we increase the efficiency of N<sub>2</sub> fixation? More-efficient rhizobia can be applied as inoculants, but they rarely out-compete indigenous strains. Plant breeding is the key, but we need to focus on improving the efficiency of N<sub>2</sub> fixation, not just allocating more resources to root nodules. Simultaneous measurements of nitrogenase activity and nodule respiration show that the same strain of rhizobia can differ at least twofold in efficiency, depending on the host (Oono & Denison, 2010). The most dramatic improvements apparently involve host-imposed eusociality, with some rhizobia in each nodule differentiating into nonreproductive N<sub>2</sub>-fixing bacteroids analogous to worker bees. This ability to impose eusociality on rhizobial symbionts has evolved at least five times in legumes (Oono *et al.*, 2010), with alfalfa, clover, pea, and peanut being examples, but it is not clear how easily this trait could be developed in crops like bean or soybean.

As an alternative to increasing the efficiency of each rhizobial strain, we can increase nodule occupancy by those strains that are intrinsically most efficient. Soybean plants monitor individual nodules and respond in ways that reduce the reproduction of rhizobia that fix little or no N<sub>2</sub> (Kiers *et al.*, 2003). These "host sanctions", if stringent enough, could enrich the soil with only the best local strains, improving N<sub>2</sub>-fixation efficiency over years. There may be considerable room for improvement in host sanctions through plant breeding, because existing cultivars often tolerate mediocre performance by rhizobia in their nodules (Kiers *et al.*, 2006). There appears to be substantial genetic diversity for host sanctions; older soybean cultivars suffer less from the presence of nonfixing rhizobia (Kiers *et al.*, 2007). We have unpublished data documenting host sanctions in alfalfa and pea as well as soybean.

A third category of tradeoff is that between individual-plant competitiveness and the collective performance of the plant community (Denison *et al.*, 2003). This tradeoff was analyzed more than 40 years ago in the famous "ideotype" paper of Colin Donald (1968). The best-known example is the poor competitiveness of high-yield dwarf wheat and rice, which divert resources from stem growth (and competition for light) to grain production. Higher-yielding grain cultivars are often out-competed by lower-yielding ones, with the former disappearing from mixtures over a few generations (Suneson, 1949, Jennings & de Jesus, 1968). Donald argued that crops with horizontal leaves are more competitive, but that they use light less efficiently for photosynthesis, a conclusion supported by various studies (Pendleton *et al.*, 1968, Kokubun, 1988). Similarly, we recently showed that solar tracking by alfalfa also sacrifices yield potential for competitiveness (Denison *et al.*, 2010). Below ground, natural selection based on individual competitiveness leads to over-investment in root, as individual plants benefit from stealing soil resources from their neighbors, but thereby reduce the productivity of the plant community as a whole (Zhang *et al.*, 1999).

Plant breeders focusing on organic farming may rightly see tradeoffs between competitiveness and yield potential differently, however. Donald advocated sacrificing competitiveness to achieve higher yields, assuming high soil fertility and excellent weed control. I have tended to agree (Denison *et al.*, 2003, Denison, 2007, Denison, 2009), but I recently pointed out that a small increase in yield potential may not justify a big decrease in competitiveness (Denison *et al.*, 2010). In organic systems, competitiveness against weeds may often be more important than yield potential. If so, then it may make sense to breed for taller crops with horizontal leaves (or solar tracking) and aggressively spreading roots.

Meanwhile, Jacob Weiner and colleagues are looking at individual-plant-versus-community tradeoffs in an exciting new way (Weiner *et al.*, 2010). They suggest that weed suppression by crops can be greatly enhanced through a combination of new management practices (increased crop density and uniformity) and plant breeding. Even with existing cultivars, weed suppression increases with crop density, especially if spacing between crop plants is similar in both horizontal dimensions (Weiner *et al.*, 2001). (Closer spacing within rows, while keeping row spacing constant, can be counterproductive.)

How might plant breeding further strengthen weed suppression, in the context of high crop density and uniformity? Weiner *et al.* (2010) note that past natural selection is unlikely to have maximized plant traits whose only effect is to suppress neighbors, because "a single plant cannot suppress its neighbors." Suppressing one neighboring competitor would help others, perhaps indirectly reducing the individual fitness of the suppressing plant. Shade-avoidance responses (e.g., growing taller in response to crowding) might have some tendency to shade neighbors, but these responses are unlikely to be optimal in terms of overall weed suppression.

Can we increase suppression of weeds by crops, without increasing suppression of crop plants by each other? Maybe not, if we grow the crops in widely spaced rows. But Weiner et al. note that, with uniform spacing of crop plants, crop seedlings would compete with and suppress weeds long before they grew large enough to compete with each other. And they would have an advantage in that initial competition, resulting from the larger size of crop seeds relative to those of weeds. It might therefore be possible to breed for increased competitiveness against weeds, while keeping mutual interference between crop plants relatively low.

Natural selection is a powerful force, but its limitations -- particularly, favoring individual fitness even when it conflicts with the collective performance of plant communities -- offer many of our best opportunities for improvement by plant breeders (Denison, 2010). Collective suppression of weeds may be an example of a trait that hasn't already been optimized by natural selection, leaving opportunities for improvement by breeding (Weiner *et al.*, 2010). Similarly, tradeoffs between the fitness of rhizobia and of their legume hosts imply opportunities to favor the legumes, in ways that can decrease reliance on off-farm nitrogen sources and increase crop diversity. Increasing crop diversity will, in turn, reduce the severity of tradeoffs between within-year diversity (e.g., intercropping) and crop diversity over years (e.g., rotation).

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## Breeding for resilience in wheat - Nature's choice

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### Introduction

Resilience, i.e. the ability of a system to remain functional when under external stress, is a property that is anticipated to be of increasing desirability in a fast changing world, especially in the face of diminishing non-renewable resources. Breeding plants for resilience aims to produce crops that can deliver high yield and quality under changing, and more marginal, resource conditions. A promising breeding approach to increase crop resilience is evolutionary breeding, as introduced by Suneson (1956). A key component of this approach is the introduction and maintenance of high genetic diversity in the field through the creation of composite cross populations (CCPs). The diversity of genotypes within the plant population means that genetically different plants can complement each other, e.g. by using slightly different resource niches, thereby increasing overall resource use efficiency. Moreover, high within-crop diversity offers the advantage that the failure of some plants under certain environmental conditions (e.g. drought) can be compensated by plants with different properties (drought tolerance). This idea leads to the hypothesis that the advantage of using crop diversity over using monocultures may increase along a gradient of increasing environmental variability. Here we confirm this hypothesis for winter wheat CCPs (high diversity) which were compared with their parents (low diversity), as well as with the physical seed mixtures of the parents (intermediate level of diversity). We further show that the yield advantage of diverse populations over monocultures is particularly prevalent under stress conditions, i.e. when the environment affects yields negatively.

### Materials and Methods

#### *Selection of parents*

Six composite cross populations of winter wheat were created in 2002. The twenty parental cultivars were selected from data of both published and unpublished studies, and from experience of the consortium partners. Key criteria for selection included a diverse genetic base and the potential for robust performance under low input agronomic conditions. The parent cultivars were selected in summer 2002 in two categories: high yielding (Bezostaya, Buchan, Claire, Deben, High Tillering Line, Norman, Option, Tanker, Wembley) 'Yield' varieties, and high bread-making quality (Bezostaya, Cadenza, Hereward, Maris Widgeon, Mercia, Monopol, Pastiche, Renan, Renesansa, Soissons, Spark, Thatcher) 'Quality' varieties. Bezostaya was included in both categories as it was known as both high yielding and high quality in Russia, where it was grown successfully over many years.

#### *Creation of CCPs and mixtures*

All 20 parents were crossed together in a complete half-diallel to produce all individual 190 F<sub>1</sub> cross combinations. The F<sub>1</sub> seed was harvested, germinated and grown to maturity in a glasshouse. All ears were bagged to ensure self-pollination, and the F<sub>2</sub> seed from each of the individual F<sub>1</sub> plants of each cross was harvested and bulked for each cross. From these F<sub>2</sub> bulks three separate 'foundation' CCPs were started by bulking F<sub>2</sub> seed from the individual crosses. The first was synthesized from the 66 crosses between varieties with good milling potential (Q), the second synthesized from the 36 crosses for varieties identified as having high yield potential (Y) and a third synthesized from the 99 crosses between Y and Q parents (YQ). In addition, male

sterile CCP populations (CCPms) were generated by artificially hybridizing all the above parents to characterized genetic male sterile lines (as females) obtained from two sources (RAGT and CIMMYT). The F<sub>2</sub>s of these crosses were bulked as above to create QCCPms, YCCPms and YQCCPms, which together with QCCP, YCCP and YQCCP, gave six CCPs available as starting material for field evaluation.

In order to compare the performance of mixtures of homozygous lines with that of heterozygous populations, parental seed of equal proportions was also mixed in the same categories as those used to create the populations to provide a Yield, Quality and Yield-Quality Mixture. Unfortunately the Norman seed that was used in the mixtures subsequently turned out to have been contaminated. Therefore the Yield and the Yield-Quality Mixtures contained a small amount of an unknown genotype.

The CCPs, mixtures and all parental varieties (apart from Norman) were drilled in single replicate plots of varying size at four locations in October 2003. There was enough seed available in autumn 2004 to begin replicated field trials.

#### *Field trial site description*

Trial sites consisted of two organic sites (Wakelyns Agroforestry (WAF), in Suffolk (52°39'N, 1°17'E); Sheepdrove Organic Farm in Berkshire (SOF) (51°41'N, -1°52'E)) and two non-organic sites (Metfield Hall Farm (MET), continuous wheat, adjacent to WAF in Suffolk (52°41'N, 1°29'E); and Morley Farm (MOR), an experimental farm in Norfolk managed by The Arable Group (TAG) (52°56'N, 1°10'E)). Experiments took place over three years (2004/5 to 2006/07) in different location within each farm. This set up resulted in 12 field trials or environments (3 years x 4 sites).

Soil type (clay content 13-40 %) was medium to heavy at Metfield, Morley and Wakelyns and light to medium at Sheepdrove. The preceding crop was always grass-clover or grass-vetch-clover ley at Wakelyns; grass-clover at Sheepdrove; winter wheat at Metfield; and winter oil seed rape at Morley.

#### *Data analysis*

Here we report results for the categories Q and Y only, since the overlap of parents between YQ on the one hand and Y and Q on the other means that the results of YQ are not sufficiently independent from the results of Y and Q, respectively. The programme R was used to analyse all data.

We used four steps to test our hypothesis that diverse wheat might show a stronger yield increase over wheat monocultures in variable environments.

(1) For each of the 12 field trials (environment E<sub>j</sub> with j = 1...12), we first calculated the relative yield difference D(E<sub>j</sub>)<sub>CCP</sub> between the CCPs and the average of the monocultures, with

$$D(E_j)_{CCP} = [y(E_j)_{CCP} - y(E_j)_P] / y(E_j)_P * 100 \quad (\text{eqn. 1})$$

Accordingly, D(E<sub>j</sub>)<sub>Mix</sub> is the relative yield difference between the mixtures and the average of the monocultures:

$$D(E_j)_{Mix} = [y(E_j)_{Mix} - y(E_j)_P] / y(E_j)_P * 100 \quad (\text{eqn. 2})$$

where y(E<sub>j</sub>)<sub>CCP</sub> and y(E<sub>j</sub>)<sub>Mix</sub> are the yields of the CCPs and the mixtures, respectively, averaged for each environment E<sub>j</sub>, and y<sub>p</sub>(E<sub>j</sub>) is the average yield of all respective parents (Y or Q) for each environment.

(2) We then determined the *within*-environment variability W(E<sub>j</sub>) of each of the 12 trials by calculating their coefficients of variation:

$$W(E_j) = [1 / (n_j - 1)] * [\sum (y_i - y(E_j)_P)^2]^{0.5} / y(E_j)_P * 100 \quad (\text{eqn. 3})$$

where y<sub>i</sub> are yields of parents of individual plots i, y(E<sub>j</sub>)<sub>P</sub> are again the trial averages as above, and n<sub>j</sub> is the number of plots in each environment.

This measure of variability contains all sources of variation within the environment (block, variety, experimental error). It measures how predictable the yield performances of individual plots are when the overall average of the environment is known.

(3) Finally, we performed an analysis of covariance to determine the relationship between the relative yield differences D(E) and the variabilities W(E).

(4) In a complementary analysis, we investigated the relationship between the yield effect of diverse wheat and *between*-environment variation B(E). For this purpose we calculated the relative yield increase or decrease for each environment compared to the system average:

$$B(E_j)_{Org} = [y(E_j)_P - y(S_{Org})_P] / y(S_{Org})_P * 100 \quad (\text{eqn. 5})$$

$$B(E_j)_{Con} = [y(E_j)_P - y(S_{Con})_P] / y(S_{Con})_P * 100; \quad (\text{eqn. 6})$$

Where y(S<sub>Org</sub>)<sub>P</sub> is the overall yield average of the parents from the six organic trials and y(S<sub>Con</sub>)<sub>P</sub> the respective average for the conventional trials.



Here, we were particularly interested in negative values of  $(B(E_j) < 0)$ , i.e. when the yield of  $E_j$  is below the average of the system across the 2 sites and 3 years.  $B(E)$  was then subjected to a covariance analysis against  $D(E)_{CCP}$  and  $D(E)_{Mix}$ .

### Results

The differences  $D(E)$  between populations and monocultures are plotted against the measure of within-environment variability  $W(E)$  as determined in step (2) (Figure 1). This shows a significant association between high variability and the difference between populations and monocultures (slope =  $0.71 \pm 0.17$ , adjusted  $R^2 = 0.477$ ,  $p = 0.0001$ ,  $df = 22$ ). There were no significant interactions between this effect and the system (organic vs. conventional). For the mixtures, the relationship between  $D(E)_{Mix}$  and variability  $W(E)$  was also significant but slightly weaker than for the CCPs (slope =  $0.54 \pm 0.26$ , adjusted  $R^2 = 0.131$ ,  $p = 0.0462$ ,  $df = 22$ ).

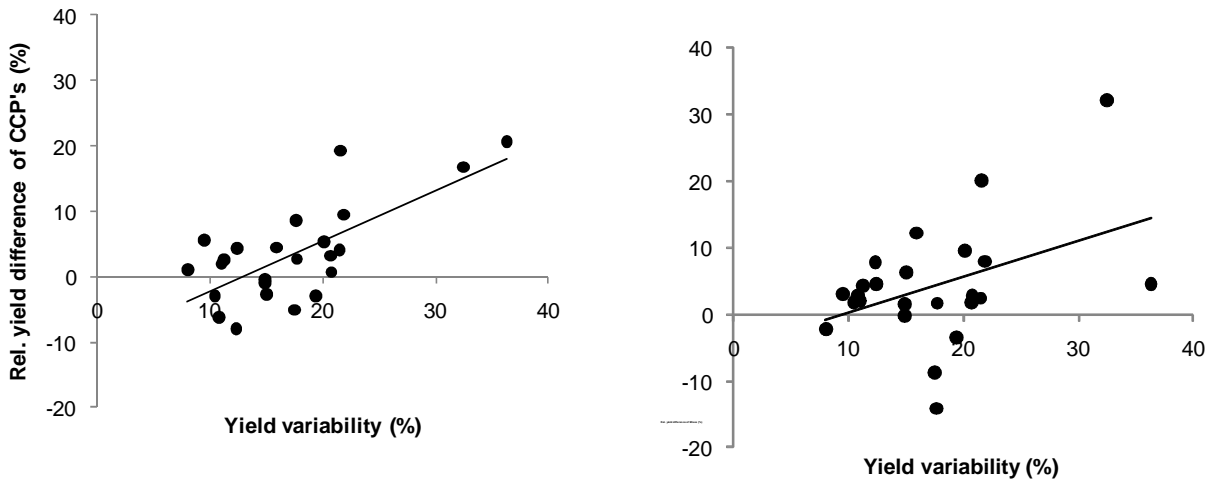


Figure 1: Relationship between yield variability (coefficient of variation  $W(E)$ , x-axes) and relative yield difference  $D(E)$  of composite cross populations (left) or variety mixtures (right) compared to monocultures (y-axes).

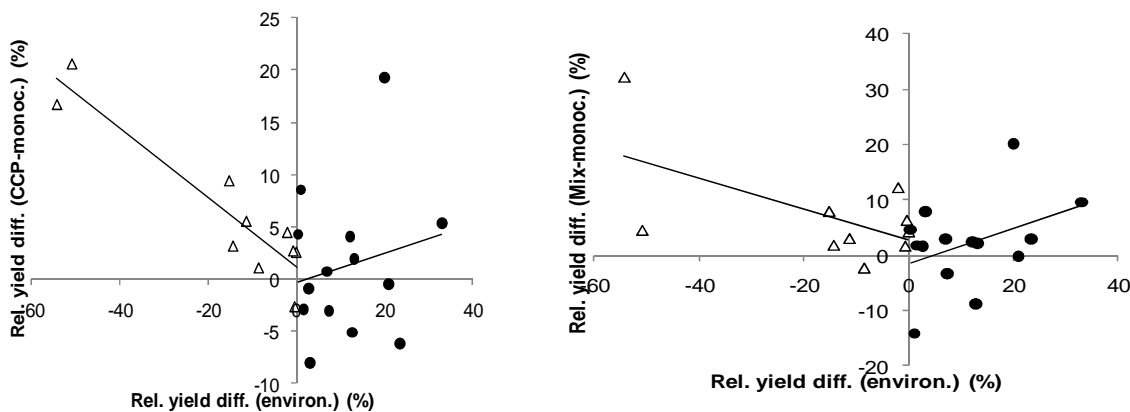


Figure 2: Relationship between a measure of between-environment variability ( $B(E)$ , x-axes) and relative yield difference  $D(E)$  of composite cross populations (left) and variety mixtures (right) compared to monocultures (y-axes). Open symbols for negative values of  $B(E)$ , filled symbols for positive values of  $B(E)$ .

When the average yield of an environment was *below* the system's average ( $B(E) < 0$ ), the yield effect of the populations was negatively correlated with  $B(E)$  (Figure 2), i.e. the lower the yield of the environment was

below the expectation, the higher was the advantage of the populations (adjusted  $R^2 = 0.844$ ,  $p = 0.0001$ ,  $df = 8$ ). For the mixtures, this relationship was non-significant but pointed in the same direction (adjusted  $R^2 = 0.268$ ,  $p = 0.072$ ,  $df = 8$ ). For positive values of B, i.e. above-average yielding environments, neither the CCPs nor the mixtures showed any significant correlations with B(E) ( $p = 0.501$  and  $p = 0.163$ , respectively).

### Discussion

Traditional plant breeding programmes rely on selecting genotypes in optimal conditions: in the absence of weeds, diseases and pests and with peak nutrient availability. This breeding approach has produced many successful pedigree line bred varieties for high-input production systems. However, low input agriculture has suffered from a lack of varieties adapted to the environmental variability on low input farms (Phillips and Wolfe, 2005). Furthermore, there is a need to reduce costly inputs in high input agriculture and to prepare for the impacts of climate change. In low input conditions, the variability of the environment has a far greater influence on yield than the choice of variety. This can lead to a lack of stability in performance that has been demonstrated in many studies (e.g. Soliman & Allard, 1991). Physical mixtures of complementary varieties provide an improved ability of a crop to buffer variation in soil, climate and disease and weed pressures (Wolfe, 2001). The advantage of diversity in cereal variety mixtures has been well demonstrated (Finckh and Wolfe, 2006). However, there are practical limits to the number of genotypes that can be used, usually no more than three or four, which limits the potential for buffering against environmental variability. This project was designed to overcome the problem of limited genetic variability by developing crop material directly from segregating populations in the form of evolutionary breeding (Suneson, 1956).

The populations, mixtures and monocultures, while all containing the same genetic material, exhibit widely different degrees of genetic diversity in space. Our results show that in more variable environments, the advantage of highly diverse populations over monocultures was greater than in less variable environments. While the variety mixtures showed the same relationship, it was weaker than in the populations, indicating that the level of diversity determines the degree of resilience. Furthermore, we found that high within-crop diversity is particularly effective under stressful conditions, when environmental effects push yields below expectations.

We conclude that both theory and empirical evidence suggest that, even for different farming systems, high levels of within-crop diversity offers the possibility of improving crop performance and reliability in highly unpredictable environments.

### Acknowledgements

We are grateful for funding of this project from Defra, UK (project AR0914).

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## Population developments from the F<sub>5</sub> to the F<sub>9</sub> of three wheat composite crosses under organic and conventional conditions

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*Keywords:* Evolutionary breeding; weed competitiveness; disease;

### Introduction

The evolutionary processes in pathogen populations leading to resistance breakdown are mutation, recombination, adaptation, and selection. All these processes, in particular adaptation and selection processes are critically influenced by environmental conditions affecting the pathogen population directly or via the host and the genetic structure of the host population. The beneficial effects of diversity for resistance in space and time, reducing diseases are well known and a multitude of mechanisms has been identified contributing to these effects (see Finckh and Wolfe, 2006 for review). Resistance gene management on the population level will be an important component of future sustainable agricultural systems as well under organic as conventional conditions. For long-term sustainability it will be crucial to reintroduce diversity for resistance into modern varieties to prevent resistance breakdown.

Alternative approaches to pure line breeding have been developed by using composite crosses, top crosses and population breeding (e.g. Murphy et al., 2005; Wolfe et al., 2008). The “modern landrace” or “evolutionary breeding” approach aims at combining the advantages of the breeding success of the last century and the use of genetic variation producing high yielding and high quality but genetically diverse crop populations that can be adapted to local conditions by the farmers. The overall aim is to provide materials for the development of locally adapted populations and varieties (Murphy et al., 2005).

Although wheat is an inbreeding crop observations on wheat landraces indicate that outcrossing can be as high as 11% (Dreisigacker et al., 2005). A French study shows that overall genetic diversity was conserved within subpopulations (Goldringer et al., 2006). In addition, it was found that the mutation rate in these wheat populations were exceptionally high possibly due to the initial large crossing design and thus contributes to the continuous production of new genetic variation (Raquin et al., 2008). In the F<sub>5</sub> of wheat composite crosses grown in Germany in 2005-2006 diversity for resistance to brown rust (*Puccinia recondita*) was still exceptionally high. We tested individual plants from single seed progenies for their resistance to three different rust races. The progenies segregated in 22 out of 23 cases into two to eight resistance phenotypes (Finckh et al., 2009).

The purpose of this paper is to present some results from three wheat CC populations that were maintained since the F<sub>5</sub> without conscious selection at the University of Kassel under organic and conventional growing conditions and one population grown without weed control under organic conditions since the F<sub>8</sub>.

### Materials and methods

Three winter wheat CC populations were created in 2001 in the UK with the aim to produce materials for use in the development of highly adaptable modern wheat populations and possibly modern landraces. The CCs were produced either by intercrossing 12 high quality parents (Q) (Bezostaya, Cadenza, Hereward, Maris Widgeon, Mercia, Monopol, Pastiche, Renan, Renesansa, Soisson, Spark, Thatcher), 9 high yielding parents (Y) (Bezostaya, Buchan, Claire, Deben, High Tiller Line, Norman, Option, Tanker, Wembley), or all 20 modern wheat parents (called A Population).

Since the F<sub>5</sub> (2005-2006) the populations have been grown at the University of Kassel under organic and conventional conditions in well separated large (>100m<sup>2</sup>) plots in two parallel sets (12 populations total) without

conscious selection applied. In the conventional system no fungicides or insecticides are used to expose the populations to natural pest and disease pressure. Several popular winter wheat varieties grown in the same fields next to the populations were included as reference varieties since the F<sub>6</sub> (organic) and since the F<sub>8</sub> (conventional) to compare the yields of the CC populations to typical yields attained in the given environment. Currently, in 2010/2011, the F<sub>10</sub> generation is being grown.

In addition to the 12 populations described, in 2008/2009 (F<sub>8</sub>) a sample of the A population was broadcast sown and grown without additional mechanical weed control under organic conditions and resown under the same conditions in the F<sub>9</sub>. The purpose is to determine if a population adapted to growing without mechanical weed control can be created. This population is currently in the second year of adaptation.

Yields were taken from predetermined plots of 12-15m<sup>2</sup>. Since the F<sub>8</sub> two replicate samples are taken per CC population to determine the variability. In addition, resources permitting, leaf and foot diseases, plant heights and other morphological parameters were assessed on 30-50 single haulms or stems of the CCs and 10-20 single haulms of reference varieties in pure stands. Single plant harvests from the F<sub>7</sub> and F<sub>8</sub> are presented in a separate poster (Weedon et al. this conference).

In 2009 and 2010 the parental varieties were grown in an organic field adjacent to the organic CCs (but different rotation) in single plots to increase the seed for future experiments. In 2010 the lines were also assessed for their foliar and foot diseases.

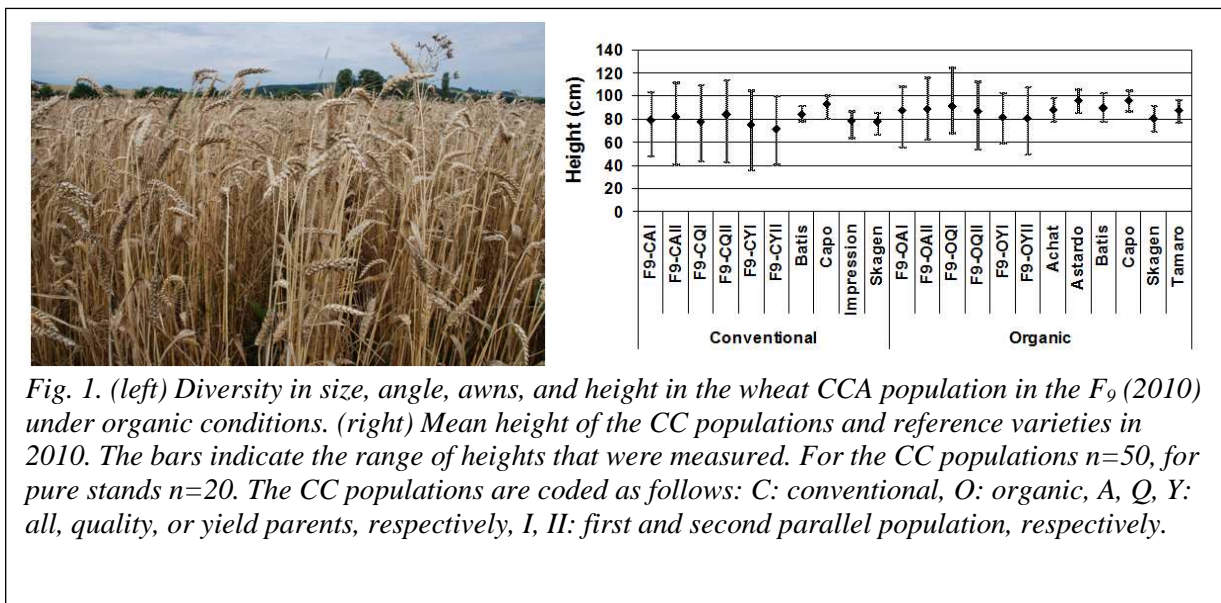


Fig. 1. (left) Diversity in size, angle, awns, and height in the wheat CCA population in the F<sub>9</sub> (2010) under organic conditions. (right) Mean height of the CC populations and reference varieties in 2010. The bars indicate the range of heights that were measured. For the CC populations n=50, for pure stands n=20. The CC populations are coded as follows: C: conventional, O: organic, A, Q, Y: all, quality, or yield parents, respectively, I, II: first and second parallel population, respectively.

## Results and discussion

### Morphological diversity

The morphological diversity is still very high in the F<sub>9</sub> with large variations in leaf angles, height, color, head shape and awns, among others (Fig. 1).

The Q populations were consistently taller than the Y populations, an effect still visible in 2010. Variations in height were still huge in the CCs compared to the pure stand references.

### Yields F<sub>5</sub> – F<sub>9</sub>

Especially in the year 2007 yields were generally low at our site. The different yield levels in the organic and conventional fields reflect site differences and an overall lower yield potential of the conventional site (Fig. 2). Overall, yields of the CC populations were comparable to the yields of the reference varieties in the given years. However, the range of yields of the reference varieties was often much greater than that observed in the CCs. While it appeared that yields of the Y populations originating from the high yield parents were somewhat higher in the first two years under organic conditions than the yields of the other populations, this pattern disappeared in later years.

The A population sown broadcast and grown without mechanical weed control performed equally well as the population sown in rows and managed with harrow and hoe in both years (Fig. 2a). It was especially

conspicuous that no more weeds were visible in the broadcast sown plots than in the plots with mechanical weed control (Fig. 3).

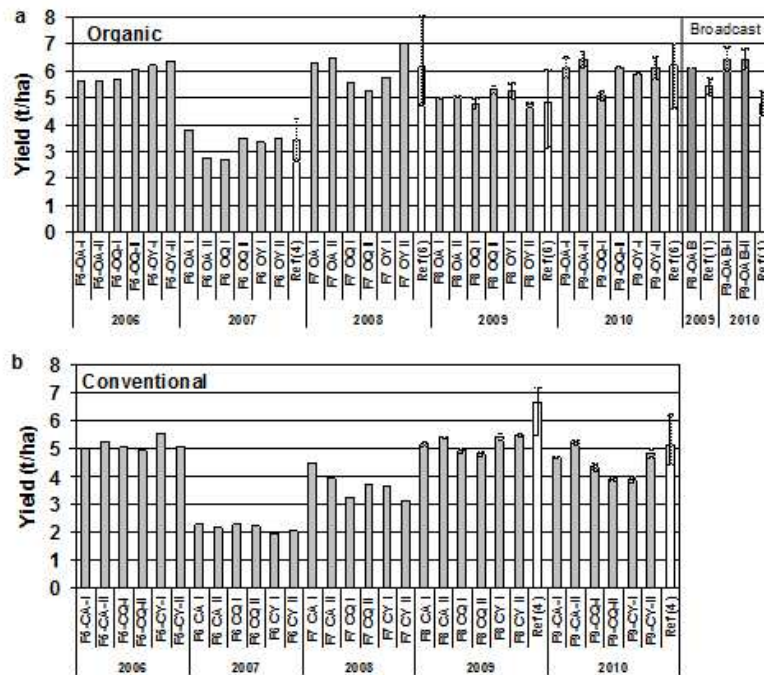


Fig. 2. Yields of the CC populations and reference varieties 2006-2010. Plots of 12-15m<sup>2</sup> were harvested. Since 2009 replicate plots in CC populations. The number of reference varieties (white bars) is given in parentheses. Error bars represent the range (min/max) of yields measured. For population codes see Fig. 1. In addition, in (a) the yields of the CC that were broadcast (OAB) compared to the reference variety Capo in the same system are shown



Fig. 3. (left) CC grown with 30sm row distance with harrowing and hoeing for weed control and (right) CC grown broadcast without mechanical weed control before harvest 2009.

### Diseases

Disease pressure in the area varied considerably among years but was generally low as well under organic as conventional conditions. In 2009, brown rust (*Puccinia recondita*) severity on the flag leaf of a susceptible pure line was 14% diseased leaf area while in the CC populations mean severity did not exceed 5% diseased leaf area. In 2010, leaf disease pressure again was low.

In contrast to foliar diseases, in 2009 and 2010 foot disease pressure was moderate to high depending on the field. The reference varieties were grown adjacent to the CCs in fields with the same rotation. In contrast, the parent varieties of the CCs were grown in an organic field with another rotation. Therefore, it is not possible to compare them in detail. The variation in disease severity was considerably higher in the reference varieties compared to the CCs. Among the parental lines variation was also high (Fig. 4).

### Conclusions

Overall, the yield performance of the CCs is comparable to standard varieties albeit less variable. The very good performance of the broadcast population in two years is encouraging as it suggests that it should be possible to adapt the population to lower inputs than common in organic farming with respect to weed control.

The high morphological variation observed in the F<sub>9</sub> suggests that overall diversity is still high. Repeats of the tests for heterogeneity as in the F<sub>5</sub> (Finckh et al. 2009) in single seed progenies would be highly interesting in one of the next generations.

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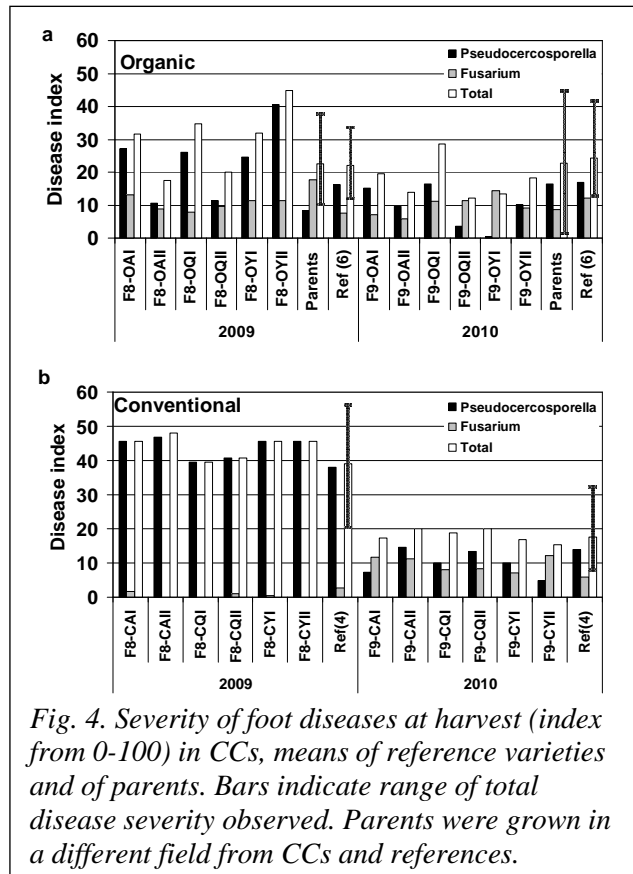


Fig. 4. Severity of foot diseases at harvest (index from 0-100) in CCs, means of reference varieties and of parents. Bars indicate range of total disease severity observed. Parents were grown in a different field from CCs and references.

## Breeding rice cultivars to enhance positive feedbacks between soil and crops?

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*Keywords:* agroecology, ecological engineering, earthworm, biochar, rice, crop-soil positive feedback

### Introduction

Tropical soils are considered to be particularly vulnerable to fertility losses because of their low capacity to retain organic matter and mineral nutrients. This prompts the development of “new” agricultural practices to manage mineral nutrients and organic matter in a more sustainable way while relying less on inputs of fertilizer. Two methods pertaining to ecosystem engineering and agroecology have already been tested with some success. The first is based on the addition of biochar to the soil and has led to the creation of long-lasting fertility spots by pre-Columbian populations (Marris 2006). The second is based on the maintenance of higher earthworm densities (Lavelle et al. 1989). Earthworms have indeed been shown to increase crop production by their effects on plant growth (Scheu 2003). However, crops have to be adapted to agricultural practices and particularly to the soil conditions they lead to and common cultivars might not be adapted to new practices. In particular, modern cultivars have been selected in soils deprived of biochar and with usually low earthworm biomass (Edwards and Lofty 1982, Mäder et al. 2002). Using rice as a model plant, we thus compared in a greenhouse experiment the responsiveness to biochar and earthworms of five rice cultivars. These rice cultivars were chosen to represent a wide gradient of origins, between a very local landrace to common modern cultivars.

### Material and methods

*Experimental design.* The experiment was conducted at CIAT (International Center for Tropical Agriculture) in Cali, Colombia. Plants were submitted to the four possible combinations of two factors: with (five adults of *Pontoscolex corethrurus*) and without earthworm (respectively noted E, NE) and with (25 g of biochar per kg of dry soil) and without biochar (respectively B, NB). All treatments combinations were implemented for the African rice (*Oryza glaberrima*, IRGC 103544) and four Asian rice cultivars (*Oryza sativa*): cv. *Line 30* (accession CIRAD 409), *Azucena* (accession IR64), *Nipponbare* (accession IRGC 12731) and *Donde lo tiren*. For each combination of treatments (E x B x rice cultivar), two fertilization treatments were implemented: without or with mineral fertilization. Five replicates were implemented for each treatment, resulting in 200 microcosms containing each 1500 g of an inceptisol (see Supplementary Method 1). Rice was grown in a greenhouse for four months (relative humidity 65-95%, temperature 27-29°C, light intensity 600  $\mu\text{mol, m}^{-2} \text{s}^{-1}$  and a 12 h photoperiod).

*Measurements.* After sixteen weeks of growth, plants were harvested and separated into leaves, grains and stems. Roots were collected by wet sieving. Subsamples of each plant material were analyzed for total carbon, and total nitrogen using a ThermoFinnigan Flash EA 1112 elemental analyzer (ThermoFinnigan, Milan, Italy).

*Statistical analyses.* We base the analysis of our results on the effect sizes of the different treatments (Nakagawa and Cuthill 2007). This approach allows focussing on the magnitude of the effects of the different treatments comparing the magnitude of these effects relatively to the control treatment, in different cases: here the rice cultivar and the fertilization treatment. We used a standardized statistics, Cohen's *d*. Effect sizes were calculated separately for each rice cultivar and for each fertilization treatment (with and without).

## Results

The full statistical model (four-way ANOVA with “earthworm”, “biochar”, “fertilization” and “rice cultivar” as factors and all the interactions between these four factors) showed that for most output variables (total biomass, root biomass, grain biomass, shoot/root ratio, leaf C/N) there were significant interactions between rice cultivar and biochar, and between rice cultivar and earthworms. This proves that different rice cultivars have different responsiveness to biochar, earthworms and the combination of both. Overall, the positive effect of mineral fertilization was of the same amplitude as earthworm and biochar effects. Hereafter, we base the description of our results on the calculation of the effect sizes (ES, see Materials and methods) that denote the strength of the effects relatively to the control treatment (within a fertilization treatment). The effect sizes are given together with relative percentage changes.

In all cases, i.e. all cultivars and with or without fertilization, treatments increased the total rice biomass (Fig .1, positive ES). This biomass tended to respond more to earthworms and biochar without fertilization than with fertilization. With fertilization, the strongest effect size was obtained with both biochar and earthworms in *Nipponbare* (+128%, ES about 5) and *O. glaberrima* (+44%, ES about 5). Without fertilization, the strongest effect was obtained with earthworms in *O. sativa* cv *Azucena* (+233%, ES about 7) and *Donde lo tiren* (+119%, ES about 7) and with both biochar and earthworms in *Donde lo tiren* (+171%, ES about 9). Effects of earthworms and biochar on the shoot/root ratio were different with and without fertilization. The other documented variables displayed different patterns but in all cases the responses of the different cultivars were different and varied with the fertilization treatment.

The complete mechanistic interpretation of the observed effects on rice growth and resource allocation goes beyond the objective of the present article. Another experiment (Noguera et al. 2010) focussing on one rice cultivar (*line 30*) but comparing different soil treatments, suggested that earthworms and biochar mainly act through their positive effect on the availability of mineral nutrients. However the earthworm effect could not be fully understood without assuming that they lead to the release of plant growth factors in the soil through the stimulation of particular bacterial groups (Muscolo et al. 1999, Blouin et al. 2006). Here, the key point is that the pattern of response of rice cultivars is complex: the responsiveness to biochar, earthworms and the combination of the two generally depended on the cultivar, and was often different with and without fertilization. Beyond this complexity it is noteworthy that: (1) The same maximum total biomass could be obtained with (in *O. glaberrima*) or without fertilization (in *Donde lo tiren*) but with both earthworms and biochar; (2) The highest absolute grain biomasses were obtained with the combination of fertilization, earthworms and biochar (in *O. glaberrima* and *Line 30*); (3) With fertilization, choosing the best combination of cultivar and treatment allowed a 353% relative increase in the total grain biomass (biochar and earthworms in *Nipponbare*); (4) Without fertilization, choosing the best combination of cultivar and treatment allowed a 437% relative increase in the total grain biomass (biochar earthworms in *Azucena*); (5) Overall, choosing the best combination of treatments and cultivar multiplied by more than four the mean relative increase in grain production calculated over all the combinations of treatments and cultivars (with and without fertilization).



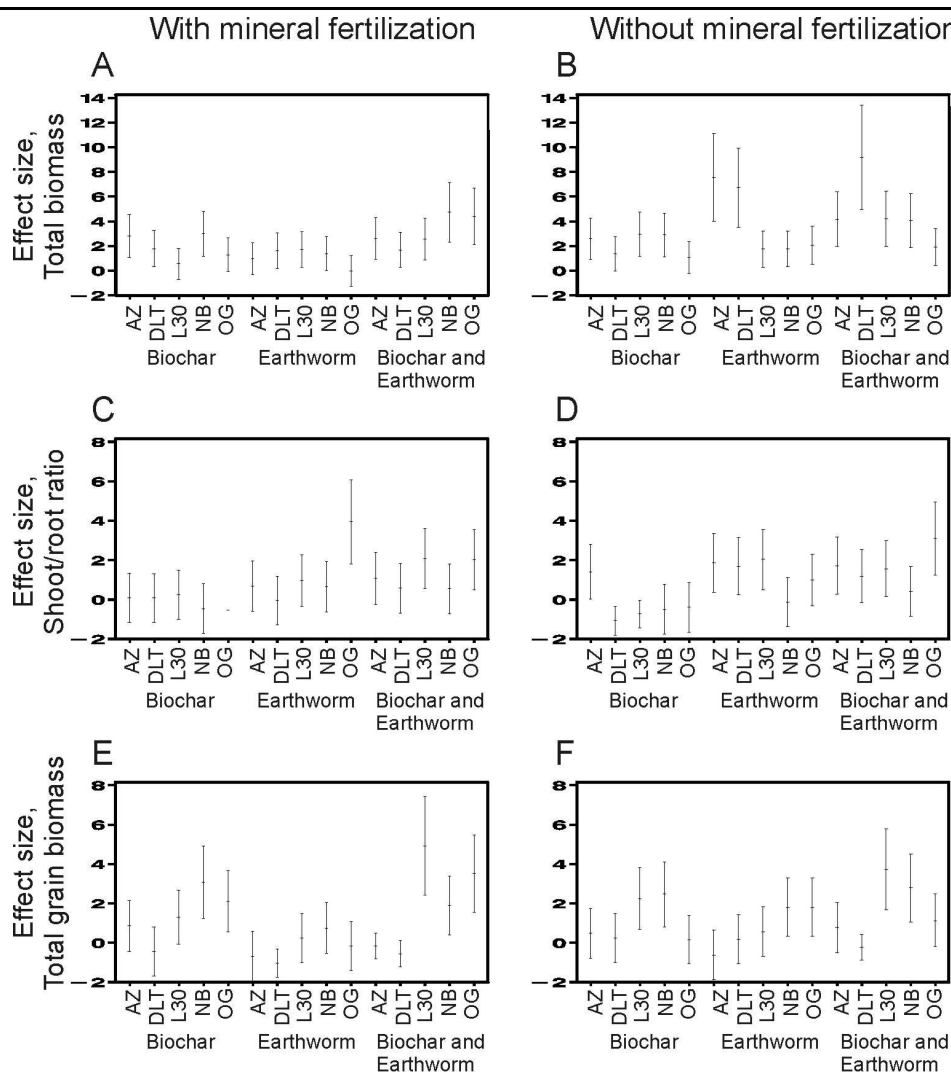


Fig. 1 Effect sizes of the different treatments (biochar, earthworm, and combination of biochar and earthworm) on the total biomass (A-B), the shoot/root ratio (C-D) and the total biomass of grains (E-F). In each case, effect sizes are displayed for the mineral fertilization treatment (left-hand column of panels) and the non-fertilized treatment (right-hand column of panels), and for each Asian rice cultivar (AZ, Azucena; DLT, Donde lo tiren; L30, Line 30; NB, Nipponbare) and the African rice (OG, *Oryza glaberrima*).

## Discussion

Why focus on cultivar responsiveness to biochar and earthworms? For biochar, the answer is very clear: if we want to develop new agricultural practices, we also need to develop the suitable cultivars that highly benefit from these practices. For earthworms, the answer is threefold. First, modern agricultural practices such as tillage and the subsequent negative effect of these practices on soil organic matter content have often a negative impact on earthworm populations (Fragoso et al. 1997). Conversely, alternative agricultural practices aiming at increasing sustainability often have a positive impact on soil fauna and earthworms (Mäder et al. 2002). Second, practices are proposed to directly increase earthworm biomass to restore soil fertility (Lavelle et al. 1989). All these practices would particularly benefit from the use of responsive cultivars. Third, soil organisms are more and more thought to play an essential role in the sustainability of soil capacity to support vegetal production because of their involvement in positive feedback loops between soil properties and plant production (Lavelle et al. 1989, Wardle et al. 2004). It might thus be possible to foster further these positive effects on sustainability through the selection of new responsive cultivars. Such an approach is currently in development for mycorrhizae (Sawers et al. 2008). They are an obvious place to start because of their symbiotic association with plants. However, most soil organisms interact directly or indirectly with plants (Wardle et al. 2004). Therefore, this approach might be extended to many other organisms. This would be a way to green the green revolution and to favour the ecological intensification of crop production (Cassman 1999).

The next key issue is to find genotypes having a high responsiveness to the desired organisms. It is probable that intensively selected modern cultivars have lost some of the traits allowing natural species to interact efficiently with soil organisms and fine-scale sources of soil heterogeneity (such as biochar). Indeed agricultural practices tend to be unfavourable to many groups of soil organisms and modern cultivars have been developed to grow efficiently when provided with an abundant mineral fertilization that tends to homogenise soil fertility (at all scales). Consequently, subtle mechanisms allowing plants to access mineral nutrients through complex rhizospheric interactions (involving interactions with soil organisms, see for example Bonkowski 2004), might have been selected against or might have been stochastically lost during the selection of high-yielding cultivars. A solution might thus be to find lost traits in local landraces or non-domesticated ancestors (McCouch 2004). Our results support these views in the sense that *Donde lo tiren* is the more rustic rice cultivar of our experiment and responds well to earthworms in terms of total biomass without mineral fertilization (+119%, ES about 6, Fig. 1). However, due to interactions with the resource allocation strategy, *Donde lo tiren* does not respond well to earthworms in terms of grain biomass. On the contrary, a modern cultivar such as *Nipponbare* increases very significantly its grain biomass in presence of earthworms, even without mineral fertilization (+93%, ES about 2).

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## Session 2 - Posters

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### 11. Evolutionary dynamics of cycle duration in pearl millet: the role of farmer's practices and gene flow

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One of the major preoccupations today is to understand the effect of environmental modifications on biodiversity and how human societies are going to face these changes. Our research aims to study the consequences of environmental and social changes on the genetic diversity of Pearl millet (*Pennisetum glaucum*), a major low-input crop for human consumption in the Sahelian region. In this region Pearl millet presents many varieties based on candle's shape and size, seed color and size, and taste. Sahelian farmers use to grow early and late flowering varieties in order to face rainfall variation. Two main types can be distinguished. The early type : 75 to 95 days (sowing-maturation) ; the late type : 120 to 150 days of cycle length. Usually both varieties are sown at the same date. In south-west Niger, because the two types of varieties are adapted on different type of soils, they were used to be grown on well isolated pieces of land. However, recent environmental (the important drought from 70's and 80's) and social changes (especially the very strong demographic increase) have modified these practices. The lack of available soils forces sometimes farmers to cultivate early and late flowering pearl millet varieties in adjacent parcels. Moreover, it happens that farmers sow lately in the season (mid-july) due to irregular rains at the beginning of the season, which modifies the delay between sowing and flowering, especially for photoperiodic late flowering varieties. All these changes open the question of a possible overlapping flowering period and hence gene flow between the two types of varieties.

In order to evaluate the possibility of gene flow between early and late flowering pearl millet four villages in south west Niger were chosen. The first two villages SNK and KAN represent the case where pearl millet varieties are grown in parapatric parcels; while in the other two TSZ and BOB parcels of late and early varieties are still distant in most cases. Firstly we observed flowering periods of the two types of varieties in several pearl millet fields in two villages (SNK, TSZ) showing contrasting parcel/variety management situations. Secondly, we assess the level of genetic differentiation between 24 late and early populations sampled from the 4 villages, using 15 microsatellite loci fairly distributed on the genetic map of this species.

Results of the field study show large flowering overlapping between early and late varieties in SNK and KAN villages while in the two other villages flowering are well separated in time, although observations suggest that asymmetrical gene flow (mainly from early to late) could occur.

Genetic data support strongly our field's observations:

- The genetic differentiation between early and late varieties ( $F_{st}$  levels) is low between the couple of varieties in SNK and KAN in comparison with those of TSZ and BOB.
- Admixture levels assessed by Bayesian analysis also support our hypothesis of asymmetric gene flow from early to late variety.

Altogether, the results suggest that an evolution (a shortening) of the cycle duration of late varieties of pearl millet could occur because of recent changes in farmer's practices.

Additional experiment is ongoing in order to evaluate the level of genetic differentiation between the two types of varieties at the phenotypic level ( $Q_{st}$ ).



## 12. Variation in number of seeds per plant and seed size from the F7 to the F9 of three wheat composite crosses under organic and conventional condition

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**Keywords:** Composite cross, evolutionary breeding, effective population size

Alternative approaches to pure line breeding have been developed by using composite crosses, top crosses and population breeding. The “modern landrace” or “evolutionary breeding” approach aims at combining the advantages of the breeding success of the last century and the use of genetic variation producing high yielding and high quality but genetically diverse crop populations that can be adapted to local conditions by the farmers. The overall aim is to provide materials for the development of locally adapted populations and varieties.

An important question when allowing populations to adapt in response to natural selection is to maintain an appropriate population size. This is determined based on the effective population size, a function of the number of genotypes in a population, the breeding structure and the variation in number of offspring produced per individual.

Three winter wheat composite cross (CC) populations were created in 2001 in the UK consisting either of 20 modern wheat parents (called A Population), a subset of 12 high quality parents (Q), or a subset of 9 high yielding parents (Y). Since the F<sub>5</sub> the populations are grown at the University of Kassel under organic and conventional conditions in well separated large (>100m<sup>2</sup>) plots in two parallel sets (12 populations total) without conscious selection applied. In the conventional system no fungicides or insecticides are used to expose the populations to natural pest and disease pressure. The large plot size is used to avoid genetic drift due to uneven contributions of different genotypes in an inbreeding population to the next generation.

From the F<sub>7</sub> (2008) to the F<sub>9</sub> (2010) between 50 and 100 single plants were harvested from each of the 12 populations by digging them up and separating them carefully before threshing. Plants were threshed separately and the seed number and seed size per plant determined.

Overall, the number of seeds contributed to the next generation by single plants varies hugely with some plants contributing only a few seeds while others contribute several hundred seeds. Seed sizes varied greatly among the two years with overall seed weights considerably higher in 2007 (F<sub>6</sub>) than in 2008 (F<sub>7</sub>). Also, in the F<sub>7</sub>, but not in the F<sub>8</sub> some plants with exceptionally big seeds (TGW 74g) were found. The data for 2010 are in the process of being collected and more details about the results will be added to the abstract in September.

## 13. Overcome drought in chickpea (*Cicer arietinum* L.) imitating natural life cycle of progenitor

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**Keywords:** Chickpea, *Cicer arietinum*, cold, drought, ascochyta blight, *Ascochyta rabiei*, large seed size, mutation breeding. Considerable yield loss in chickpea (*Cicer arietinum* L.) occurs when it is subjected to drought accomplishing heat (Canci and Toker 2009). The most efficient practical and economical solution to overcome drought in West Asia, North Africa and south Europe including Mediterranean region is imitated natural life cycle of progenitor *C. reticulatum* Ladiz. This is through the utilization of cold tolerance during vegetative period and ascochyta blight [*Ascochyta rabiei* (Pass.) Labr.] resistance when sowing time is shifted from spring to autumn (Toker et al. 2007, Toker and Yadav 2010). Aims of the study are to pyramid of cold tolerance during early stage, ascochyta blight resistance and to improve large seed size characteristics in eight chickpea genotypes. Air dried seeds were irradiated with 200, 300 and 400 Gy gamma rays (Toker et al. 2005). A single seed descent set of two sets in M<sub>2</sub> generation was used. Mutated populations sown early autumn were screened for cold tolerance under natural freezing and ascochyta blight resistance under artificial epidemic conditions from M<sub>3</sub> to M<sub>6</sub> generations at highland of Anatolia. The same materials were sieved prior to sowing to improve seed size and over 9 mm sieve was selected for large seed size. In the M<sub>6</sub> generation, desirable mutants were selected as bulk for resistance to ascochyta blight, cold tolerance and large seed size from a single seed descent set. Considerable genetic gain was obtained for mentioned three characteristics when mutant are compared to checks and parents. One of these mutated populations is selected via farmer participating breeding and grown by farmers at the highland of west Mediterranean region of Anatolia.

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## 14. Breeding Research Program Green Breeding 2010-2020 for sustainable Agriculture in the Netherlands

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*Keywords:* breeding, research programme, organic and low-input agriculture

The Dutch Ministry of Agriculture, Nature and Food Quality has launched a new Research Program ‘Green Breeding’ to support plant breeding for sustainable, low-input agriculture with 1 million euro per year. As breeding is a long lasting activity, the Programme is intended for a period of ten years. This program aims to support classical plant breeding as requested by a motion of the Dutch parliament and at the same time aims to bridge the gap between conventional and organic agriculture. The focus of Green Breeding is on breeding research to develop selection tools and genitors for improved pest and disease resistance with emphasis on insect resistance and for improved adaptation to lower input conditions for food crops. Results of the projects should be applicable in and serve both organic and conventional agriculture.

More and more the Dutch Ministry of Agriculture, Nature and Food Quality is requiring active participation of the breeding companies as one of the preconditions, including 33% in-kind and/or in-cash contribution. For the first phase of four years, four research projects have been granted, focusing on: organic potato breeding (late blight resistance (*Phytophthora infestans*) and low-input), spinach (horizontal resistance to downy mildew (*Peronospora farinose*) and nutrient-use efficiency), leek (resistance to thrips (*Thrips tabaci*)) and tomato root-stock improvement for nutrient use efficiency.

In this program several research centres participate: Plant Research International, Wageningen University, Louis Bolk Institute, Centre for Genetic Resources and University Groningen. In total six potato breeding companies, seven spinach breeding companies, six leek breeding companies and two tomato breeding companies are involved. The potato breeding is set up as a participatory breeding program including farmer breeders. The program is coordinated by Olga Scholten and Edith Lammerts van Bueren.



## 15. Development of different *Triticum turgidum* x *T. monococcum* synthetic hexaploids to improve the adaptability of cereals to changing environmental conditions.

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**Keywords** : organic breeding, genetic variability, synthetic hexaploids, environmental tolerance

Einkorn wheat (*Triticum monococcum* ssp. *monococcum*) is one of the most valuable source of resistance genes for cereal breeding. Its excellent resistance for fungal diseases becomes more important at organic or low input conditions. Several organically selected einkorn populations and genotypes have good agronomic performance and some of them show excellent frost resistance, allelopathy and straw strength. The high tocol and carotenoid content of the best einkorn lines make them promising sources of functional food production. Nevertheless their uses in wheat breeding programs are highly limited, because they are hardly crossable with cultivated cereals.

We have examined the crossability of several einkorn gene bank accessions and breeding lines with durum (*Triticum turgidum* ssp. *durum*) and emmer wheat (*Triticum turgidum* ssp. *dicoccon*). According to the recent results, the original einkorn accessions are completely incompatible with the tetraploid genotypes tested; meanwhile some new selected semidwarf einkorn lines are showing a relatively high crossability with all the other parental lines. After the genome duplication of the cytologically identified triploid seeds, highly fertile synthetic hexaploids were developed. In their progeny the genome structure (ABA) were cytologically confirmed using molecular cytogenetic methods (multicolor FISH).

Out of the different combinations the progenies of the durum x einkorn and emmer x einkorn crosses were studied for their agronomic performance, resistance character and genetic variability under organic management conditions in comparison of the conventional low input management conditions. According to the results obtained, practically all the developed lines and genotypes showed excellent resistance against wheat diseases, except the fusarium, and showed very good adaptability to both management conditions. Comparing the two synthetic hexaploid combinations, the durum x einkorn originated lines (SH lines) had better straw strength and earlier heading than that of the emmer based SHs. Studying the newly generated diversity of the SHs, we had found, that the durum based SHs are showing a very high level of genetic diversity, which were confirmed both at phenotypic and cytogenetic level. According to the phenotypic performance high variability were identified in case of plant height, in flowering time, ear structure and form, and also in plant growth habit. On the contrary, in the case of emmer based SHs, no visible phenotypic variability was observable. The molecular cytogenetic analysis confirmed the data obtained in phenotypic studies, showing high instability of chromosome pair formation, and the irregularities are suggesting the high level of recombination between the genomes of different origin.

Selected SH lines are recently using in the development of new interspecific composite cross populations and also in bread wheat improvement.

Acknowledgement: The recent research was financed by the research funds of the ALKOBEEER project of the National Science and Technology Office, Hungary and by the European Commission FP7–KBBE No. 245058 "SOLIBAM" projects.



## 16. Evolution of a mixed wheat population in response to dynamic management on farm with contrasted environments and farming practices.

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The genetic diversity of a species is necessary for the development of local adaptation and the potential evolution in case of changing of environmental conditions. In dynamic management (DM) of cultivated populations, plants of the initial composite population are grown for successive generations in several contrasting environment. This meta-population is submitted to evolutionary pressures such as natural selection and drift. This conservation permits both the maintenance of gene combinations adapted to the local environments and the creation of new variation by mutation and recombination (Goldringer et al. 2001).

The meta-population studied is an association of four wheat varieties cultivated in a farmer network: Touselle Anone, Touselle Blanche Barbue, Touselle Blanche de Provence and Touselle Sans Barbe. With 26 samples of 80 individuals from different farms and years and 32 individuals of each original variety, this population was used to study the influences of environment and farmers' practices on the genetic diversity and phenotypic evolution of the meta-population with a special focus on earliness traits evolution, as their are involved in climatic adaptation.

Interviews with the farmers allowed to characterise each sample by its origin and year of reception, the year of harvesting, the farm location and farm management practices. Phenotyping was done on the populations grown in a polytunnel at the research station. Plant height, heading date, and viability were measured. Genotyping was done with 21 neutral microsatellite markers and with 4 markers of major genes involved in flowering time: VRN1A promoter, VRN1A exon 7, VRN1D and VRN3A. Flowering time is a major adaptive trait for wheat and has been shown to evolve quickly under dynamic management (Goldringer et al. 2006). The microsatellite markers were used to study the evolution of neutral diversity and the markers within earliness genes were used to study evolution of traits hypothesized to be subject to selection pressure.

The objectives are to study the correlation between phenotypic and genotypic diversity, and to analyse spatial and temporal evolution of these populations at the genetic and phenotypic level in order to identify possible links between environments or management practices on diversity evolution.

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**Session 3**  
**New insights into the mechanisms of adaptation**  
**to local conditions and organic farming**  
**Thursday, December 2 nd - 13:30 am**

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**Building resilience in organic and low-input farming systems - an ecological geneticist's vision**

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*Keywords:* biomass, diversity, fertility, model, species richness, yield

**Introduction**

There is a long running controversy in ecology (Craine 2007; Grime 2007; Tilman 2007) surrounding the relationship between diversity and productivity. The debate has primarily focused on biomass production in improved versus unimproved grasslands. The disagreement is so fundamental that it includes the precise nature of this relationship, with some researchers reporting a positive relationship (Tilman et al. 1996; Hector 1999; Loreau and Hector 2001a,b) while others claim that the highest levels of biomass production are associated with high fertility, low diversity grasslands (Rusch and Oesterheld 1997; Grime 1998). Of these contrasting opinions the first has received most attention perhaps because it offers the attractive if theoretical possibility for simultaneously enhancing agricultural production while supporting biodiversity and reducing inputs. Over the last 30 years the majority of dramatic increases seen in agricultural yields have been attributed to the genetic improvement of crops (Mackay et al. 2010). Gaining a better understanding of the yield-diversity relationship may allow ecology the opportunity to contribute to future increasing yields. Theoretically as more species are added to a system, niche complementarity should ensure that the efficiency of resources exploitation increases, effectively increasing the fertility of the system by increasing the amount of resources utilized, and thus facilitating greater biomass production. Much of the difficulty in understanding the relationship between yield and diversity arises because it is typically studied in an agricultural context by comparing grasslands that differ in their soil fertility, evolutionary history and management. To avoid the complications that arise from these confounding factors we used an evolutionary ecological model of plant interactions to investigate the nature of the yield-diversity relationship. Specifically we investigated the potential for increasing yields and yield-resilience in reduced input farming systems by exploiting more diverse agricultural systems.

**Materials and Methods**

*Model*



To investigate the yield-diversity relationship we used Evolve - an agent based modelling system that we have applied successfully in several earlier studies (Warren & Topping 1999, 2001, 2004). The model simulates plants interactions in a temporally and spatially explicit arena. Individual plants are defined by life-history traits that are analogous to genes. Seventeen such parameters regulate each plant's ability to grow tall, wide, reproduce, allocate resources to defence etc. The individual plants interact indirectly by modifying their environment, by extracting nutrients and occupying space and thus limiting the growth of surrounding plants. The environment is defined by another set of 14 parameters, which define, how the vegetation is cut or grazed, soil fertility levels, disturbance etc. (see Topping 2010 for full documentation). A key feature of the Evolve model is that life-history traits can be allowed to mutate. If the simulations are run for long enough, ecological and evolutionary processes can both be observed as emergent properties (Warren & Topping 1999, 2001, 2004).

### *Simulations*

The Evolve model was used to perform two sets of simulations. These were carried out to investigate the relationship between soil fertility and species diversity and the relationship between diversity and yield; free from the historic constraints of confounding in real agricultural grasslands. The simulations were based on grazed grasslands of varying soil nutrient status. In the first set of simulations the effect of the nutrient status of the soil on the ability of diversity to evolve was investigated. The simulations comprised of 10 different environments, which varied in fertility status, ranging very low fertility and limiting to growth through to saturation levels (N=15 to 225 in arbitrary units of soil fertility). These were designed to mimic levels in real grassland types from pioneer sand dunes through to improved agricultural grasslands. Each simulation was replicated 10 times. All simulations were run for 10,000 simulated years and all started with 100 propagules of each of the same six species of plant based on typical grassland species (two grasses, two forbs and two legumes). Mutations in life-history traits parameter values were allowed to occur in 1 in every 500 propagules. In simulation 1, diversity was allowed to evolve under intense grazing. At the end of the simulations, the number of species that had evolved was plotted against the soil nutrient status (Fig 1.). In addition, the mean system yield estimated as above ground biomass production in June was plotted against mean species richness over the 10 soil fertility treatments (Fig 2).

In simulation 2, the community of species that had evolved at the end of each of the first set of simulations were transplanted into a fertile environment (N=225). Each of these communities was then allowed to interact for a further 100 years, but this time with no mutations occurring. In the final year of the simulation, the above ground biomass of the grassland was recorded on a month by month basis. The sward yield estimated as biomass production in June was plotted against the species richness of the community (Fig. 3). In addition, biomass production over the entire year was plotted for contrasting sampled communities with high and low species richness (Fig. 4).

### *Results*

At the end of the first set of simulations, more species diversity was seen to evolve in the highest fertility grasslands (Fig 1). Almost three times as many species evolved in the most fertile grasslands than were observed in the low fertility ones. These high fertility and high diversity grasslands were observed to produce high levels of biomass (Fig. 2), while the low diversity, low fertility grasslands produced almost no biomass yield.

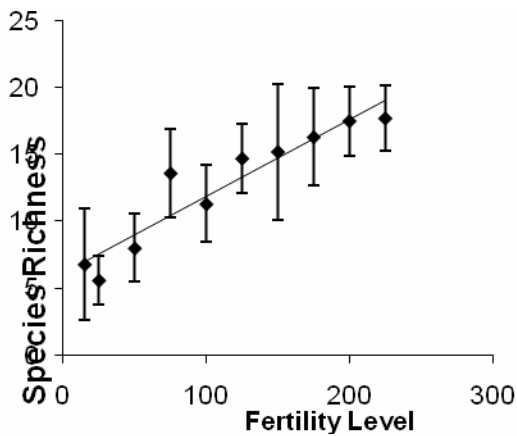


Figure 1. At the end of simulation 1 species richness was observed to be positively correlated with soil fertility status

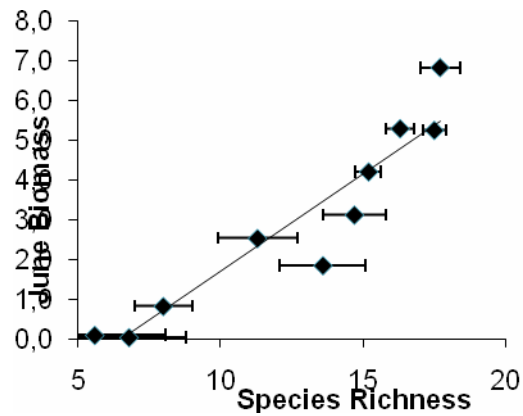


Figure 2. At the end of simulation 1 yield was observed to be positively correlated with species richness

After the 100 years plant competition in the second set of simulations June biomass production was found to be weakly negatively correlated with species richness (Fig. 3). However, when total annual biomass production was considered, highest yields were observed to be associated with the most diverse grasslands (Fig. 4). These diversity communities were seen to be more consistently productive across the entire year than were low diversity grasslands.

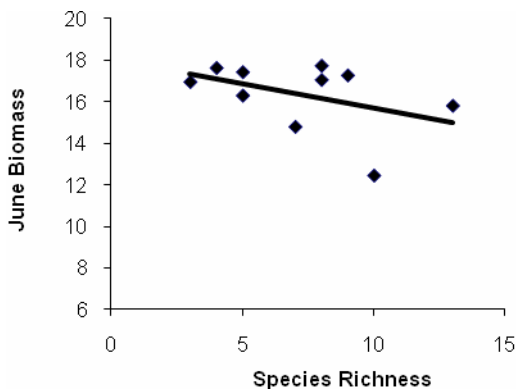


Figure 3. At the end of simulation 2 June biomass production appeared to be inversely correlated with system species richness

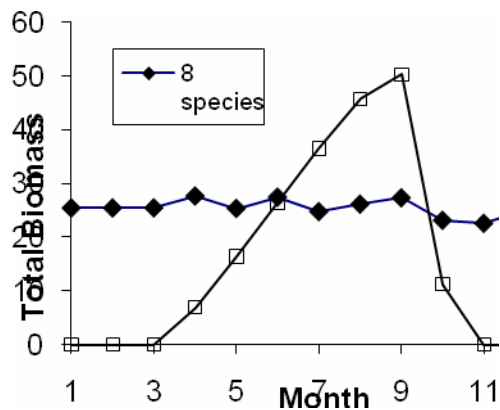


Figure 4. Total biomass production over the entire year at the end of simulation 2, is higher in more diverse systems, although peak production is highest in low diversity grasslands

### Discussion

At the heart of the long running controversy surrounding the relationship between biomass, diversity and fertility is the uncertainty surrounding the low levels of diversity associated with high fertility. Is species richness limited by the lack of opportunity for it to evolve or is high diversity incompatible with high nutrient status and high biomass production (Smits et al. 2008)? Our previous studies (Warren et al 2009) have shown that it is possible to generate the humped back relationship between diversity and fertility by transplanting some communities into high fertility conditions. However, the first set of simulation results presented here suggests that if sufficient time is available then diversity may evolve under high fertility conditions and that such systems are likely to be high yielding. These high yields associated with artificially high fertility (Figs. 1 & 2) are of little interest to organic or low input agricultural systems, whereas the results of the second set of simulations are more directly relevant.

When fertility levels are kept constant, figures 3 and 4 show that while low diversity grasslands may be more productive at peak productivity, higher diversity systems are more productive over the entire year. This has clear implications for yield resilience. High diversity agricultural systems are likely to remain more productive, if one or two species fail because of disease or because of the predicted increasing vagaries of the climate.

These predictions point the way to a new agriculture, one which will be scientifically difficult to develop and currently only existing as computer simulations. Even so new developments in gene expression are providing better insights into ecological interactions such as competition and the vision gets closer to becoming reality every day.

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## Breeding onions for low input agriculture: Genetic analysis of the interaction between *Allium* species and arbuscular mycorrhizal fungi

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*Key words:* Onion, *Allium cepa* L., arbuscular mycorrhizal fungi, low input farming

### Abstract

Arbuscular mycorrhizal fungi (AMF) play an important role in the uptake of nutrients and water from soil. Some crops, as for example onion, *Allium cepa* L., have a poorly developed root system. As a result, onion plants need a lot of fertiliser for growth, and they are sensitive to drought. The aim of this project was to study the beneficial effects of mycorrhizal fungi on the growth and development of *Allium* species and to determine whether it is possible to improve onions for better mycorrhizal responsiveness by breeding. In two subsequent years experiments were carried out with plants of *A. cepa* L., *A. roylei*, *A. fistulosum*, and the interspecific hybrid *A. fistulosum* x *A. roylei*. The tri-hybrid population of *A. cepa* x (*A. roylei* x *A. fistulosum*) was used to study the genetic basis for response to AMF. Plant response to mycorrhizal symbiosis was expressed as mycorrhizal responsiveness, which is the difference in plant weight of non-mycorrhizal and mycorrhizal plants divided by the weight of non-mycorrhizal plants. As the plants grew very poorly under non-mycorrhizal conditions, selection of plants with high mycorrhizal responsiveness would result in selection of plants with poor growth under non-mycorrhizal conditions. To improve the beneficial response of onions to AMF, mycorrhizal responsiveness was considered not to be a useful trait in breeding onions for low input agriculture. Two other variables were considered, absolute responsiveness and average plant performance. These variables were positively correlated with biomass of non-mycorrhizal and mycorrhizal plants. QTLs contributing to mycorrhizal response were located on a linkage map of the *A. roylei* x *A. fistulosum* parental genotype and coincided with QTLs for plant biomass. As onion breeding focuses on improvement of plant yield, these findings suggest that breeding did not select against the response to AMF. In addition, a positive correlation was found between high number of roots, biomass and large response to AMF for close relatives of onion opens, indicating possibilities for breeding onions for low input agriculture to obtain more robust cultivars.

### Introduction

Arbuscular mycorrhizal fungi (AMF) are plant associated microorganisms that occur naturally in soil. They play an important role in plant growth since they contribute to the uptake of nutrients and water from soils (Ryan and Graham, 2002). Onion (*Allium cepa* L.) is an important vegetable crop worldwide, but one of the major challenges in onion cultivation is to provide the plants with sufficient nutrients (Brewster, 1994). Large amounts of fertiliser are needed, but, because of the poorly developed root system (Portas, 1973), much of the applied nutrient is not used. For low-input systems, plants have to be good nutrient scavengers. Therefore, productivity and stability of onion production in such systems can be particularly problematic (Greenwood et al, 1982).

From earlier studies, it is known that onion plants associate with AMF (Hayman and Mosse 1971; Stribley, 1990; Charron et al., 2001; De Melo, 2003). The aim of the present research was to study the beneficial effects of arbuscular mycorrhizal fungi on the growth and development of *Allium* species, and to determine whether it is

possible to improve onions for mycorrhizal responsiveness by means of breeding. Related species of onion, such as *A. roylei* and *A. fistulosum* L. are known to possess interesting traits to breed robust onion cultivars (Kik 2002). Genes from *A. fistulosum* can be introgressed into onion germplasm via a bridge cross with *A. roylei* (Khrustaleva & Kik 2000).

### Materials and methods

A tri-hybrid population, the *A. cepa* × (*A. roylei* × *A. fistulosum*) was developed as described by Khrustaleva and Kik (1998) by crossing an onion with one plant obtained from a cross between *A. roylei* and *A. fistulosum* (RF hybrid). Experiments were carried out in 2006 and 2007 in a climate-controlled greenhouse (day/night 22/17 °C), and comprised the population, the parental species and the RF-hybrid. The experiment comprised 77 tri-hybrid genotypes in 2006 and 83 in 2007. Each genotype was multiplied vegetatively, and transferred to individual pots containing a mixture of sterilized clay soil, sand and perlite (6:1:1, v/v/v). AMF species *Glomus intraradices*, kindly provided by Dr. Y. Kapulnik, Volcani Centre, Israel, was added to the plant hole just before transplanting. Per genotype, six replications were inoculated with AMF (treated plants, AM) and six with sterilized AMF (control plants, NM). Of eight tri-hybrid genotypes, parental species and the RF-hybrid extra plants were transplanted to evaluate whether genotypes differed in colonization rates. Three replicates were evaluated seven weeks after inoculation. Root colonization was estimated for each individual plant, applying the magnified intersection method, after staining with trypan blue. For each sample, 100 observation points were evaluated (McGonigle et al. 1990).

Plants were harvested thirteen weeks after transplantation. During growth, and at harvest, several characteristics of the plants were measured, including total fresh and dry weight, and their partitioning into leaves, bulb or stem, and roots. Numbers of leaves, stems, and roots was also recorded, as well as plant height. Mycorrhizal responsiveness was calculated as the increase in plant weight compared to the non-mycorrhiza treatment (Baon, 1993). Responsiveness was considered significant when AM and NM treatments were statistically different ( $p < 0.05$ ). In addition, absolute mycorrhizal responsiveness (R), the difference in weight between mycorrhizal and non-mycorrhizal plants (Sawers et al., 2010), and the average plant performance under both treatments were calculated. AFLP® markers were obtained as described by Vos et al. (1995). A linkage map was calculated using JoinMap 3.0 (Van Ooijen and Voorrips, 2001). Quantitative trait loci (QTLs) were obtained using MapQTL® 4.0 (Van Ooijen et al., 2002).

### Results and Discussion

In both years, AMF had a strong significant effect on plant dry weight of the tri-hybrid population. Mycorrhizal responsiveness was strongly determined by the weight of plants under non-mycorrhizal conditions. Breeding for improved mycorrhizal responsiveness leads to breeding for plants that have poor weights under non-mycorrhizal conditions, irrespective of the effect that mycorrhizal fungi may have on plant growth and crop yield. This is illustrated in Fig. 1, which shows the hypothetical situation in which mycorrhizal fungi have a positive, a negative or no effect on plant weight under mycorrhizal conditions compared to non-mycorrhizal conditions and the resulting calculation of mycorrhizal responsiveness.

Absolute mycorrhizal responsiveness and average plant performance were regarded as better traits for breeding. Both traits were highly correlated with biomass of AM plants (resp.  $r = 0.91$  and  $r = 0.97$ ). Two QTLs from *A. roylei* were detected on chromosomes 2 and 3 for absolute mycorrhizal responsiveness, average plant performance and biomass of mycorrhizal plants. A QTL from *A. fistulosum* was detected on linkage group 9 for average plant performance, biomass of mycorrhizal and non-mycorrhizal plants, and number of stem-borne roots. Coincident QTL regions for plant biomass, absolute mycorrhizal responsiveness and average plant performance indicate that selection for plant biomass may also select for enhanced beneficial effects of AMF.

With absolute mycorrhizal responsiveness a breeder looks at the beneficial effect obtained directly from the mycorrhizal fungi. In this case, it is still possible that plants will be selected that grow poor under non-mycorrhizal conditions. The advantage to look also at average plant performance is that plants will be selected that grow well under all conditions, even for example in years or areas, when amounts of mycorrhizal fungi present in the soil are low. For breeding onion under low-input conditions there is no reason to carry out experiments under non-mycorrhizal conditions, as results of the beneficial effect of AMF are highly correlated with results obtained under mycorrhizal conditions. These results can only be explained by the fact that onions are highly mycorrhizal dependant.

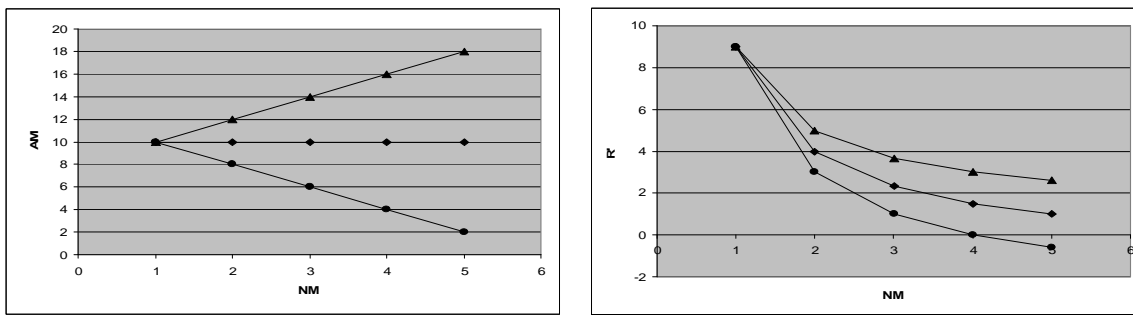


Figure 1. Hypothetical effect of mycorrhizal fungi on plant weight, expressed as a positive or negative or no effect (left) and the mycorrhizal responsiveness, calculated as the difference in plant weight between the mycorrhizal and the non-mycorrhizal condition divided by the weight under non-mycorrhizal conditions. This figure illustrates why mycorrhizal responsiveness is not a good trait to be used in breeding.

Tri-hybrid genotypes differed in early colonization, with values ranging from 33 to 73% (REML analysis,  $p < 0.05$ ). Genotypes with high levels of colonization had larger absolute responsiveness for total dry weight in 2006 and 2007. Results suggest that differences in early colonisation could be translated into differential growth performance. Further research is needed to establish the importance of early colonization in *Allium*.

As a follow-up of this research, the population has been transplanted into the field under high and low phosphorus conditions, to study whether genotypes differing in response to mycorrhizal fungi also differ in their ability to grow under low and high P.

#### Acknowledgments

This work is funded by the Dutch Ministry of Agriculture, Nature and Food quality as part of Programme 388-II Breeding for Organic Farming. Dr. Galván Vivero thanks the Alban Programme of the EU, PDT-Uruguay, and the NFP for financial support.

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## Genetic variation for nutrient use efficiency in maize under different tillage and fertilization regimes with special emphasis to plant microbe interaction

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### Introduction

Conservation tillage (no-till and reduced tillage) brings many benefits with respect to soil fertility and energy use, but it also has drawbacks regarding the need for synthetic fertilizers and herbicides. To promote conservation tillage in organic farming systems, crop rotation, fertilization and weed control have to be optimized. In addition, crop varieties are needed with improved nutrient use efficiency (NUE) and high weed competitiveness or tolerance. As soil temperature rises slowly in reduced tillage systems in early spring, nitrogen mineralisation is often delayed resulting in temporary shortage of nitrogen (N) supply. Symbiosis with arbuscular mycorrhizal fungi (AMF) play an important role for nutrient acquisition of many crop plants, especially for insoluble elements like phosphorus (P) and zinc (Zn). Crop management that favours native AMF populations or inoculation of selected AMF strains might thus increase crop nutrient uptake. In the scope of an European project for improving nutrient use efficiency in major European crops (NUE-Crops) we studied the effect of different agronomic practices on the NUE and plant microbe interaction of maize (*Zea mays* L.) as well as the effect of AMF inoculations on maize yield.

The objectives of the first study were (i) to quantify the NUE of maize genotypes under different tillage regimes and fertilization levels, (ii) to compare the effect of slow releasing organic *versus* easily soluble mineral fertilizer on dry matter yield and weed coverage, and (iii) to assess the correlation of root colonization by arbuscular mycorrhizal fungi (AMF-RC) and NUE. The main objective of the second study was (iv) to quantify the effect of AMF seed inoculation on grain yield and nitrogen use efficiency of maize elite lines under different fertilization levels.

### Material and Methods

In order to determine the genotype x crop management interaction, we tested in 2009 six maize varieties with different NUE under conventional (CT) and reduced tillage (RT), applying five different fertilization regimes (unfertilized, slurry with either 85 or 170 kg/ha total N, mineral NPK fertilizer with either 85 or 170 kg/ha total N) with four replications. The field trial was carried out on an organically managed arable field with loamy soil in Muri, canton Aargau, Switzerland. Weed pressure, male and female flowering time, plant height, chlorophyll content and corn borer infestation was assessed during the vegetation period. At silage harvest, dry matter content and dry matter biomass of maize was determined as well as silage quality parameters including netto energy lactation (NEL) and N and P concentration for all 240 plots. In addition, root capacitance measurements, shoot and root biomass, N and P concentration in the shoots as well as AMF symbiosis was determined at flowering stage for selected plots. The percentage of AMF-RC was assessed on fine roots derived from two different soil layers (0-10 cm and 10-20 cm) by grid intersect method after bleaching and staining the mycorrhizal structures with Trypan-blue. The presence and quantity of different AMF species (*Glomus mosseae*, *Glomus claroideum*, *Glomus intraradices*, *Gigaspora margarita*, *Scutellospora pellucida*, and *Diversispora celata*) was determined after DNA extraction by real-time quantitative polymerase chain reaction (RT-PCR).



In order to assess the effect of mycorrhizal seed inoculation on nutrient uptake and grain yield of maize, two different sets of maize genotypes were tested. Set 1 consisted of 100 testcrosses of doubled-haploid lines derived from old flint landraces including elite checks, while set 2 consisted of 100 testcrosses of elite maize lines. Seed inoculation was performed by the company AMYKOR, Germany, using *in-vitro* produced AMF spores. The 200 testcrosses were examined in 2009 with and without artificial inoculation at two locations in Germany (Bernburg, Grucking) applying mineral N at two levels (0 and 160 kg N/ha).

### Results

The six different maize varieties tested in Muri under different tillage and fertilization regimes had a significant effect on almost all traits except for weed coverage in early vegetation period. Dry matter yield (DMY) of silage averaged across management techniques ranged from 15.4 to 18.6 t/ha for the tested genotypes. The produced energy for animal feed (NEL) per acreage ranged from 9.5 to 11.3 MJ/m<sup>2</sup>. Although no significant genotype x tillage interaction were detected for these traits, the differentiation of the 6 varieties was much more pronounced under RT. Significant genotypic and fertilization effects were found for nitrogen use efficiency (Nit\_UE) measured as dry matter yield per kg N<sub>available</sub> (fertilizer + N<sub>min</sub> in soil). Nit\_UE ranged from 188 up to 232 kg DMY/kg N for the tested varieties. Mineral fertilizer resulted in significantly higher DMY (18.4 t/ha for both 85 and 170 kg N input/ha) than realized with slurry fertilizer (16.3 and 16.9 t/ha for 85 and 170 kg total N input/ha, respectively), which was significantly higher than the unfertilized control (14.0 t/ha). Tillage regime had no significant effect on DMY (16.5 t/ha for RT vs. 17.1 t/ha for CT), but strongly affected weed pressure, ripening, chlorophyll and N contents. Reduced tillage resulted in 50% increase in weed scores and higher dry matter content and reduced chlorophyll and protein contents. Increasing fertilization level resulted in shorter silking anthesis interval, higher chlorophyll content, increased plant height, delayed ripening, higher N and reduced P content, and reduced Nit\_UE. Significant genotype x tillage x fertilization interaction were found for plant height, weed scoring and Nit\_UE.

Tillage and fertilization regime had also a significant effect on AMF root colonisation. Higher AMF-RC was found under RT compared to CT and also in the unfertilized control plots compared to the mineral fertilizer treatment (Fig. 1). In addition, significant genotype × tillage interaction was detected for AMF-RC. Similar results were obtained by the DNA quantification of the different AMF species by real-time quantitative PCR. Focusing on single AMF species, significant genotypic effects were identified. While *G. mosseae* and *G. claroideum* were more frequently found on roots of variety S4 than on roots of S3, the opposite was true for *S. pellucida* and *D. celata*. AMF-RC was negatively correlated with K and N content and DMY. A weak positive correlation was found between AMF-RC and N uptake and Nit\_UE, but not with the P content. Instead a strong positive correlation was found for AMF-RC and to the sugar content of the plant at flowering suggesting a more parasitic plant microbe interaction.

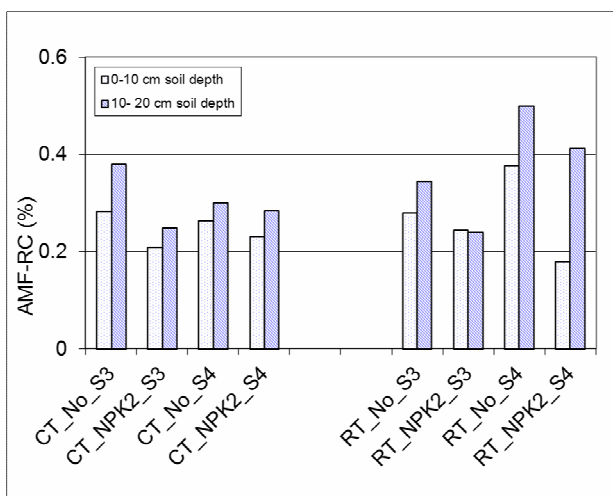


Fig. 1: AMF-RC of two different maize varieties (S3 and S4) cultivated under different tillage regime (CT and RT) and fertilization levels (No fertilizer and NPK fertilizer) in Muri during

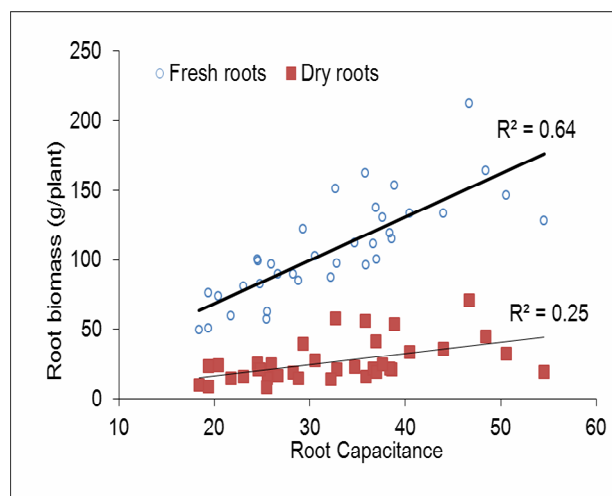


Fig. 2: Correlation of root capacitance measure with root fresh weight and dry weight during flowering in Muri 2009

*flowering time (21.07.2009)*

The measurement of root capacitance in water saturated soils was proposed as a non-destructive method indicative for the active root surface. In order to calibrate this method, the plant-soil system was measured for its capacitance in the field first and afterwards plants were uprooted to determine root fresh and dry weight at flowering stage. Significant correlations were found between the measured root capacitance and root fresh weight per plant, but only weak correlation with root dry weight (Fig. 2).

The effect of artificial mycorrhizal seed inoculation on grain dry matter yield (GDY) relative to the untreated seed varied according to the N level, the tested gene pool and individual genotypes. On average the GDY under low N conditions (no N fertilizer since several years) was reduced by 60% compared to the high N level, indicating severe N deficiency. Testcrosses of set 2 derived from elite breeding lines had under both low and high N level a higher yield potential than testcrosses of set 1 derived from old flint landraces.

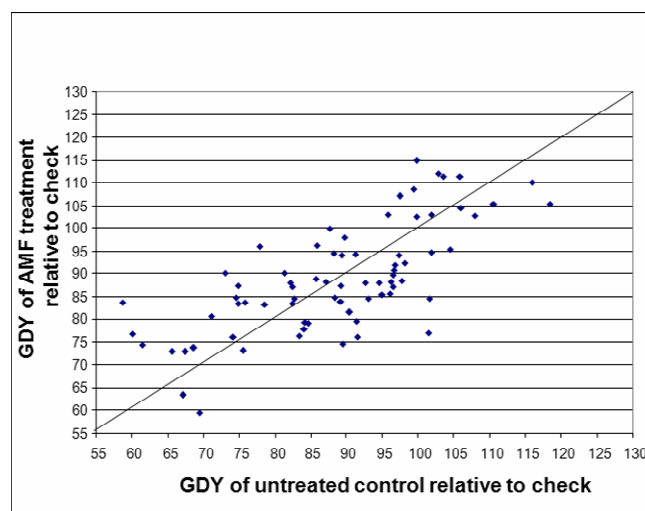


Fig. 3: Relative grain dry matter yield (GDY) of set 1 maize genotypes with (y axis) and without AMF seed inoculation (x axis) tested under low N conditions at two locations in 2009

The AMF seed inoculation of set 1 resulted in an average yield increase (GDY relative to the untreated control) of 2.9% under low N and of 1.1% under high N conditions. In contrast, set 2 showed an average yield increase of 4.9% under low N, but an average yield decrease of 1.8% under high N conditions due to artificial seed inoculation. However, the response to artificial AMF seed inoculation of individual genotypes varied a lot within each material set (Fig. 3), indicating a strong genotype x AMF inoculation interaction.

## Discussion

Although reduced tillage resulted in higher weed pressure during the whole vegetation period than conventional tillage, not significant reduction in DMY was observed. The N supply was reduced under RT which was clearly visible by the reduced chlorophyll content of the unfertilized plots. This resulted in a stronger positive response in plant height and DMY with increasing fertilizer input compared to CT. Under CT, no yield increase could be obtained by doubling the amount of mineral fertilizer or doubling the amount of slurry application. Therefore, the dosage of applied fertilizer needs to be adjusted to the respective tillage system to avoid N leaching. For the optimal choice of variety, not only the genotypic effects on DMY needs to be considered but also the significant genotype x tillage x fertilizer interactions for Nit\_UE.

There was significant variation for AMF-maize symbioses, measured by AMF-RC and DNA-based quantification of commonly occurring AMF species. While RT and no fertilization increased AMF-RC, different varieties promoted different AMF species and significant genotype x tillage interaction were encountered. Averaged across all treatments we found a negative correlation of AMF abundance with DMY of maize under the Muri growing conditions. This is in line with the yield reduction observed in the elite breeding material after

AMF seed inoculation under high N conditions. However, under stress conditions, like the severe N deficiency at the two locations in Germany, the AMF inoculation with selected strains were on average beneficial to the maize plants resulting in GDY improvement. This agrees well with the hypothesis that the maize-AMF symbiosis is of special benefit for the plant under stress conditions (mutualism), while under optimal growing conditions plant growth might be reduced due to the parasitic behaviour of the fungi.

However, these results are based on one year data and need to be verified in further field experiments that were set up in 2010.

#### **Acknowledgements**

The authors gratefully acknowledge financial support from the COOP Sustainability Fund (Klimaneutraler Acker- und Gemüsebau, KLINE AG, Switzerland) and funding from the European Community financial participation under the Seventh Framework Programme for Research, Technological Development and Demonstration Activities, for the Integrated Project NUE-CROPS FP7-CP-IP 222645.

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## Validation of associations between plant traits and Thrips damage in cabbage

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*Keywords* : Brassica oleracea var capitata, Thrips tabaci, wax, earliness

### Abstract

When cabbage is cultivated for storage in the Netherlands, it is usually harvested around mid-October. This type of cabbage crop may be severely damaged by thrips (*Thrips tabaci*). The thrips population on the plants and the more severe symptoms develop mostly during September and October. Also during cold storage symptoms continue to develop. The damage caused by thrips is due to the symptoms that develop after feeding, which are small callus-like growths that will turn brownish after some time and which may cover substantial amounts of leaf area.

Large differences exist between cabbage varieties in their susceptibility to thrips damage. Based on several years of field trials with more than 40 commercial varieties and gene bank accessions it was established that a large proportion of the variation for thrips damage could be explained by a few plant traits: the amount of leaf surface wax, earliness and Brix (Voorrips et al, 2008).

Using an F3-line population derived from a cross between a high-damage, low-wax, high-Brix, earlier heading and a low-damage, high-wax, low-Brix, later heading accession we determine whether the relations between plant traits and thrips damage can be confirmed.

### Introduction

Cabbage (*Brassica oleracea* var *capitata*) is an important field crop in the Netherlands, cultivated by conventional as well as organic growers. Cabbage cultivated for storage is usually harvested around mid-October. This type of cabbage crop may be severely damaged by thrips (*Thrips tabaci*). The damage caused by thrips is due to the symptoms that develop after feeding, which are small callus-like growths (intumescences) that will turn brownish after some time. These symptoms force growers to remove the outer leaf layers before marketing, leading to increased labor costs and yield losses.

Among red and white cabbage varieties, large differences have been reported in the amount of thrips damage (Shelton et al. 1983; Stoner and Shelton 1988; Fail and Penzes, 2001; Van de Steene et al. 2003; Voorrips et al. 2008) and in the numbers of thrips observed on the plants (Stoner and Shelton 1986; Van de Steene et al. 2003; Voorrips et al. 2008). However, the genetic background of tolerance or resistance to thrips is not clear, and breeding for resistance or tolerance to thrips is difficult. One of the problems is the large variability between years and locations in the severity and timing of thrips infestation. Also within genotypes in the same experiment, large plant to plant variations can occur in damage or thrips population size. Also, it is not easy to have all the accessions in a comparable developmental stage throughout the evaluation period, which is a requirement for reliable comparisons.

Indirect selection might help to overcome these problems. One possibility is to identify plant traits that are associated with the level of thrips damage. In previous work we have explored this approach, and found that a combination of earliness, the amount of leaf surface wax and Brix could explain about 75% of the variability in thrips damage among cultivars of white cabbage (; Voorrips et al. 2008). A problem with this analysis was that among the varieties studied we observed a significant negative correlation between earliness and the amount of leaf surface wax. Here we describe a validation of these results based on a larger set of cultivars, partially selected to reduce this correlation, and on a set of 150 F3 lines derived from a cross of two accessions contrasting for these traits.

## Materials & methods

### *Field experiments*

In 2009 field experiments with a collection of 36 varieties were conducted at two locations in the Netherlands. Small cabbage plants were planted in the field in the last week of May. The experimental fields were divided into two blocks, with one plot per accession per block. At three harvest dates between mid-August and mid-October three plants were harvested from each plot. Harvested plants were scored for several traits including developmental stage, circumference of the head (cm), leaf thickness (mm), wax layer, compactness and Brix. One half of each head was peeled and assessed for thrips damage and the number of thrips adults and larvae.

In 2010 one field experiment with a set of 150 F3 lines and a few standard accessions was conducted in the Netherlands. The field was planted in the third week of May. The experiment was divided into three blocks, with one plot per F3 line and two plots per standard accession in each block. At four harvest dates between 6 September and 2 November plants were harvested, as close as possible to commercial harvest stage; however the data for the last harvest were not available yet for this presentation. Harvested plants were scored for developmental stage, circumference of the head (cm), wax layer and compactness. One half of each head was peeled and assessed for thrips damage.

In the 2010 experiment the thrips population was increased by interplanting shallot plants that had been pre-infested with thrips for one month.

### *Plant Material*

The plant material of the 2009 experiment consisted of 36 medium to late white cabbage varieties, including mostly F1-hybrid varieties and a few classical open-pollinated varieties, as well as one glossy mutant line. The material of the 2010 experiment consisted of 150 F3 lines, all derived from one F1 plant of a cross between the OP varieties Bewama (relatively early maturing, low amount of leaf wax, high Brix, sensitive to thrips damage) and Langendijker Bewaar (late maturing, high leaf wax, low Brix, tolerant to thrips damage). Also included were six standards: the two parents, the F1 and three F1-hybrid cultivars (Bartolo, Galaxy and Zerlina).

## Results

### *Varieties experiment, 2009*

In 2009 the amount of thrips in both fields was quite low, resulting in relatively low levels of thrips damage and therefore also in relatively low correlations between the amount of thrips damage and the different plant traits. Taking the date with the most severe damage at each location as reference, 11 varieties were not significantly different from the most resistant one with respect to damage, and five were not significantly different from the most susceptible. The set of 11 most resistant varieties included the reference variety Galaxy and the resistant parent of the F3-line population: Langendijker Bewaar. The set of 5 most susceptible varieties included the reference variety Bartolo and the susceptible parent of the population: Bewama.

The correlations between thrips damage and leaf surface wax varied between harvests and locations from -0.39 to -0.45, which is lower than the values observed in previous experiments (Voorrips et al. 2008) but still significant. The same was the case for the correlations between damage and earliness. Most correlations between damage and Brix were not significant.

In spite of our attempt to select varieties with all combinations of wax layer and earliness we still observed a correlation between these traits. Part of the correlation is caused by the early maturing glossy mutant. However, there was also still a lack of late accessions with little surface wax (Figure 1).

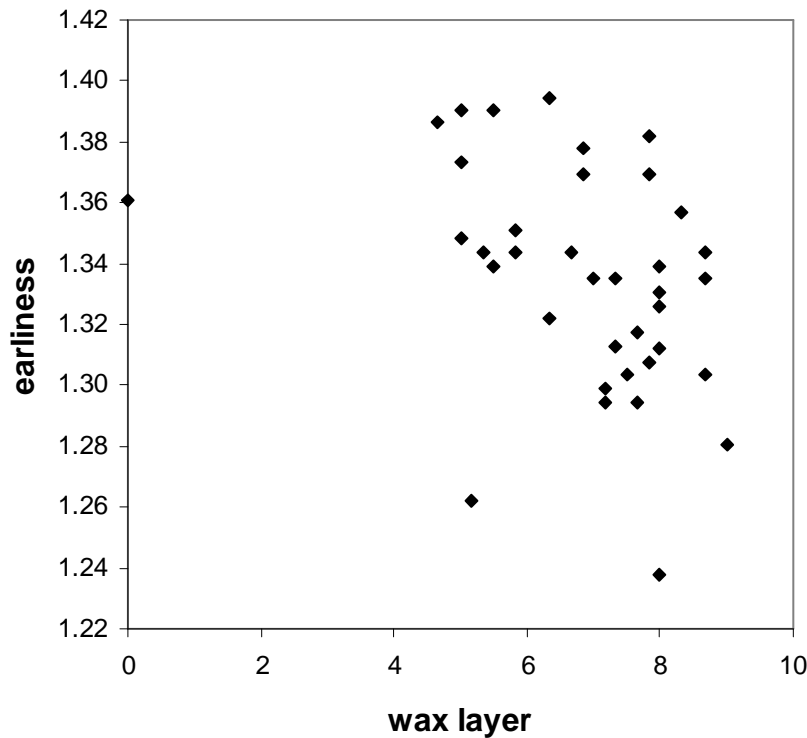


Figure 1. Scatterplot of earliness vs wax layer for 36 white cabbage varieties and accessions in the 2009 field experiment. The one accession with a 0 score for wax layer is a glossy mutant line.

#### *F3 lines experiment, 2010*

The thrips damage in 2010 was considerably higher than in 2009. No transgression was observed in the F3-line population, i.e. the parents were not significantly less resp. more damaged than the least resp. most damaged line. The same was true for the amount of leaf surface wax. However the parents were intermediate for earliness, in contrast to what was observed in earlier experiments.

We observed significant correlations of thrips damage with the amount of wax ( $R=-0.68$ ) and with earliness ( $R=0.66$ ), comparable with what was observed earlier in variety tests but higher than in the 2009 experiment. Among the F3 lines we also observed a correlation between the amount of leaf surface wax and earliness; as in the variety panel of the 2009 experiment the combination of lateness and low wax amounts was lacking.

#### **Conclusions**

Both among a set of varieties and in a cross progeny we observed a relation between the amount of leaf surface wax and earliness: in both cases the combination of lateness and little wax was lacking. This suggests that the generally thick wax layer of late varieties is not only the result of selection, but may have a biological cause.

The experiments in 2009 suffered from a low amount of thrips in the field, and therefore correlations between plant traits and thrips damage were low. However in the experiment of 2010 with 150 F3 lines from a cross progeny the thrips damage was high, perhaps because extra thrips were brought into the field by interplanting infested shallots. In this experiment the previously found relations of high thrips damage with little wax and with earliness were confirmed.

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## Wheat varieties in competition with weeds for sustainable agriculture, in particular organic farming

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*Key words:* variety, crop competitiveness, weeds, organic farming, winter bread wheat.

### Introduction

Historically winter wheat breeding programmes have mainly focused on developing varieties for high-input, conventional production systems which have improved yield, yield stability and protein content. For the last 50 years herbicides have been the way to suppress weeds, thus relative competitiveness of varieties against weeds was studied nearly exclusively in organic farming (Eisele *et al.* 1997, Drews *et al.* 2002). Indeed, in organic farming, prevention methods are used to control weed infestation, by enhancing agro-ecological principles. One mean is to optimize competition by cultivating species with a strong ability to cover the soil. Among species, the choice of competitive varieties is also important (Lecomte *et al.* 2000). The programme presented here associates organic and low input systems in order to enhance exchanges between researchers and stakeholders from both types of agriculture.

### Materials and methods

Two types of complementary trials were conducted from 2007 to 2009 in a multi-site experiment in France. The objectives were to develop a method for assessing the competitive potential of bread wheat varieties against weeds and to encourage the adoption of this criterion in selection programmes.

First and second year, 4 trials using Italian ryegrass (to simulate weed) were set up to identify the most explanatory characteristics of the competitive potential of wheat, in conventional experimental stations, in Ile-de-France (La Minière – Boigneville) and Bourgogne (Dijon). Second and third year of the programme, 8 trials were run in organic farming (table I), to test varieties in various conditions with natural infestation, in order to screen their ability to compete with weeds. Assessed varieties (table II) were selected in order to have a wide range of earliness and height. One triticale was added, as a control with a high potential of competitiveness against weeds. According to field heterogeneity, experiments were designed in randomised complete block with six replicates per environment in organic farming conditions. Organic trials were selected to have as homogeneous as possible natural weed infestation. Floras were different but dominated by monocotyledons in most places (table I).

Plots were assessed for growing stages, ground cover, wheat biomass, crop canopy height, leaf inclination, lodging and grain yield. Measures on weed (to assess weed suppression) consisted in (i) determination of weed density by visual estimation, (ii) measure of weed density, (iii) measure of weed biomass (on 1m<sup>2</sup>). Additionally, we also compared yield of varieties with or without weeds in competition. In organic trials, weeds were pulled out by hand only in Renan (high/medium competitiveness) and Caphorn (low competitiveness).



Table I: Summary of the 8 field tests in organic farming (years 2 and 3)

Trial	year	location	Sowing date	Sowing density	Inter row (cm)	N fertiliz.	Major flora
ABPC	2008	Mignaloux-B. (86)	09-nov	350	17	90 U	<i>A. myosuroides</i> , <i>Avena fatua</i>
ABPC	2009	Les Ormes (86)	27-nov	350	17		<i>Avena fatua</i>
Arvalis	2008	Josnes (41)	25-oct	400	17	0	<i>A. myosuroides</i>
Arvalis	2009	La Chapelle St M. (41)	04-nov	400	17	70 U	<i>Galium aparine</i> , <i>Papaver rhoeas</i>
CREAB	2008	Auch (32)	14-nov	350	17	0	<i>Sinapis arvensis</i>
CREAB	2009	Auch (32)	08-janv	400	17	0	<i>Sinapis arvensis</i>
INRA	2008	Sermaise (91)	05-nov	350	20	0	<i>A. myosuroides</i> , <i>L. perenne</i>
INRA	2009	Sermaise (91)	06-nov	350	20	0	<i>Agrostis</i> , <i>L. perenne</i>

Table II: List of the varieties used in organic field tests

		height		
		short	medium-sized	high
Earliness (Z55)	Early	<b>Caphorn</b>	<b>Cézanne</b> (Sumo), SUR. 233	<b>Grandval</b> (triticale)
	middle	(Boisseau)	Quebon, CF99102, <b>Renan</b>	Saturnus
	late		LD269	<b>Pegassos</b> , (Ataro)

This article presents main results from organic trials and a comparison to results obtained the previous year in experimental non organic stations.

## Results

### Impact of weeds on yield

Statistical analysis on controls Renan and Caphorn (tableau III) shows a significant effect of manual weed removal on yield, with a significant interaction between location and weeding. Yield losses are more important on Caphorn, a short cultivar with low ground cover ability.

Tableau III: Yield losses ( $t^{-1}.ha$ ) due to weeds for the varieties Renan and Caphorn in the organic field tests

Trial	Renan			Caphorn		
	with weeds	without weeds	losses ( $t^{-1}.ha$ )	with weeds	without weeds	losses ( $t^{-1}.ha$ )
Arvalis 08	49,1	52,4	-3,3	50,3	52,0	-1,7
Arvalis 09	44,9	49,1	-4,3	34,2	41,4	-7,2
CREAB 08	35,7	38,0	-2,3	35,9	38,9	-3,0
CREAB 09	21,5	23,2	-1,7	17,6	22,2	-4,6
INRA 08	60,2	56,8	3,4	58,3	59,0	-0,7
ABPC 08	20,8	23,0	-2,2	21,2	24,0	-2,8
ABPC 09	24,6	23,5	1,1	18,9	19,7	-0,8
Average			-1,1			-3,0

*Ranking of varieties according to impact on weed biomass*

We demonstrated a variety effect on weed biomass. Nevertheless, the heterogeneity in weed repartition led to end with non precise trials. We selected trials with significant biomass data (in g/m<sup>2</sup>). Trials from CREAB had to be withdrawn (lack of precision in 2008, lack of variety Pegassos in 2009), so we finally pooled 5 trials for 5 common varieties: Caphorn, Renan, Cézanne, Pegassos and Grandval (triticale). We notice a strong effect of varieties on weed biomass when the triticale Grandval (high ground cover control) is included (table IV): Grandval is the most competitive cultivar whereas Caphorn is confirmed as the less competitive one; the 3 other varieties are not statistically different. However, the effect of variety on weed biomass is low when we don't consider Grandval (probability = 0.25). Moreover, the effect of location is significant due to heterogeneity in weed infestation. The interaction variety x location is also significant due to diversity of trial sites.

Tableau IV: Ranking of the varieties according to effect on weed biomass (5 trials pooled)

	weeds biomass	Newman&Keuls 5 %	
CAPHORN	148	A	
PEGASSOS	115	A	B
CEZANNE	110	A	B
RENAN	106	A	B
GRANDVAL	63		B

*Comparison to results obtained in 2007 in experimental stations*

Then we compared the weed suppression ability of varieties, assessed in organic trials through measures of weed biomass, to the weed tolerance of varieties, assessed the previous year in analytic trials in experimental non organic stations (impact of weed on yield).

For the 5 common varieties, we have the same classification (figure 1): Grandval >> Cézanne > Renan >> Caphorn. Pegassos, initially identified for its strong ability to cover the soil, is not as well noted in the organic field tests as in the analytic trial.

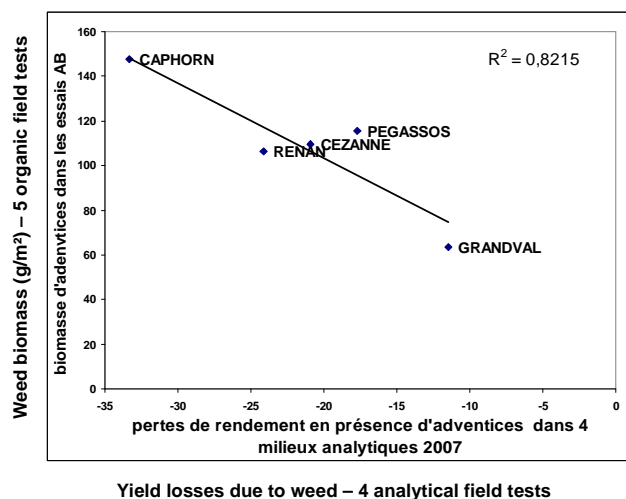


Figure 1: Comparison of the competitiveness of varieties between the analytical field tests performed in 2007 (4 trials) and the 5 organic field tests performed in 2008 and 2009

*Phenotype traits to explain weed competitiveness*

In analytic trials performed in 2007, we found that the more important wheat traits influencing shading ability and thus weed growth were the plant height and the ground cover (Fontaine *et al*, 2008). In additional trials in organic conditions performed in 2008 and 2009, height is predictive of differences of competitiveness between varieties only in 3 trials out of 7. Leaf inclination at early stem elongation and ground cover explain partially the

differences between varieties. This confirms that crop canopy height and wheat ground cover at GS34 are interesting to approach weed competitiveness using standardized variables.

### Conclusions

Experiments with simulated weed made it possible to identify the most explanatory characteristics of the competitive ability of wheat varieties: height first, followed by ground cover and leaf habit, for the conditions of the experiments. In organic conditions, these factors were nevertheless not adequate for totally predicting the competitive potential of all wheat varieties: we have significant results for extreme varieties, but we can't draw clear conclusions for intermediate varieties for which wide variations were observed from one situation to another in our experiments, certainly linked to field heterogeneities due to natural infestations and organic conditions. Yet, methodologies to assess competitive ability of varieties have been specified and clarified, as weed biomass measure. New experiments are planned to collect additional results, in order to improve assessment of all varieties with different weed populations, in particular the intermediate ones.

### Aknowledgements

This project was co-funded by FSOV-GNIS. Authors thanks all organizations and persons responsible of trials: P. Farcy and L. Falchetto (INRA UE Dijon Epouisses) S. Gilles (INRA-AO UEFMV Le Moulon La Minière), J. Daucourt, L. Poirat, L. Bonin, J.-C. Gapin, M. Bonnefoy (Arvalis Institut du végétal Boigneville, La Minière and Ouzouer le Marché), T. Quirin (Agrobio Poitou-Charentes), C. Aubert and C. Glachant (Chambre d'Agriculture 77), L. Prieur (CREAB Midi-Pyrénées). Thank to farmers who entertained trials and to breeders Saaten Union and Lemaire-Deffontaines.

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## DNA methylation patterns in the *VRN1* gene in vernalized and non-vernalized wheat

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*Keywords:* Epigenetics, sodium bisulfite treatment, vernalization, DNA methylation, winter-spring type wheat

### Introduction

Epigenetics refers to the study of any inheritable variation in the phenotype that does not involve a genetic explanation, *i.e.* that is not due to a change in DNA sequence.

Epigenetic variation is well known for its role in cell programming and tissue differentiation, but this variation is generally considered as short lived and reset in germ cells at each generation. Undervalued up to now, the inheritance of epigenetic variation, and its effect on phenotypic variation in organisms, ecological interactions and selection remains largely under explored (Rapp & Wendel 2005). Recent progress in the understanding of the molecular basis of epigenetic variation is revealing the importance of such non-genetic variations. Epigenetic variation can result in strong fitness differences, as demonstrated by example in monozygotic twins subjected to selection (Fraga et al. 2005). Several examples of stable inheritance of epigenetic modifications across generations have been recently demonstrated, and they affect key phenotypic characters such as floral shape, vegetative and seed pigmentation, pathogen resistance and general development in angiosperms (Richards 2006). Jablonka & Raz (2009) have reviewed more than 100 cases of inherited epigenetic variations in a wide range of organisms from prokaryotes to eukaryotes and from plants to animals. Among them, three examples illustrate how trans-generational epigenetic inheritance can act on individual fitness: i) epigenetic modifications at *H1l* and *pc* loci induce an increase of spike and seed sizes in *Triticum aestivum*, stable over 59 generations (Jablonka & Raz 2009), ii) similarly dwarfism and resistance to pathogen induced in *Oryza sativa* can persist over 9 generations, iii) epi-modification of *fwa* gene delay flowering in *Arabidopsis thaliana*. This last example points out a particular interest of inherited epigenetic variation: being more plastic than mutation, it provides a flexible way to allow the transmission of adaptation to local climatic conditions to the progeny of an individual.

Heading time is a character which is associated with floral transition timing in cereals and is crucial to plant breeders as it influences the adaptability of cereals to different environmental conditions (Shimada et al. 2009). Variation in heading time among cultivars and landraces is a main explanation to the worldwide cultivation of wheat in very contrasted agro-climatic conditions. Previous studies revealed that three main pathways are involved in the genetic control of flowering time: photoperiod, vernalization and earliness *per se* (Snape & Worland 2001). Vernalization is the acquisition of the competence to flower in spring following exposure to the long cold of winter. In wheat, some varieties need this cold period to perform the transition from vegetative to reproductive stage, *i.e.* winter type varieties. Other varieties, referred to as “spring types”, do not need this cold period for their transition. This vernalization response is mediated by the stable induction of the *Vrn1* gene, which encodes a FRUITFULL-like MADS-box transcription factor involved in the reproductive developmental initiation at the shoot apex (Murai et al. 2003; Shitsukawa et al. 2007), and which expression induces to flowering (Danyluk et al. 2003; Trevaskis et al. 2003).

Winter type wheat varieties, normally sown in late summer or autumn, are vernalized during winter and flower in spring. These plants “remember” the prolonged cold period (vernalization) and flower in spring when exposed to the long days (Sung & Amasino 2005). For *Vrn1*, a vital gene in vernalization pathway (Murai et al. 2003; Shitsukawa et al. 2007), expression is induced by vernalization, and is maintained posterior to the

vernalization period, while its initial expression level is reset in the next generation. These facts are favouring the hypothesis of an epigenetic mechanism controlling cold memorization in the plant. Several epigenetic molecular modifications can alterate gene expression, such as those involving DNA methylation, histone modifications and regulatory noncoding RNAs. In DNA methylation, a 5-methyl group is attached to the Cytosine nucleotide by methyltransferase enzymes. In plants, presence of this 5-methyl group in promoters sometimes correlates with repression of gene expression, while methylation within the gene body correlates with gene expression. As for histones, main modifications rely on protein methylation and acetylation. Histone methylation and DNA methylation are interlinked with each other (Stancheva 2005 ; Henckel et al. 2009; Tariq et al. 2003). Histone methylation of *Vrn1* gene has been reported to respond to vernalization, and subsequently control *Vrn1* gene expression in barley (Oliver et al. 2009). Since DNA methylation is becoming a common tool in population studies (populational epigenetics), and that *Vrn1* DNA methylation has never been studied, we focussed our study on the description of gene methylation patterns. The main aim of this study is therefore to get insights into the variation of DNA methylation patterns within *Vrn1* between different wheat haplotypes and to explore to which extent these patterns are affected by the influence of vernalization treatment.

### Material and Methods

On the basis of earliness and winter/spring type, eleven wheat (*Triticum aestivum* L.) lines of a Dynamic Management Program presenting 6 different allelic compositions for the three *VRN1* genes (A-B-D) were selected, in order to compare the effect of vernalization treatments between and among the different haplotypes. Five of these 6 genotypes are “Spring types”, with different earliness explained both by their *Vrn1* allelic combinations and variation at other QTLs.

To assess the effect of temperature on the methylation pattern of *Vrn1*, four sets of plants, each composed of the 11 selected genotypes, were produced, corresponding to two vegetative stages (2 and 4 leaves) submitted or not to a vernalization treatment (couple of vernalized/non vernalized sets). Three sets were sown at the same time and kept in glasshouse, and the fourth set was sown afterwards.

Sampling from Sets 1 and 2 was done at 2 leaves stage, with or without vernalization treatment respectively (45 days in a vernalization room at 5°C and 8-10 hr of light for set 2).

Sampling for Sets 3 and 4 was done at 5-6 leaves stage, with or without vernalization treatment. Vernalized set (Set 3, treated as Set 2 by 45 days incubation in a vernalization room at 5°C and 8-10 hr of light) was transferred to the glasshouse at 2-3 leaves stage, allowing the growth of the fourth leave in non vernalizing conditions. As vernalizing signals are maintained despite this increase in temperature, this treatment allows us to differentiate cold induced patterns from the ones specific to vernalization.

DNA was extracted and sodium bisulfite treatment was performed using the EZ DNA Methylation-Gold Kit. This treatment converts non-methylated cytosines into thymines but does not affect the methylated cytosines, thus enabling the identification of methylated sites on sequencing chromatogram.

Primers were designed using the Methyl Primer Express software, which integrates the bisulfite-treated DNA constraints to efficiently design primers. Common wheat is hexaploid (AABBDD) so specificity of the designed primers to one of the three homeologs has also been carefully checked.

Three regions (Figure 1) have been studied up to now: a) promoter, b) initial part of intron 1 and c) end of the intron 1. As shown in the figure 3, the *Vrn-1A* gene has a very large intron 1. Review of previous literature shows that either the promoter or intron 1 is responsible for the behavioral change of the gene in barley, so these regions were selected for initial methylation profiling.

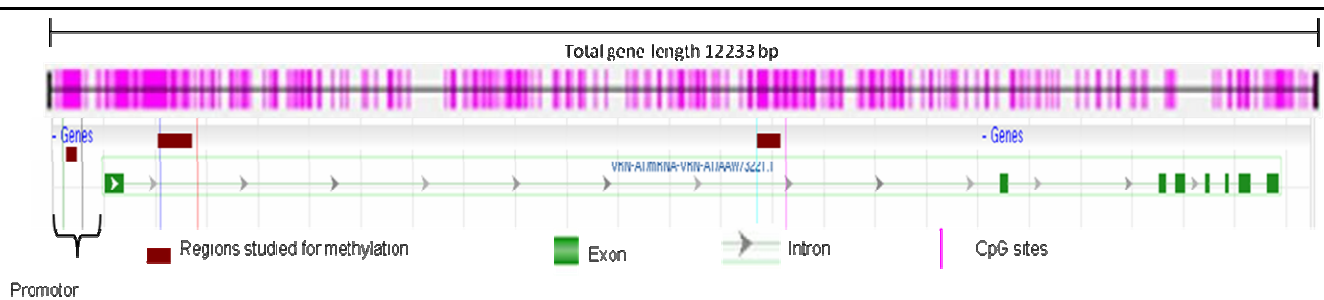


Figure 1: Structure of the *Vrn-1A* gene. Small green boxes represent exons position in the gene and the other regions with light green lines represents introns. The pink lines represent the CpG sites. The brown boxes represent the regions studied for methylation.

## Results and Discussion

DNA was successfully extracted from the different genotypes and treated with bisulfite. Three different primers per target sequence were ordered and tested, allowing the identification of a primer combination per area giving good PCR amplifications. To get a first overview of epigenetic variation, we performed a direct sequencing of PCR products, without cloning.

In the promoter region, designed primer pairs amplified non specifically all the three genomes. Sequence analysis reveals no methylation, but few double peaks corresponding to polymorphic site between A/B/D homeologs where observed, as expected due to absence of specificity of the designed primers.

In the first part of intron 1 (near exon 1), the designed primers specifically amplified the *Vrn1-A* homeolog. Different haplotypes with and without vernalization treatment were sequenced and analyzed. Only one site (Figure 2, methylated site underlined) was found to show some methylation variation (i.e., presence of double C/T peaks). Comparing the data available for both vernalized and non vernalized plants, the results show that this methylation signature variation is more often observed (3 times over 5) in non vernalized winter haplotypes as compared to the spring haplotypes, which show non-methylated pattern (5 times over 8) for both vernalized and non-vernalized plants. These preliminary results give the impression that, at this site, the non-vernalized plants show methylation signals while the vernalized winter type plants exhibit non-methylation pattern, as well spring genotypes.

The third region under consideration is the last part of intron 1 (near exon 2). With 18 CpG sites, this sequence appears to be globally highly methylated in both spring and winter haplotype, irrespective of vernalization treatment. Interestingly, two sites which are not located in CpGs, display some variation in methylation intensity in response to vernalization treatment. At the first methylation site (Figure 3) non vernalized winter types give two peaks (C/T) as compared to vernalized one which shows higher C compared to T. At the second site the non vernalized plant show only T as compared to the double peaks (C/T) in vernalized plant.

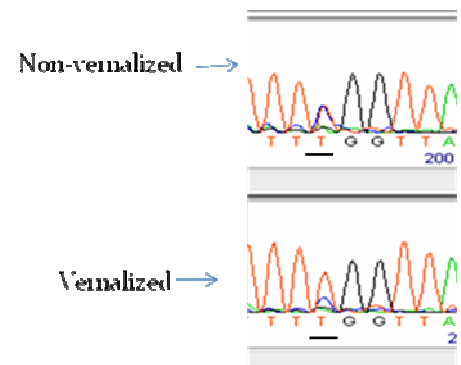


Figure 2: Chromatogram showing the methylation site (underlined) of vernalized and non-vernalized plant of winter haplotype in initial part of intron 1 of *Vrn-1A*.

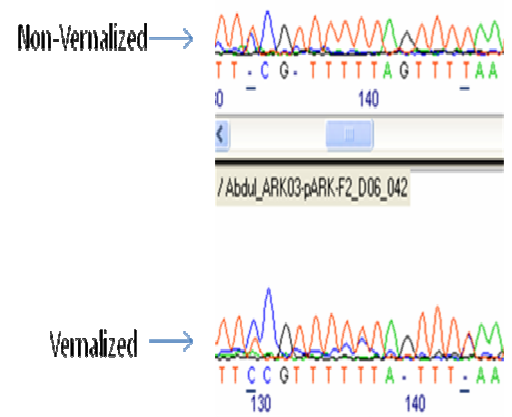


Figure 3: Chromatogram showing the methylation sites (underlined) of vernalized and non-vernalized plant of winter haplotype in intron 1 near exon 2 of *Vrn-1A*.

In conclusion of these preliminary results, patterns of methylation seem to be variable along the promoter and intron 1 of *Vrn1-A* gene, the beginning of the gene presenting very low methylation, at the opposite of the end of the intron 1 (also corresponding to the end of the gene). Few sites were present variation in methylation according to vernalizing treatments, and further analyses need to confirm the reliability of the signals detected so far. Study of other parts of the gene will confirm the gradient in methylation that seems to exist in *Vrn1-A*. Some slight variation in the DNA methylation profile was also detected between different genotypes. This specific variation might be the trace of the presence of epigenetic regulation of the expression of *Vrn1* between lines. If confirmed, the presence of stable inheritable epigenetic variation in *Vrn1* gene among varieties could explain part of the variation observed in wheat earliness. Thus, epigenetic variation could provide an additional source of variation that may be triggered by selection just as DNA variation is. Being more flexible than DNA changes, DNA methylation changes may be particularly interesting for local adaptation to climatic conditions.

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## Session 3 - posters

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### 17. Searching for crop characteristics correlated with nitrogen use efficiency in potato (*Solanum tuberosum* L.)

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*Keywords:* Nitrogen use efficiency, low input, potato, genetic variation

In organic farming, nitrogen fertilizer rates are low as part of a farming strategy in which crops are stimulated to rely on their own resource capture. In future, nitrogen application will also be reduced in conventional farming systems because of the restrictions in nitrogen use to avoid nitrate leaching. Therefore, breeding for nitrogen efficient varieties is necessary. This is especially true for potato which has a rather high nitrogen demand and a low capacity to capture this resource. However, breeders lack knowledge about genetic variation in nitrogen response of potato under low input growing conditions, about relevant crop traits associated with variation in nitrogen response and the physiological mechanisms behind such variation. The objective of our research is to identify plant traits that are correlated with the ability to deal with low availability of nitrogen. These traits will be transformed into selection criteria which can be used by potato breeders to develop nitrogen efficient potato varieties.

Four field trials (on an organic site and a conventional site, in 2008 and 2009) were conducted with nine (2008) or six (2009) varieties and three nitrogen input levels in a split-plot design with four replicates. Several plant traits were assessed. The soil cover was assessed twice a week, and leaf area, tuber bulking and nitrogen accumulation were measured at two intermediate harvests and at the final harvest.

We found genetic variation for nitrogen response under low input conditions. This variation seemed closely related to the maturity type of the variety. Late varieties seemed more efficient in nitrogen uptake and early varieties more efficient in nitrogen use (i.e. produced more tuber dry matter per amount of nitrogen taken up). Also for other traits such as yield, dry matter production, harvest index (HI), leaf area index (LAI) and soil cover we found genetic variation. Preliminary results showed that the level of maximum soil cover, the duration of maximum soil cover and LAI could be candidate traits correlated with nitrogen use efficiency. In the coming two years these traits will be validated in subsequent field trials at two organic locations with 18 varieties.



### 18. Analysis of the relationship between root development, field performance and resource capture of 4 contrasting lettuce cultivars under transplant stress

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In conventional agriculture, breeding of modern lettuce cultivars (*Lactuca sativa* L.) has been focusing mainly on types with high shoot:root ratios, providing high yields, but with minor demands on the roots as conventional farming systems are able to supply a constant flow of water and nutrients. Organic agriculture aims at optimizing the production system rather than the individual crop, and thus has fewer means to control growing conditions. Improving the root system of lettuce is thought to provide varieties better adapted to the fluctuant growing conditions of organic/low input systems. This study was undertaken to investigate the effect of transplant stress on the relationship between root development, nitrate and water uptake, and product quality of 4 contrasting lettuce genotypes at 3 transplant ages (i. e. 3 shoot:root ratios). Three field trials were performed at 2 locations (Wageningen, NL, in spring 2009 and 2010, and Voorst, NL, in spring 2009).



## 19. Effect of genotype and nitrogen input level on nitrate and nitrite contents of sweet corn kernels

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**Keywords:** sweet corn, nitrate, nitrite, mineral N fertilization, genotype

In sweet corn it is quite expectable that, in poor postharvest condition of ear storage, a smaller or a larger part of the kernel nitrates be converted into nitrites. The consumption of 3-4 freshly boiled ears/day adds up to an ADI of  $\text{NO}_2^-$ -N which should be seriously taken into consideration. Five sweet corn hybrids were tested in field trials on four levels of mineral N fertilization (0 kg N/ha; 50 kg N/ha, specific to organic and low-input agricultural practices, and 100 kg N/ha; 150 kg N/ha customary with conventional agricultural technologies) applied to soil as  $\text{NH}_4\text{NO}_3$  on a general organic fertilization of 30 t/ha manure applied once in three years. The trials were organized in three locations with obvious different soil and climatic conditions and in two years (2007 and 2008). At technical maturity, ten ears were harvested at random from each experimental plot and kernel samples of 20 g of each variant were taken on the day of harvest and analyzed for nitrates and nitrites. Both the cultivar and the level of mineral N fertilization have significantly influenced the nitrate content of kernels. The average value of nitrate (34.41 mg/kg product) and nitrite (1.18 mg/kg product) contents are not very high and are far from being alarming as compared to ADI (Admissible Daily Ingestion) values recommended by the UE Scientific Committee for Food in 2007 (0 – 3.7 mg/kg b.w. of  $\text{NO}_3^-$ -N and 0 – 0.1 mg/kg b.w. of  $\text{NO}_2^-$ -N). The registered values of nitrite content in sweet corn kernels are, nevertheless, alarming since they were found following a rather proper handling and storage of harvested ears and kernel samples.



## 20. Phenotyping of an extensive potato germplasm set for nitrogen use efficiency: nitrogen effect on physiological model parameters for canopy development.

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**Keywords:** Nitrogen use efficiency, Phenotyping, Potato, Canopy development

Nitrogen (N) is an essential nutrient for crop growth and its use has great economical and environmental impact. Proper nitrogen management is especially essential in potato production because of the high demands of the crop and its small and shallow root system. Moreover, the EU nitrate directive (91/767/EEC) makes nitrogen use efficiency (NUE) a very important trait and will force to reduce N-supply, thus affecting the physiological and agronomical performance of the potato crop. The challenge is to know which varieties are performing well under low input and to understand the genetics of high NUE under low N. We used an eco-physiological model to analyze canopy cover as a tool to identify and quantify genetic variation among potato varieties. A diverse set of 200 potato genotypes was planted in 2009 and grown under two levels of available nitrogen (75 and 180 N kg/ha) at the breeding station of Agrico Research (Bant, Noordoostpolder, The Netherlands) on a light clay soil. To apply the physiological model for canopy development, the percentage of soil covered by green potato leaves (soil coverage, SC) was assessed weekly throughout the growing season. Using the hourly temperature, obtained from a nearby meteorological station, days after emergence were converted into thermal days to describe crop development as a function of temperature using the beta function and the three cardinal temperatures for potato. Relevant curve fit parameters estimated from the model for each individual genotype showed large differences between nitrogen levels, with lower values for the low level of N input. Especially the maximum value of SC and the period over which this maximum was maintained were strongly affected by nitrogen. These effects resulted in large differences in light interception, yield, tuber size distribution and nitrogen uptake. Differences between genotypes were large and partly associated with maturity type. The results will be confirmed in a second trial in 2010. An association mapping analysis will follow in the second phase of the project to identify candidate genes related with NUE in potato.

## 21. Screening methods for drought tolerance in cereals

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**Keywords:** Drought tolerance, durum wheat, measurement methods, root system, screening, *Triticum durum*  
Improving drought tolerance is a key breeding target for many agricultural regions of the world and is likely to become a main challenge for agronomy under global change. Revealing the physiological basis of drought tolerance in plants can result in more targeted crop improvement strategies for water limiting conditions. Plant responses to drought in terms of drought avoidance can be roughly grouped into minimization of losses and maximization of uptake according to Levitt's (1980) classical scheme. While the first strategy is mainly linked to leaf parameters, the latter relates to the root system. The present research evaluates the usability of several physiological and agronomic measurement methods for the purpose of screening crop traits of potential interest for improved water stress tolerance in breeding trials. Measured parameters were initial soil coverage, root capacitance, stomata conductance, chlorophyll concentration, leaf area index, time to heading, plant height and harvest index.

A core set of 50 durum wheat cultivars was used in order to reveal the existing variability in the aboveground and root parameters. Furthermore the most appropriate experimental design is evaluated in terms of time-efficiency and representativity of data for characterizing and discriminating among the cultivars. The physiological parameters are then analysed for their predictive relation to biomass growth and yield. The results should provide an indication on advantages and problems of methodological approaches for revealing distinct physiological plant strategies for drought avoidance.

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## 22. Effects of genotype, environment and crop management on biotic and abiotic stress responses of potato

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**Keywords:** Genotype by environment interaction, organic farming, potato tuber diseases, *Solanum tuberosum*  
A database on tuber diseases of table potato (*Solanum tuberosum*) which were delivered to LAPRO for conditioning and packaging was established from 2002 onwards including all information concerning producer, production area and variety. The potato lots were scored for bacterial diseases, i.e. bacterial soft rot (*Erwinia carotovora*), bacterial brown rot (*Ralstonia solanacearum*), fungal diseases, i.e. common scab (*Streptomyces* spp.), silver scurf (*Helminthosporium solani*), black scurf (*Rhizoctonia solani*), viral diseases, i.e. potato virus Y, damages by wireworms (Elateridae larvae) and miscellaneous diseases and disorders, i.e. stem-end browning, internal brown spot, elephant hide, hollow heart, unspecific grub and percentage of over- and undersized potatoes.

From the huge dataset relevant information was extracted to enlighten the influence of variety, environment (producer, area of production, year) and crop management (organic, conventional) on the most important diseases and disorders in the main potato growing areas of north-eastern Austria. Generally, organic potato lots showed a higher percentage of deficits. This higher percentage was mainly due to biotic diseases (wireworm, common scab, black scurf) and to a higher amount of undersized potatoes. In contrast conventionally produced potatoes exhibited significantly higher physiological disorders (deformed and oversized potatoes, elephant hide, hollow heart). Despite this general trends significant interactions between varieties, environments and/or crop management were discovered.

**Session 4**  
**Breeding for diverse environments and products**  
**Thursday, December 2 nd - 4:30 pm**

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**Breeding for Genetic and Market Diversity: Examples from wheat, quinoa and buckwheat**

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**Abstract**

Evolutionary Participatory Breeding (EPB) is a breeding method designed to maximize genetic diversity in elastic self-pollinating crop populations developed through farmer-breeder collaborative selection. An example of EPB in wheat is discussed to illustrate the concept and potential of this breeding strategy. Recently, two alternative grain crops (buckwheat and quinoa) were identified by organic farmers in Washington State, USA, as unique in their potential to diversify and improve existing small to mid-scale farming systems. Buckwheat and quinoa have the potential, to differing extents, to: diversify cropping systems with under-represented grain crops destined for local markets; function as valuable high-biomass cover crops; provide locally grown options for nutritious food for humans, particularly those with gluten intolerance or wheat allergies; and, serve as a high protein, nutritious animal feed. Farmers across Washington State are interested in growing these crops for different reasons and their respective environments dictate that different traits be targets of selection. EPB methodology will differ for these two crops based on their pollination strategies as quinoa is predominantly an inbreeding species, while buckwheat is a cross-pollinating species. Diversification of crops subject to farmer-breeder breeding collaboration will increase on-farm genetic diversity and encourage greater market diversity options to be utilized by farmers.

**Evolutionary Participatory Plant Breeding: A Brief Overview**

A farmer-breeder collaborative approach to organic wheat breeding combines the well-established methods of participatory plant breeding with the selection strategies for fitness traits that have proved successful over decades of evolutionary breeding. This method is referred to as evolutionary participatory breeding (EPB) within the context of this paper. EPB optimizes the power of natural selection in farmers' fields to select genotypes with specific adaptation to their local environment and farming systems. EPB promotes the development of modern landraces or variable, genetically diverse populations which are adapted through natural selection to a very specific local ecosystem.

Farmers generally choose the parental cultivars to be cross-fertilized based on their intimate knowledge of varietal performance over time on their farm. Initial crosses between parent lines are often conducted at research facilities (either by breeders or farmers) to help ensure reproductive success. Seed is then increased from the F<sub>1</sub> to the F<sub>2</sub>, after which F<sub>3</sub> seed is distributed to the grower for several generations of natural and farmer-assisted selection. Examples of farmer-based selection include negative selection, such as roguing of poor performers or elimination of disease susceptible genotypes, and positive selection, including screening of grain for larger seed

size and higher test weight. Depending on end-use quality needs, market opportunities, and the improvement of specific value-added traits, breeders or farmers can periodically isolate potentially high-performing plants and conduct cultivar trials and generate pure lines and synthesize varietal blends. However, farmers should continue on-farm EPB to continue to improve upon and develop heterogeneous, high-performing populations.

The need for breeding and selection within organic systems is clear. There is abundant evidence in other crops, ranging from wheat to lentil to maize, emphasizing the importance of direct, system-specific selection for optimization of yield, disease resistance and quality in organic farming systems. EPB methodology is particularly suited to organic agriculture, because it decentralizes the selection process and compensates for the heightened inter-farm environmental variances which emerge with decreased inputs. When using EPB methods, plant breeders are aware that the same natural forces that present the greatest challenge to organic cultivation can also offer a natural means of selecting plants capable of handling these biotic and abiotic pressures.

### **A Case Study for EPB in wheat: Lexi's Project**

The most advanced example of EPB in Washington State revolves around a traditional wheat farming family's search for a variety of winter wheat that would be adapted to their harsh environmental conditions and provide yield stability over their 4000ha farm and across climatic changes over the years. The farmer, Jim Moore, and his twelve year-old granddaughter Lexi Roach, decided that breeding their own wheat variety would be a worthwhile project for Lexi's class project. Lexi contacted Dr. Stephen Jones at Washington State University to assist with this effort. She then selected parents with promising traits for her family's farm and made initial crosses for three distinct populations. Within 2 years Lexi and Jim were cultivating the F3 populations on the family farm. Choosing emergence as one key selection criterion, Lexi planted the seed very deep, over 200 mm. Only the plants with the appropriate genetics for emerging from these challenging conditions grew and flourished each year. Additionally, Lexi and her family removed lines that were severely infected with stripe rust (*Puccinia striiformis*) annually, harvested in bulk and planted a random sample of the next generation. She maintained three populations from the three separate crosses on approximately 1 hectare each year over a period of 6 years (Figure 1). In 2010, one of Lexi's bulk lines, WA0008094, was the highest yielding wheat in the official Washington State University wheat yield trials for St. Andrews, WA in Douglas County (about 160 kilometers from their farm). WA0008094 topped 59 other lines from 11 public and private breeding programs in the US. WA0008094 performed well at several other locations and was statistically equivalent to all the top yielding varieties except for 'Xerpha' across the low-rainfall precipitation zone (<300mm/yr) in Washington State. This project, carried out by a grade-school student and her grandfather, has so far delivered impressive results and illustrates the potential of utilizing EPB methods for optimizing specific adaptation in unique environments.

### **Quinoa and Buckwheat: Mixed and cross pollination strategies**

Quinoa (*Chenopodium quinoa*) and buckwheat (*Fagopyrum esculentum*) are ancient grains that have been grown for thousands of years in their native regions. They have served well as highly nutritious staple food sources but have been largely neglected by research universities in the US, where research overwhelmingly focuses on the major cereal and legume crops. Both buckwheat and quinoa are in high demand from consumers, can be grown in Washington State, are currently predominantly imported from abroad or other regions of the US, and would fill an under-represented niche in local and regional farming and marketing systems.

Quinoa (Figure 2) is predominantly an autogamous (self-pollinating) species, with outcrossing rates typically ranging from ~0.5 to 10%. However, extreme cases of complete self-pollination and obligate outcrossing by self-incompatibility and male sterility have been reported. Most quinoa varieties are drought resistant, tolerant to light frost and require short daylengths and cool temperatures for good vegetative growth and high seed-set. Identifying genotypes with lygus bug (*Lygus* spp.) and aphid resistance, early maturity and a determinant growth habit will be among the most important selection criteria in the northwest region of the US.

Buckwheat is a short season crop that thrives on well-drained soil, and typically does well on low-fertility or acidic soils. Challenges in buckwheat agronomy will include identifying early maturing cultivars that can set uniform seed prior to the first frost in the fall. Buckwheat cultivars currently grown in Washington State are primarily adapted to regions of North America with significantly more heat units per year, especially when compared to the climate in western Washington. However, developing buckwheat varieties through mass selection is fairly straightforward (T. Bjorkman, pers. comm.) and an example of low-hanging fruit that needs to be exploited to benefit regional farmers.

Based on our 2010 variety trials for quinoa and buckwheat across different agro-ecological zones in North Dakota and Washington State, diverse base populations are currently being developed through wide and narrow crossing schemes. Training in EPB methods for a dedicated group of farmers is occurring simultaneously during the off-season, and populations will be distributed to farmers in contrasting sites of each target region. The choices of parental cultivars are dependent upon cooperating farmer preference, and populations are being formulated from two-parent straight crosses, three to six-parent poly-crosses, and 10 to 30 parent composite crosses.

#### **Marketing Opportunities in the US: Enhanced Nutritional Value and Gluten-Free**

Apart from agronomic considerations, alternative grains can be more profitable to farmers through the exploitation of value-added traits. For example, nutritional value is a primary reason many consumers choose to buy organic food. A 1999 Organic Farming Research Foundation survey reported that 56% of respondents ranked determining the relationship between organic farming practices and the nutritional value of food as the highest research priority for organic field crops.

Almost two million North Americans suffer from gluten intolerance. Both quinoa and buckwheat are gluten-free and often purchased by health-conscious consumers. The gluten-free food market was worth almost \$1.6b last year and had an annual growth rate of 28% over the last four years. Replacing standard gluten-free flours with quinoa improves intakes of protein, iron, calcium and fibre. Quinoa is a highly nutritious food with excellent protein quality and quantity, very high lysine and a balanced amino acid content. Quinoa also has been shown to be higher in calcium, phosphorus, magnesium, potassium, iron, copper, manganese, and zinc than wheat, barley or corn. Buckwheat contains high concentrations of all essential amino acids, especially lysine, threonine, tryptophan, and the sulphur-containing amino acids. Additionally, buckwheat is rich in iron (60-100ppm), zinc (20-30ppm) and selenium (20-50ppm). These traits can be enhanced by plant breeders and used to add marketing value to particular varieties/populations of these crops. Because EPB is especially well-suited to increasing values of fitness-related characteristics, parents chosen for crossing schemes should exhibit excellent value-added quality and nutritional traits that will hopefully remain with the population over years of selection.

#### **Expanding the scope of EPB beyond wheat**

Quinoa and buckwheat are the choice of many organic farmers in Washington State and other regions in the northern US to be the focus of an expanded EPB program. Expansion beyond wheat despite fewer funding sources is appropriate due to the relative ease and economy of this breeding method. The development of a crop non-specific breeding program based on assorted participatory breeding methods will encourage improvement and farmer adoption of a greater diversity of non-traditional crop species. Through this process, both within-crop and on-farm genetic diversity will increase and provide greater access to and opportunities for expanding local markets and food and farm stability.

## Breeding forage and grain legumes for adaptation to specific agroclimatic regions and cropping systems: opportunities and limitations

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*Key words:* drought tolerance, genotype × environment interaction, mixed cropping, organic agriculture, plant competition

### Introduction

A major issue for breeding programmes is whether selecting material that is widely adapted to environments of the target region, or breeding distinct varieties for specific mega-environments (alias subregions) as represented by different geographic areas, farming systems (e.g., organic or conventional) or crop managements (e.g., pure or mixed cropping). Crucial aspects for choosing either adaptation strategy are the size of the selection gains that they can offer for same overall selection costs, and the market size for specifically-adapted varieties. For a given level of phenotypic variance, the selection gain is proportional to the heritability ( $h^2$ , in the broad sense for inbred lines and the narrow sense for open-pollinated populations: Simmonds, 1979). Indicating by  $s_g^2$ ,  $s_{ge}^2$  and  $s_{err}^2$  the genetic, genotype × environment (GE) interaction and experimental error variances, respectively, breeding widely-adapted inbred lines or clones requires to minimize  $s_{ge}^2$  over the target environments:

$$h^2 = s_g^2 / (s_g^2 + s_{ge}^2 + s_{err}^2).$$

Breeding for specific adaptation can capitalize on the portion of GE interaction accounted for by the different mega-environments subjected to specific breeding, as its overall selection gain is maximized by maximizing the genotype × mega-environment interaction variance ( $s_{gm}^2$ ) and minimizing the GE interaction variance within mega-environments ( $s_{ge(m)}^2$ ):

$$h^2 = (s_g^2 + s_{gm}^2) / (s_g^2 + s_{gm}^2 + s_{ge(m)}^2 + s_{err}^2).$$

The same conceptual framework also applies to open-pollinated populations, but  $s_g^2$  and  $s_{gm}^2$  at the numerator relate to the additive genetic variance in this case (Annicchiarico, 2009).

Only the adaptive responses to factors that can be controlled or predicted prior to sowing can be exploited by selecting and growing specifically-adapted cultivars. This is not the case for the time factor, as the climatic conditions that generate year-to-year environmental variation are not known in advance. This variation can be dealt with in terms of genotype yield stability. Breeding for resilience equals to increasing Type 1 or Type 4 stability (Lin and Binns, 1988; Annicchiarico, 2009) by exploiting GE effects associated with stress tolerance which lead to relatively better cultivar response in unfavourable years or cropping conditions.

GE interaction may arise from several reasons, including the genetically based trade-off between yield potential and tolerance to major stresses (e.g., Ludlow and Muchow, 1990). It also affects marker-assisted selection, which can conveniently be embedded also in specific-adaptation strategies by exploiting the possibly large portion of useful QTLs and markers which are environment-specific (e.g., Messmer et al., 2009). GE effects of practical importance are of cross-over type, implying different genotype ranking across environments.

This work aims to summarize the main findings on GE interaction patterns and adaptation strategies issued by research work carried out on forage and grain legumes at CRA-FLC.

### Forage legumes

The results of different experiments reported by Pecetti et al. (2009) and Annicchiarico and Pecetti (2010) are jointly analyzed in Fig. 1, to compare the relative extent of GE interaction across different cropping systems and geographic areas. Cultivar responses were substantially consistent across conditions of high and almost nil weed competition (reproducing organic and conventional systems, respectively), suggesting no need to breed specific varieties for organic farming. Conversely, large GE interaction of cross-over type occurred across geographic areas because of specific adaptation of variety or landrace germplasm to its area of selection or evolution, suggesting to breed distinct material for each geographic area.

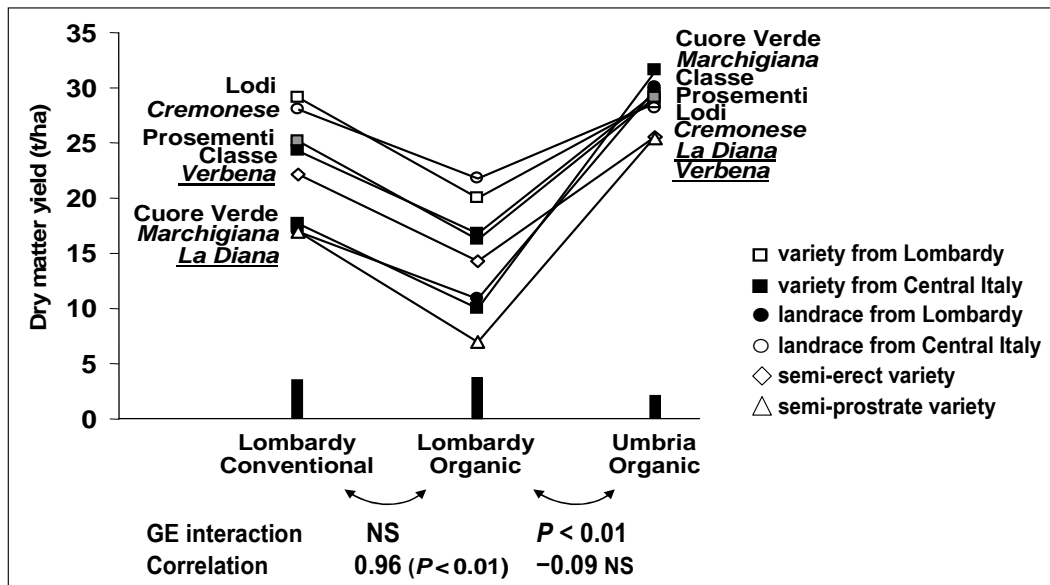


Figure 1. Forage yield of eight lucerne cultivars across conditions reproducing organic or conventional cropping systems in two geographic areas, and genotype  $\times$  environment (GE) interaction and phenotypic correlation for entry yield across environments

Competitive success of genotypes belonging to erect, vigorous forage species in relatively favourable environments relies on their scale foraging ability as determined by greater relative growth rate (Campbell et al., 1991). This feature also implies greater DM yield potential in the absence of competition, justifying the consistent response of lucerne cultivars across high and almost nil weed competition in Fig. 1. For the same reason, consistent genotype responses were observed across pure and mixed cropping in lucerne and other erect, vigorous forages (Rotili, 1985; Piano and Annicchiarico, 1995), implying no need to breed specific varieties for legume-grass associations of this species. Conversely, sizeable GE interaction across pure stand and mixed stand with vigorous associated species was found in a little vigorous, subordinate species such as white clover, whose genotype competitive ability mainly relied on precision foraging (i.e., the ability to make fine-scale exploitation of light and nutrients from undepleted zones by morphological plasticity) (Annicchiarico and Piano, 1994). Selecting white clover in the target mixed-stand environment provided distinctly greater yield gains than selecting in pure stand both on the basis of predicted gains (Annicchiarico, 2003) and in practise (Annicchiarico and Proietti, 2010), allowing to reach the minimal clover content of 30% on the annual dry matter which was required for sufficient N fixation and forage quality.

Large cross-over genotype  $\times$  location interaction for forage yield of lucerne cultivars occurred not only between Lombardy and Umbria (Fig. 1) but also across two geographic areas of northern Italy featuring, respectively, sandy-loam soil with limited drought stress mainly due to irrigated cropping (north-western Po valley), and silty-clay soil with severe summer drought due to rainfed cropping (south-eastern Po valley) (Annicchiarico, 1992). Four artificial environments created by the factorial combination of drought stress (almost nil or high) and soil type (sandy-loam or silty-clay) successfully reproduced the adaptive responses across agricultural sites of a set of reference varieties (Annicchiarico and Piano, 2005). They also revealed the close relationship between drought tolerance and drought-stress level in the evolution environment of a set of farm landraces. Specific breeding for each mega-environment provided distinctly greater forage yield gains than breeding for wide adaptation, both in the artificial environments (Annicchiarico, 2007a) and in agricultural sites

(unpublished data). Other work unveiled adaptive traits related to specific-adaptation patterns (Annicchiarico, 2007b). The EU-funded project PERMED revealed large GE interaction for yield of lucerne varieties or landraces also across sites of the western Mediterranean basin, suggesting to breed distinct varieties for: i) rainfed or irrigated environments with limited spring-summer water (< 350 mm) and nil/low soil salinity; ii) salt-stress environments; iii) fairly favourable environments with high number of harvests (Annicchiarico et al., 2011).

Cross-over GE interaction emerged also for response to exploitation mode of contrasting morphological types of lucerne, suggesting to select: i) material with rhizomatous, prostrate habit, for continuous grazing; ii) semi-erect material, for rotational grazing; and iii) erect material, for the conventional exploitation through mowing (Annicchiarico et al., 2010b).

Large GE interaction is likely to occur in lucerne and other forage legumes because of long-standing, site-specific selection pressures that acted on landraces or natural populations and the large use of such material for variety selection. Major objectives of our future research work are the identification of molecular markers associated with lucerne adaptation to specific growing conditions, and the evaluation of the impact of semi-hybrid variety type and wide genetic diversity on lucerne yield potential, adaptability, and resilience.

### **Grain legumes**

Specific breeding for organic systems may offer greater scope for grain crops, whose traits potentially related to competitive ability against weeds (e.g., tall stature) may differ from those related to high yield in the absence of competition (Donald and Hambling, 1983). This was confirmed indirectly by the usefulness of a pea ideotype devised theoretically for organic systems of northern Italy (Annicchiarico and Filippi, 2007). An index of variety suitability for organic farming was defined as the average entry rank for seven traits related to competitive ability, grain yield and stress tolerance assessed under conventional farming. Attika, which ranked first for index value (seventh for grain yield) among 50 recent varieties, outperformed Metaxa (a recent variety marketed in EU for organic farming, eleventh for index value) for grain yield and competitive ability against weeds in four organically-managed environments.

Large cross-over GE interaction emerged for grain yield of faba bean cultivars (Annicchiarico and Iannucci, 2008) and white lupin breeding lines (unpublished data) across Italian subcontinental- and Mediterranean-climate areas, mainly because of subregion-specific optimal earliness of cycle and its impact on escape from the locally-prevalent stress (winter cold or terminal drought). Especially for white lupin, its modest cropping area may nevertheless justify its selection for wide adaptation. At the larger regional scale of south-western Europe, the extent of GE interaction in a global collection of white lupin landraces definitely supported the breeding of distinct varieties, especially for cold-prone autumn-sown areas on the one hand and spring-sown areas on the other (Annicchiarico et al., 2010a).

Major targets for our future research are the identification of markers associated with pea adaptation to specific environments, the definition of a pea ideotype for mixed cropping with cereals, and the study of bulk breeding schemes allowing natural selection for abiotic stresses.

### **Acknowledgements**

This work carried out within the project “Plant Genetic Resources - FAO Treaty” funded by the Italian Ministry of Agricultural, Food and Forestry Policies.



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## Grain quality in traditional maize varieties for bakery under organic conditions

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*Key words:* maize, organic agriculture, bread, quality

### Introduction

Maize is used for making bread and several derived recipes that are made traditionally with whole flint maize kernels in Spain and Portugal (Revilla et al., 2008; Vaz Patto et al., 2007). In the northwest of Spain traditionally maize was stored in a special type of drier called “hórreo” in order to protect the ears from animals and to dry gradually. That traditional drying method allows the attack of insects that reduce grain safety. Furthermore, the grain is usually harvested with high moisture that produces pericarp fissures and subsequent fungal infections that could be dangerous for human health (D’Mello et al., 1999). Although bakery quality has not been defined for maize flour, some criteria could be kernel weight, uniformity, density, and undamaged and healthy grains (Alonso-Ferro et al., 2008; Watson, 1988).

In previous projects we evaluated a collection of autochthonous varieties under organic farming and choose some varieties with higher yield and bakery quality (Revilla et al., 2008). Those varieties included Sarreaus, with yellow kernels and early maturity, Meiro, with black kernels and late maturity, and Rebordanes, with white kernels and medium-early maturity. These traditional varieties have been improved through two cycles of S<sub>1</sub> intra-population recurrent selection for yield and milling test and were used for checking genotypic and environmental effects as well as genotype x environment interactions on grain quality.

### Materials and methods

A breeding program for yield and quality was carried out from the local populations Rebordanes, Meiro and Sarreaus. Their first cycles of selection were evaluated in 2008 and 2009 in four environments consisting of two-year trials in two locations under organic conditions. Field trials followed a randomized complete block design with 3 replications and harvest was made in three dates: early, normal and late. The traits recorded were grain yield, grain moisture, milling test, kernel weight, severe and slight pericarp damage and germination. Data were analyzed using the procedure GLM of the software program SAS 9.1 (SAS Institute 2005). Means of main effects were compared with the Fisher’s protected LSD at P=0.05 and those of the interactions by using least square mean comparisons.

### Results

Differences among environments were significant for yield and slight pericarp damage. Harvests differed significantly for moisture, slight pericarp damage and germination. Populations were significantly different for moisture, milling test, and severe pericarp damage. The population x environment interaction was significant only for slight pericarp damage. The interactions environments x harvests, and populations x environments x harvests were significant for milling test. Grain density was not significantly different between sources of variation or interactions and none of the interactions was significant for 100-kernel weight. The population x environment interaction for milling test, the population x harvest interaction for germination, and the populations x environments x harvests interaction for grain yield and milling test were mainly of magnitude rather than of rank.

Early harvest had higher grain moisture, higher slight pericarp damage and reduced germination than normal harvest (Table 1). Although differences were not significant for other traits, as harvest time advanced there was a

reduction in milling test values and on severe pericarp damage. Differences among populations were more often significant than among harvest times. Differences among populations were generally consistent with the maturity rating, i.e. populations with longer maturity had higher grain moisture, milling test, severe pericarp damage, and grain yield (although differences were not significant for yield) as well as lower grain density and germination.

### Discussion

In previous projects, we have identified autochthonous populations with the best performance under organic conditions and adequate quality for bakery, including Sarreaus (yellow kernel and early maturity), Meiro (black kernel and late maturity), and Rebordanes (white kernel and medium-early maturity). These varieties were the base material for breeding programs for increasing yield and quality (Revilla et al. 2008). The lack of significant differences for several traits among the improved populations can be explained by the reduction of variability due to the selection program, because the original populations were significantly different for yield in previous evaluations (Revilla et al., 2008). However, differences were significant for moisture because the growth period of these populations has not been modified through selection and were quite diverse originally. The population x environment interactions was as rare as in the previous report. Obviously, the variability detected among populations and the importance of environments and population x environment interactions depends on the germplasm and the environments used for each study, as shown by the discrepancies among several authors (Duarte et al., 2005; LeFord and Russell, 1985; Malvar et al., 2008).

Among quality traits, purity of color is a major issue for international standards (Serna-Saldivar et al., 2001) and the white flint corn populations from this region do not fulfill this requirement, on the contrary their endosperm is creamy rather than pure white. Moreover, yellow, orange, and black kernels are preferred in some areas. From the nutritive perspective, yellow and orange endosperms are healthier because they are sources of antioxidants (Kean et al., 2008; Perichart-Pereira et al., 2010; Troyer, 1999). Nevertheless, from a hedonic perspective, colored maize is less appreciated due to the degradation of pigments during baking or frying that causes strong aromas and flavors (Poneleit, 2001). Grain hardness depends on the relation between horny and floury endosperms and secondarily on the structure and compacting of cell components and thickness of the pericarp. Hardness is highly correlated with other quality traits, especially with density. Genotypic effects were important only for yield, while moisture was more affected by harvest time, which subsequently influenced pericarp damage and germination. Other environmental effects were minor as were the genotype x environment interaction.

The practical conclusions from these results are that the key points for producing high quality traditional maize is to choose the appropriate population for each area, based on adaptation, yield and quality. Harvest should be made when grain moisture reaches the standard level, which is above 20% for the humid Spain.

*Table 1. Means of three maize populations evaluated in 4 environments under organic conditions and harvested early, on time, and late.*

Sources of variation	Grain Yield	Grain moisture	Grain density	Milling test	100-kernel weight	Severe pericarp damage	Slight pericarp damage	Germination
	Mg ha <sup>-1</sup>	g kg <sup>-1</sup>	G cm <sup>-3</sup>	g	g	%	%	%
Harvests								
Early	5.36	23.7a	1.18	76.8	35.1	6.67	24.0a	61.5b
On time	5.36	21.9b	1.17	72.6	35.1	4.97	18.1b	75.8a
Late	5.10	20.2c	1.17	68.2	35.7	3.26	19.0b	84.4a
Populations								
Meiro	5.87	24.3a	1.16	75.5a	35.1	6.97a	16.4	68.4
Rebordanes	5.04	21.3b	1.17	71.0b	36.0	4.71b	23.6	76.8
Sarreaus	4.91	20.3c	1.18	71.3b	34.8	3.36b	21.1	76.6
LSD (0.05)		0.61		1.33		1.41	2.53	9.3
Means followed by the same letter, for each source of variation, are not significantly different. Letters are only shown when there were significant differences for a trait in the analysis of variance.								

### Acknowledgements

This research was supported by a grant from the *Xunta de Galicia* (07TAL011E).

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## Chinese cabbage variety ‘Atsuko’ as an example of biodynamic vegetable breeding with Kultursaat association, Germany

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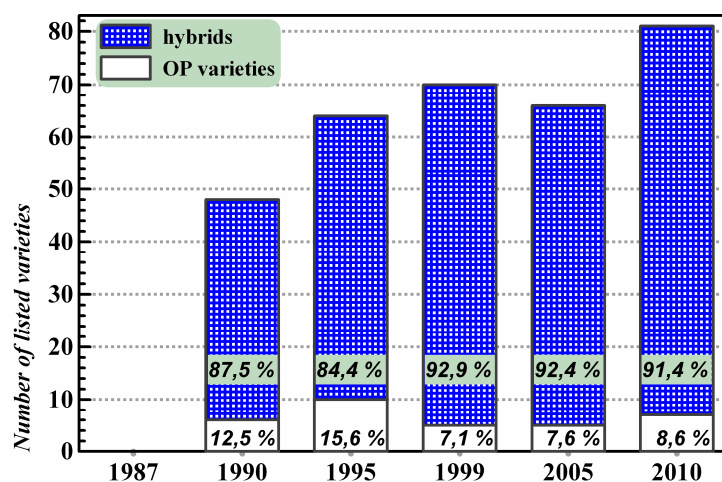
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**Keywords:** on-farm breeding, Ohio-method, robustness, taste

### Introduction

Over the last decades the percentage of hybrids in the assortment of all important vegetable crops has strongly increased and today for many varieties only hybrids are available (Tay 2002, Maggioni 2004, Stadlander 2005). As hybrids are not stable the possibility of conservation as well as developing and adapting varieties - not only under organic farming conditions - are increasingly limited. Even worse, many new hybrids especially in the *Brassica* family are so called CMS-hybrids that are not even fertile.

In very general terms popularity of hybrids is e.g. due to their vigour, uniformity, earliness and high yields. From the breeders' point of view hybrid breeding helps to control intellectual property rights through control and protection of the parental lines. However hybridisation requests either extreme inbreeding or sterility factors to allow homozygous parental lines and maximum degree of cross-pollination. Therefore hybrid breeding is considered inappropriate with biodynamic breeders. Since Chinese cabbage has been listed in the Common catalogue of vegetable species in the late 1980s, about 90 % of the registered varieties are hybrids (fig. 1). Within *Brassicaceae* many “successful” interspecific and intergeneric fusions are known (Navrátilová 2004), but information about commercially available Chinese cabbage CMS-hybrids made by cell fusion techniques<sup>1</sup> is rare.



*Figure 1:*

*Development of open pollinating (OP) varieties (white bars) and hybrids (dark bars) of Chinese cabbage listed in the Common catalogue of vegetable species, source EU (several volumes 1987 to 2010)*

On this background the ‘Atsuko’ breeding programme was Kultursaat association’s effort to develop an OP variety especially for quality oriented organic and biodynamic cropping.

<sup>1</sup> Such CMS-hybrids derived from cell fusion are banned by several organic farming associations and the IFOAM decided at its General Assembly 2008 in Vignola/Italy, that cell fusion techniques do not comply with the principles of Organic Farming (IFOAM 2008).

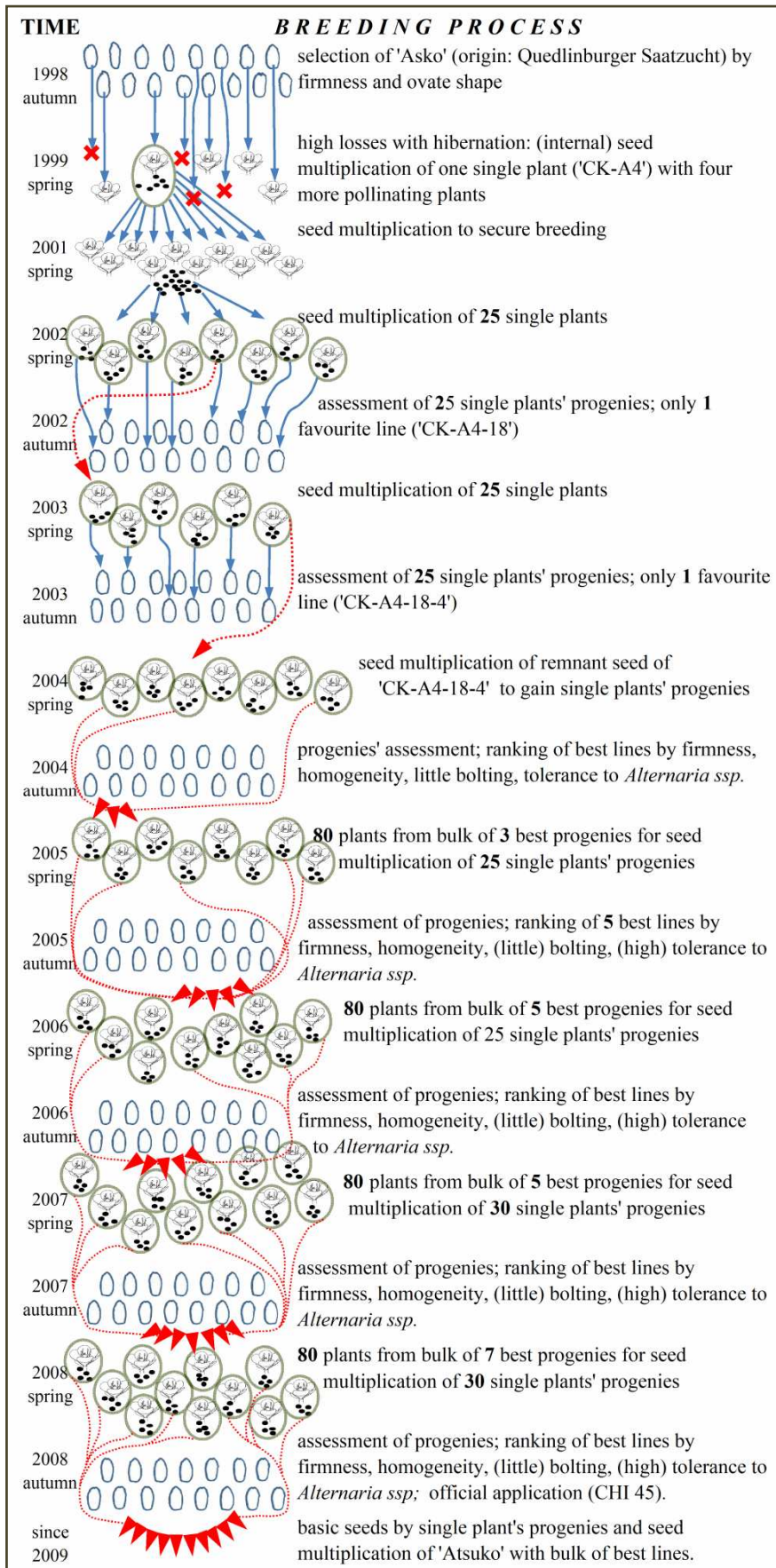


Figure 2. Breeding of head of 'Atsuko' shape and texture, as well as overall healthiness and vigour. In spring up to 100 plants were sown for seed multiplication in spring 2001 to secure sufficient breeding material. In the following work the remnant seed procedure was used. Very early sowing of Chinese cabbage during the end of January/early February allows seed production in time for summer sowing. This technique allows for seed production from individual plants and offspring selection already in autumn of the same year. Seeds from plants displaying the best morphological characteristics were selected, mixed to a bulk and used for early sowing in the following year. This procedure was used continuously up until 2008 (fig. 2). With early sowing the plants do not form heads and selection has to be limited to the stage of rosette growth (up to BBCH 19) using following criteria: firmness, size, shape, weight (1.0 to 1.5 kg) and organoleptic quality. 75 % of these elite plants died during the subsequent winter illustrating the risky hibernation of Chinese cabbage which is related to the high water content of the cabbage head. Of the five flowering elite plants only one produced seeds which formed the base for all further breeding work (fig. 2).

### Materials and Methods

'Atsuko's' origin is the open pollinating variety 'Asko' from the Quedlinburger Saatzeit which is no longer listed in the Common catalogue. Starting point of this *on-farm* breeding programme was a screening of ovate heads building accessions from the German gene bank (Gatersleben) under biodynamic conditions in autumn 1998. Despite significant inhomogeneities and a harvesting rate of just 20 % 'Asko' displayed the highest potential for commercial use. 20 elite plants were selected from a total of 250 plants using criteria such as

firmness, size, shape, weight (1.0 to 1.5 kg) and organoleptic quality. 75 % of these elite plants died during the subsequent winter illustrating the risky hibernation of Chinese cabbage which is related to the high water content of the cabbage head. Of the five flowering elite plants only one produced seeds which formed the base for all further breeding work (fig. 2).

Seeds were sown for seed multiplication in spring 2001 to secure sufficient breeding material. In the following work the remnant seed procedure was used. Very early sowing of Chinese cabbage during the end of January/early February allows seed production in time for summer sowing. This technique allows for seed production from individual plants and offspring selection already in autumn of the same year. Seeds from plants displaying the best morphological characteristics were selected, mixed to a bulk and used for early sowing in the following year. This procedure was used continuously up until 2008 (fig. 2). With early sowing the plants do not form heads and selection has to be limited to the stage of rosette growth (up to BBCH 19) using following criteria:

plants were used from which in the rosette stage 25 to 30 were selected for progenies' assessment. Open pollination between all plants was allowed during flowering. In autumn head shapes were critically evaluated based on early timing, firmness and homogeneity, as well as plant affinity for inflorescence emergence and tolerance against *Alternaria*. Criteria for evaluation of *Alternaria* infestation were based on intensity of both, outer leaves and head inner part. For continuation only such progenies were used that performed better than average on all criteria. At the beginning the number of breeding lines used for seed production was only three to five, later up to 14 lines were used annually.

Sensory quality was determined using a tasting test with an ordinal scale based on a procedure originally developed for carrots (Fleck 2009). Criteria employed were aroma and sweetness on mature leaves that had achieved good to very good texture. Visual evaluation by picture forming methods (here Rising pictures ("Steigbild")) unveiled the good forming character and ripeness of 'Atsuko' when compared to 'Askö' and hybrid varieties (fig. 3).

Kazumi F1 (common hybrid)	CK-A4 ('Askö') one step of organic seed production (F1)	CK-A4-18-4 ('Atsuko') bulk of best progenies (F8)
<ul style="list-style-type: none"> <li>♦ little pronounced drips' line („Tropfengirlande“),</li> <li>♦ average form intensity,</li> <li>♦ some indication of roughness and deterioration.</li> </ul>	<ul style="list-style-type: none"> <li>♦ average drips' line,</li> <li>♦ uneven forms,</li> <li>♦ slight deterioration.</li> </ul>	<ul style="list-style-type: none"> <li>♦ most expanded drips line (&gt; indication of ripeness),</li> <li>♦ superior form intensity,</li> <li>♦ smooth, „moved“, leaf-typical, “vegetatively vital” characteristic without any indication of deterioration.</li> </ul>

*Figure 3: Rising pictures (“Steigbild according to Wala”) and important patterns of ‘Kazumi F1’ (commonly used hybrid as reference), ‘Askö’ (one-time biodynamically farm saved seeds (F1) from the original OP variety) and ‘Atsuko’ (F8 generation’s selection of ‘Askö’ (breeding progress by Ohio-method)); samples from growing season autumn 2007, qualitative assessment: 2010-11-13 to 11-15.*

Using tests with mid-scale organic growers during 2007 and 2008 a 70-90% harvesting rate was obtained, which is a high level and sufficient for commercial purposes. In autumn 2009 DUS test resulted in the variety recognition through Bundessortenamt (Federal Office of Plant Varieties) on the denomination ‘Atsuko’ (CHI 45).

## Results & Discussion

Starting with an inhomogeneous cultivar with a high head shape variation and a poor harvesting rate a new commercially viable variety using individual selection was successfully developed. Compared to hybrids the new variety displays an ample homogeneity, excellent heads' firmness and a sufficient harvesting rate. Compared to the initial variety the field resistance is increased markedly although it is not resistant to fungal decay or bacterial rot. Following the UPOV variety description ‘Atsuko’ displays following characteristics: Low to medium plant height with egg shaped (ovate), intermediately wide, and short heads of yellow-greenish colour and whitish inner colour; medium number of blisters, small in size on upper side of outer leaf, excellent heads' firmness and early harvesting time. Resistance and disposition to inflorescence emergence were not used in the variety accreditation.

During production and storage of Chinese cabbage infection with *Alternaria* ssp. forms a major problem: early infection during production leads to low returns and high storing losses occur if *Alternaria* infested harvest is stored. Compared to hybrids, 'Atsuko's sensitivity against *Alternaria* is comparable (ranges in the middle). In addition, infested plants can easily be cleaned on the field so that only healthy plants are used for storage. So far, storage life was successfully tested up to December. Extended storage tests are still ongoing.

For 'Atsuko's maintenance breeding the Ohio-method is continuously used. The number of individual plants should exceed 30 to circumvent genetic restriction that could cause inbreeding depression. Breeding experiments will continue to optimise *Alternaria* tolerance and disposition to inflorescence emergence. Identification of tolerant lines is enabled as the breeding area is characterised by a high risk of *Alternaria* infection.

Compared to usual hybrids 'Atsuko' has a smaller head, a fact this is often seen positively for marketing to the end consumer. The increased disposition to inflorescence emergence might present a problem. It occurs more frequently with early sowing, at more northern sites and with increased drought stress. So far, commercial tests evaluated the new variety mainly positively.

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## Evaluation of potato breeding clones in organic and conventional growing conditions

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*Keywords:* potato, breeding, organic farming.

### Introduction

The desired traits for potato breeding for organic farming are adaptability to organic fertilization, ability to give yield in a short growing period (Tiemens-Hulscher *et al.*, 2003), resistance to pests and compliance to marketing. More detailed: varieties for organic farming should be nutrient efficient, able to build up leaf coverage (canopy) comparatively fast and to set tubers early and to complete acceptable yield shortly (Lammerts van Bueren *et al.*, 2008). A part of traits are included in conventional potato breeding programmes, but some of the characteristics are particularly significant for organic growing conditions. As conventional and organic growing conditions are different, requirements to varieties are different, too. Breeding for organic agriculture could take an advantage that the expression of many traits is highly correlated between conventional and organic agriculture (Loschenberger, 2007). The correlation between starch content and tuber yield assessments in organic and conventional growing conditions were detected in previous research (Skrabule, 2008). One of the ways is to start breeding for organic farming in a conventional programme, using so called indirect selection, and at a defined generation evaluate potentially acceptable clones in organic conditions. Because of the expected large plant – environment – management interactions under organic conditions the most efficient way is to start selection in organic field as early in the selection process as possible (Lammerts van Bueren, 2002). The collaborative breeding strategy, where breeding companies, organic farmers and breeding scientists are involved, started to work in the Netherlands. The experience and breeding material from conventional breeding programmes has been tested and selection of most acceptable genotypes has been done in organic farms (Lammerts van Bueren, 2010).

The aim of the study was to compare evaluation and selection of selected clones from conventional breeding programme in conventional and organic growing conditions, with a purpose to estimate the possibility of carrying out part of the breeding programme for organic farming in a conventional breeding programme, including selection in conventional growing conditions. The correlation between assessments in both environments was evaluated.

### Materials and methods

Potato clones from conventional breeding programme were evaluated in organic and conventional growing conditions for four years (2006-2009). The clones were selected from existing potato breeding programme according assessment of leaf coverage, foliage resistance to late blight and maturity under conventional growing conditions. Nine potato clones were evaluated in 2006 and 2007, 12 clones in 2008 and 15 clones in 2009. The evaluation of potato clones was done during growing period and after harvesting. The medium late variety 'Brasla' was used as standard variety. The leaf resistance to pests was assessed visually (% of damaged leaves area) during disease development in the field. The length of growing period was determined counting days from emergence (80 % of planted tubers have sprouted) to the end of vegetation. After harvesting yield and tuber size distribution were measured. The starch content in tubers was determined indirectly via specific gravity as percentage of fresh weight. The boiled tuber taste was assessed by expert panel using a 9 point scale (9 – very tasty, 1- very nasty). The results of trait assessment in both environments were compared. The main stress was on selection of clones suitable to organic growing conditions.

The obtained data were analysed using descriptive statistics and Pearson correlation coefficient. The significance of differences between means of samples was assessed using T-test.

The soil type in organic and conventional fields was mostly sandy loam. The soil acidity ( $\text{pH}_{\text{KCl}}$ ) in organic field was in range from 5.6 – 6.5, but in conventional fields – 5.3-6.2. The amount of organic matter (humus) in organic fields varied from 1.9- 2.7%, but in conventional fields – 1.2-2.5%. The estimated amount of nitrogen content in organic fields before potato planting was from 38  $\text{mg kg}^{-1}$  to 60  $\text{mg kg}^{-1}$  depending on fields used each trial year. The potato crop providing with nitrogen was low in organic field, but during growing period it could be improved due to decomposition of organic matter in soil. The coverage of phosphorus and potassium was satisfactory in organic fields (P 34-61  $\text{mg kg}^{-1}$ , K 61-121  $\text{mg kg}^{-1}$ ). There was not detected nitrogen content in conventional fields, so only added nitrogen with fertiliser was taken in account. Fertilisation provided high availability of nitrogen (N 55-65  $\text{mg kg}^{-1}$ ), phosphorus (including soil composition P 107-179  $\text{mg kg}^{-1}$ ) and potassium (including soil composition K 151-142  $\text{mg kg}^{-1}$ ) in conventional fields. The winter cereals were used as pre-crop in each field.

Comparing rainfall during growing period of trial years, the lowest amount of precipitation was in 2006 (total precipitation from May to August - 141 mm). The weather was dry and warm, average day temperature during growing period was 16.4°C. Next year was quite warm as well (average day temperature 15.9°C), but after dry beginning heavy rainfall followed at the second part of growing period (total precipitation from May to August – 381.2 mm), what was favourable for late blight distribution in this particular year. The average day temperatures were mostly similar during growing period of 2008 and 2009 (14.8°C and 14.6°C respectively), but comparatively high rainfall level was observed in August in 2008 (total precipitation from May to August 2008 - 295 mm) and in period of June and first decade of July in 2009 (total precipitation from May to August 2009 - 351 mm).

The potato clones were planted in plots 10  $\text{m}^2$  in 4 replications in both environments. The seed material planted in organic field was pre-sprouted with purpose to shorten time of crop being in field conditions. Potatoes were planted in the second decade of May and harvested in last decade of August or 1<sup>st</sup> decade of September. The spraying with fungicide was used 2-3 times in conventional field after potato flowering for foliage diseases limiting in trial years.

## Results and discussions

*Resistance to diseases.* The foliage resistance to early blight (*Alternaria solani* Sorauer) was assessed in 2006 as late blight did not appear in the field. The evaluation of damages on leaves was started on August 1<sup>st</sup>, when early blight spots covered 0-5% of leaf area depending on genotype. After a week damages did not exceed 10% in both environments. Spraying with fungicide was not effective in conventional field, maybe due to hot and dry weather conditions and only genotype resistance protected crop in both environments. But significant correlation was not found between assessments in both growing environments ( $r = 0.21 < r_{0.05,10} = 0.63$ ).

Potato clones resistance to late blight caused by *Phytophthora infestans* (Mont.) de Bary was assessed next three years. The first diseases damages in organic fields were observed 7–10 days earlier than in conventional fields in 2007 and 2008. Late blight significantly destroyed foliage (30-100%) in organic field 10 to 14 days before it happened in conventional fields. The first diseases spots on potato leaves were observed in the same time on both environments in 2009, but significant foliage damages (5-100%) were assessed after 10 days in organic field and only after 24 days in conventional field. The application of fungicide delayed late blight development in conventional field and saved crop vegetation for longer time. The late blight development was faster in both years - 2007 and 2008 than in 2009 due to more favourable weather conditions (more rainfall during August) in 2007 and 2008. The precipitation in August 2009 was approximately two times less than in two previous years. The significant correlation coefficients between clone's resistance assessments in both environments during the diseases epidemic development stage were not found (2007 -  $r=0.42$ ; 2008 –  $r=0.39$ ; 2009 –  $r=-0.01$ ). It means that genotype assessment in conventional field with fungicide application does not tell us about genotype resistance to late blight in organic conditions where fungicide was not applied. The evaluation to disease resistance has to be done in organic field to get reliable data. The late blight damages are important factor for yield limiting in organic field. The importance of fast tuber bulking turns important for genotype with low resistance to disease.

*Growing period.* The potato plants emerged 2-3 days earlier in organic field than in conventional each year due to seed tuber pre-sprouting. But growing period in organic field was shorter for about ten days than in conventional field. The late blight damages were reason for shorter growing period in organic field. Used fungicides protected potato plants from infection and so prolonged growing period. Differences of length of

growing period between clones in particular environment depended on maturity and resistance to disease. The average growing period varied from 48.8 days to 63.7 days in organic field and from 75.6 days to 87.9 days in conventional field depending on year. The difference between average data of both environments in each trial year was significant (Table 1.). Correlation was not found between lengths of growing period in both environments (Table 2.). The growing period in organic environment was more affected by weather conditions and late blight distribution than in conventional environment. The estimation of this trait preferably would be done in organic growing conditions.

Table 1. The significance level (T-test) between mean trait assessments in organic and conventional growing conditions (2006-2009).

Trait	t <sub>fact</sub> between means in organic and conventional fields			
	2006	2007	2008	2009
Growing period	2.62*	3.02	10.69	23.13
Tuber yield	6.22	6.97	2.58	5.97
Percentage of tubers larger than 50mm in yield	ns**	4.13	3.85	2.49
Starch content	2.88	2.72	ns	5.93
Boiled tuber taste	ns	ns	ns	ns

\*- significant, confidence level 95%, \*\* - not significant

*Yield.* The average tuber yield varied from 16.1 to 31.8 t ha<sup>-1</sup> in organic growing conditions and from 24.6 to 44.8 t ha<sup>-1</sup> in conventional growing conditions. Tuber yield in organic growing conditions was lower than in conventional growing conditions each trial year. The growing period in conventional growing conditions was longer and nutrition supply was better than in organic growing conditions. It was the reason of larger yield formation in conventional field. Comparing average data of potato yield in organic and conventional field a significant difference was found in each trial year (Table 1.). Correlation between yield assessments in both environments was significant in two trial years and was not significant in two following tears (Table 2.). Some potato clones exceeded standard variety 'Brasla' and average yield level in the same year in both environments, so those clones could be acceptable for selection. But others were outstanding only in one environment, most of them only in conventional growing conditions. Those clones had acceptable yield in conventional field and comparatively low yield in organic field. If selection was done only in conventional field, those clones could be accepted as suitable for organic farming. But in organic growing conditions part of them turned out unsuitable. It means that genotype yield was significantly differently in different environments. Few clones yield was acceptable for selection only in organic field. Those clones possibly had some features better adapted to organic growing conditions, which were not tested in the trial: for example, a better root system that is more tolerant to changeable moisture and nutrient uptake. If selection was done in conventional fields, those clones could not be selected. The selection of potentially high yielding potato clones for organic growing conditions could be done in conventional breeding field, but promising clones should be tested in organic field because predictions do not always turn out to be true. Beside that, some promising clones would be lost, because genotypes were not outstanding in conventional field.

Table 2. Correlation between trait assessment in organic and conventional growing conditions, 2006-2009.

Trait	Correlation coefficient (r)			
	2006	2007	2008	2009
Growing period	ns**	ns	ns	ns
Tuber yield	0.72*	0.65	ns	ns
Percentage of tubers larger than 50mm in yield	0.66	ns	0.65	ns
Starch content	0.89	0.96	0.73	0.79
Boiled tuber taste	0.72	ns	ns	ns

\*- significant, confidence level 95%, \*\* - not significant

*Amount of large tubers (> 50 mm) in the yield.* The genotype's ability to form bigger size tubers was assessed evaluating the percentage of large tubers (> 50 mm) in yield. Quantity of large tubers in yield varied

from 20% to 41% in organic growing conditions and from 25% to 62% in conventional growing conditions. The significant difference between means of both environments was detected in three trial years. The tubers size in different farming systems is mostly determined by genetic factor (Sawicka *et al.*, 2007). Although the significant impact of growing environment was observed in performed trial. The significant correlation between traits expression in different growing environments was detected only in two trial years (Table 2). The amount of large tubers mostly depends on genotype environment interaction in particular growing conditions. The ability of genotype – very fast tuber bulking – would be preferable.

*Starch content.* The starch content in tubers of potato clones was higher in organic field than conventional in 2006. The late blight did not destroy foliage this year. The starch content was lower in organic growing conditions in 2007 and 2009; the difference between means was significant during all three years. The average starch content was similar in both environments only in 2008. Starch content in tubers was mainly determined by genotype. The restrictions of starch accumulation could be caused by late blight damages. The extra week with green foliage in conventional field was a chance to accumulate more starch in tubers utilising sun energy for a longer time. The development of tuber yield was continuing simultaneously, so starch content in tubers did not increase relatively so greatly. The significant correlation was found between results in different environments each trial year (Table 2). The genotype's feature to accumulate comparatively higher amount of starch could be assessed in any environment because the potato clones ability to accumulate starch in tubers was relatively similar in different growing conditions and environments.

*Taste of boiled tubers.* The assessment of the taste of boiled tubers did not differ a lot depending on growing environment in trial years. There was not found a significant difference between taste assessments average of clones in both environments during four trial years (Table 1). The average assessment varied from 6.8 points to 7.3 points. Probably the impact of year conditions was more relevant than growing environment. The significant correlation between assessments in different growing conditions was detected only one year. This trait depends mostly on genotype expression in different growing conditions including the weather. The selection for taste of boiled tubers successfully could be done in any environment.

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Participation in Conference has been funded by EU SF LATVIA, Project Nr. 2009/0218/DP/1.1.1.2.0/09/APIA/VIAA/099.

## Urgent need for new apple breeding methods better adapted to low-input agro ecosystems

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**Keywords:** Fruit breeding, organic farming, low-input, hardiness, disease tolerance

### Abstract

Due to its perennial statute, fruit sector is the first consumer of plant protection products per hectare. This may also be a direct consequence of a historical cultivar selection that neglected the tree rusticity for years, and mostly encouraged high yield, common quality and aesthetical properties. The ecological footprint of fruit cultivation is therefore very high, and not sustainable. Many fungicides and insecticides shall be used, even in organic farming, to face sanitary problems. Considering the time necessary to create new cultivars, an urgent change of paradigm is needed. The French Ministry recently launched a mission involving many scientists active in this field, to start integrating environmental purposes into the vegetal selection devices. Discussion is ongoing, and may lead to major changes in the current organization of the French system. Some experiment already started in Belgium, Spain or France to provide more suitable cultivars, meanwhile decreasing the fruit production cost, as cultivars should require less attention in the orchard. It appears that evaluation of the cultivars shall be made at the basin scale, and not for a whole country, since their behaviour is variable, and characteristics (such as adaptation to climate change) may not be suitable everywhere. Fruit growers could also be part of the selection process as they better know the key-points to keep or reject a new hybrid. One could think of a core group of growers and technicians, skilled in hybridization, for a first screening of seedlings in a limited number of orchards, and a second wider group, active in a second step on a shorter screening but in more diversified places.

### Are the current grown commercial cultivars well suited for low-input agriculture ?

Due firstly to its perennial status, secondly to its monoculture and high density orchard design and lastly to the very high disease susceptibility - especially scab - of the currently grown commercial cultivars (cvs), apple growing sector is one of the most intensive user of plant protection products per hectare (Sauphanor *et al.* 2009). It is probably the consequence of the golden period of the modern phytopharmacy and mineral fertilizers that cultivar selection has neglected, for years, traits like durable disease resistance, tree rusticity and adaptability, for mainly to concentrate efforts on high yield and fruit aesthetical properties. The ecological footprint of intensive commercial fruit growing is therefore very high, and not sustainable; in such context, even in organic growing systems, very intensive spray schemes still need to be applied to face sanitary problems (Jamar *et al.* 2010). Considering the period of time necessary to create new cvs, an urgent change of paradigm is needed.

### **Need of new breeding methods for selecting cultivars better adapted to low-input growing systems.**

Like all responsible of an economic enterprise, fruit growers have the tendency to grow cvs that are required by the market, that reach the highest prices and in most of the cases, even if such cvs are highly dependent on high inputs of plant protection products, fertilizers,...(e.g. 'Cripps Pink'). Therefore, even if new cvs meet the requirement to be adapted to new low-input fruit production systems; they will have to be adapted to the market demand and to satisfy to high fruit quality.

Considering that low-input fruit growing production systems based on new resilient agro ecosystems become the new rule, some key requisites of new breeding methods developed to obtain adapted cvs care listed as follow:

- Pay attention to more durable disease resistance, even breeding for disease *tolerance* is a new features;
- Adapted screening methods for selecting cvs with lower and more durable production inputs: easy tree training – adapted tree architecture - low tendency to biennial yield, good yield, a plant physiology adapted for a good efficiency to low fertilizer level, easy and long conservation capacity,...
- Better adaptation to abiotic stresses induced by e.g. climatic changes : e.g. better drought tolerance, better local adaptation, frost tolerance,...

Such changes firstly bring about the need to enlarge the genetic bases of our breeding programmes by making better use of original parents and/or local genetic resources that are well evaluated and characterised. This is applicable even for the diversification of sources of disease resistance that express different defence pathways for a more durable partial resistance as for tree architecture, high physiological efficiency, abiotic resilience. At the level of scab resistance it is obvious that new strategies are under development for a more durable resistance than it was the case with the *Vf* gene (Dapena & Blázquez, 2004, Gessler *et al.* 2006, Lateur *et al.* 2009).

Secondly, our selection methods need to be in line with the fixed objectives e.g. by application of extra low-input growing methods inside our experimental plots, by testing breeding lines in a diversity of cultivation basins or in contrary in specified local place instead of at national or even world-wide scale and thirdly and finally, by promoting the participative breeding method with motivated fruit growers that are involved as expert stakeholders.

Further cvs evaluation and final selection trials would be organized in organic orchards with new designs that integrate a larger functional biodiversity.

### **Some examples of fruit breeding methods to obtain proper cultivars for low-input agriculture**

Currently, some original breeding programmes – mostly linked with the use of local fruit genetic resources as parents - already started in Belgium, Spain or France with the aim to provide more suitable cvs adapted to low input that meanwhile helps to decrease the fruit production costs.

#### *The Belgian example*

Since the early 90<sup>th</sup> started at Gembloux an apple breeding program based on the use as parents of local old 'landrace' cvs (Lateur & Populer, 1994) with the objective to enlarge de genetic diversity of the common breeding work especially focused on quantitative traits linked with scab tolerance, long natural keeping ability, flesh firmness and good flavours. As landraces were formerly grown in high stem standard pastured orchards without both any plant protection scheme and mineral fertilizing mass selection were applied that screened for disease tolerance, hardiness and low dependence for fertilizing. All these factors are positively influenced by the former use of vigorous rootstocks.

Concomitantly research inside this breeding program has developed adapted selection methods that fits with the objectives of firstly quantitative scab and powdery mildew tolerance (Lateur *et al.* 1999; Lateur *et al.* 2000; Lefrancq *et al.* 2004; Lefrancq & Lateur , 2006) and secondly, with low-input organic production (Lateur & Doucet, 2006; Lateur *et al.* 2009). Specific attention is given on the early selection procedures such as to pay attention on the inoculum diversity, the applied doses inside the resistance screening tests and the use of quantitative assessment scales that allow selecting genotypes with a good level of disease tolerance. Further studies indicated that there was a satisfactory correlation between the early greenhouse scab selection procedure on seedlings and their further behaviour under field conditions. It is illustrated by the results obtained on a population of 1200 selected genotypes where, after at least four years of selection pressures, 87 % of them were still ranked inside the first four classes – on a 1-9 scale - of low scab susceptibility (Lefrancq *et al.* 2006, Lateur *et al.* 2009). A complementary selection pressure is applied in the selection plots in both stages of on own roots and grafted plants where plants are selected without any plant protection spray, nor with mineral fertilisation

Such hard conditions help to select the few more robust plants that aim to be better adapted to low-input agro ecosystems.. The first 65 better performing selection numbers are currently under evaluation inside two plots, one in IPM and the other in Organic Production. A new POMINNO project is currently focused on the enhancement of dietetic compounds in apple and on the identification of new minor scab resistance genes present in old cvs emerging from our breeding program.

Inside a cross-border INTERREG IV BIODIMESTICA project in collaboration with northern France, a cooperative apple and pear breeding program started (Lateur *et al.* 2007) with two common structured organic evaluation orchards that are planted in both regions which allow testing the capacity of adaptation of a selection of old cvs and breeding selections. One of the object concerns the comparison of cvs on different rootstocks (M9 and Supporter 4) in comparison with trees on their own roots. In 2009 started a new participative cooperative breeding program in collaboration with an organic grower. Four crosses with Belgian pollen applied on French old cvs have already been done. Seeds are collected, they will be sown in Gembloux for further being submitted to the early scab selection. The next selection steps will be organised by the grower himself. Such collaborative projects are very creating a good synergy between the partners that leads to positive sharing strategic ideas, potential good parents and finally sharing the tasks. The last steps of selection and releasing material with the management of the breeder's rights that need still to be fair solved.

#### *The Spanish example*

Asturias, in the North coast of Spain, has a high cultivar diversity linked to the traditional cider-making (Dapena *et al.*, this volume). Along with the evaluation and selection of local cvs of the Germplasm Bank (Dapena, 1996), a breeding apple programme was initiated in 1989 at Servicio Regional de Investigación y Desarrollo Agroalimentario (SERIDA) (Dapena & Blázquez, 2004). Local old 'landrace' cider-apple cvs with valuable agronomic and technological parameters were crossed with cvs of interesting characteristics in order to improve the resistance to diseases and pests and to diminish the biennial bearing which affect most of Asturian cvs. Crossings of local dessert apple cvs were also performed.

The breeding programme has four different lines:

1. Improvement of the resistance of local cider-apple cvs against scab, rosy apple aphid and fire blight (the latest is not present yet in Asturias but do on neighbour regions) by crossings with resistant cvs such as 'Florina' or 'Priscilla' (Dapena & Blázquez, 2004; Miñarro & Dapena, 2009).
2. Obtaining of scab-resistant cider-apple cvs of regular bearing by crossings of local cvs with the hybrids (kindly supplied by the INRA of Bordeaux and Angers) which apart from having the Vf system, are of regular bearing.
3. Improvement of the technological and agronomical characteristics of local cider-apple cvs, such as the phenol content, the resistance level, the rapid and abundant production or the ripening period by crossing Asturian cvs among them.
4. Improvement of the resistance, regular production and storage of local dessert-apple cvs by crossings with the hybrid H2310, resistant to scab and of regular production.

In this program attention has especially been paid to the features of the parent cvs: locally adapted cvs with partial polygenic resistance have been crossed among them or with other foreign cvs which showed high levels of resistance or regular bearing. The evaluation and selection of the cvs has being done with trees on their own roots in orchards without fungicides treatments with very low input level, similar to those in which the cvs will be used. Finally, some of the crossings performed were done attending the demand of the cider-makers who need cvs with high level of polyphenols. Therefore, this breeding program was conducted following many of the requirements proposed for new breeding programs.

Three cultivars resistant to scab, rosy apple aphid and fire blight have already been selected. Other 65 obtentions have been pre-selected.

#### *The French example*

French public fruit breeding has been strongly reduced for the last 30 years, and remains active mostly for apple. Breeding in other fruit species is either private, or is neglected. Dozens of hybrids have been obtained but not further developed because they did not fulfil conventional standards, whereas they may be very promising and suitable in low-input devices. Some of them were scab resistant (monogenic) but have been overcome.

These hybrids (see table 1), not selected for mass development, have been planted in four different orchards in 2000, among other old cvs, and observed during 8 years to collect information about their behaviour in very specific conditions.

Table 1.: List of hybrids proposed by INRA Angers, and planted in the national network

<i>Number</i>	<i>Parents</i>	<i>Number</i>	<i>Parents</i>
X3425	(Chantecler x Baujade) x Chantecler	X6807	X4355 x X4638
X3460	X3318 x (Idared x Prima)	X7368	Liberty x (Idared x Prima)
X6412	Gala x Prima	X7523	Red Winter x Liberty
X6688	Granny x X4638		

Three consecutive programs between 1999 and 2010 have been supported by French Ministry of Agriculture, and permitted the creation of a network of extensive orchards (apple, pear, apricot), and the collection of useful data on cvs behaviour under low-input cultivation, for recommendations to fruit growers (Parveaud *et al.*, 2010).

A second research program is being carried out in west of France (Conservatoire d'Aquitaine) through seedlings of old regional cultivars, and graftings in one orchard without any treatment, and one IPM orchard.

The Inter-reg program Transbiofruit conducted with CRA-W in Belgium, and CRRG in France also permitted to launch participative selection of cultivars in northern organic orchards, by motivated growers themselves, such as Jean-Yves Fillatre.

As a consequence of a new environmental context (called Ecophyto 2018), the French Ministry launched in 2009 a brainstorming involving most active people in this field (scientists, nurserers, breeders), to propose an updated breeding system in different vegetal sectors, considering the environmental patterns into the plant breeding devices, to reduce ecological footprint of the fruit sector.

### Conclusions

As explained here, some experiences and positive results have already been gathered, and will be very useful to develop other initiatives on all fruit species. Indeed, most of these works concern apple, whereas other species are also heavily concerned : peach is for example a species with a very high cultivar turn-over, but simultaneously the most difficult species to cultivate organically...

A strong commitment of fruit nurserers is needed to contribute to this ambitious goal. By proposing a wide range of cultivars and parents, and by financially support this long-term and hazardous research, they would significantly help the organic fruit sector.

Last but not least, is the methodology to be set up in order to assess the behaviour of this material under low-input: what kind of plantation (blocks, randomization...), how many trees per cultivar, how many orchards in different regions, which level of plant protection for each disease/pest...?

### Acknowledgements

The INTERREG projects BIODIMESTICA & TransBioFruit and the CRA-W POMINNO, INIA RTA2008-00120 project are acknowledged for their financial support.



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## Session 4 - posters

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### 23. Genotype x Environment Interactions of Winter Wheat

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**Keywords:** Organic farming, Organic Plant Breeding, Winter Wheat Cultivars, Nutrient Use Efficiency

Winter wheat cultivars for organic farming have to be adapted to highly variable environments. Yield and yield stability, the nutritional value and the bread making quality are here of major importance. Particularly, seed quality, tillering capacity, regeneration ability after harrowing, weed suppression ability, resistance or tolerance against diseases and improved nutrient use efficiency (NUE) under limited nutrient conditions need to be improved by specific breeding efforts (Kunz et al., 2006; Löschenberger et al., 2008).

Ten winter wheat cultivars were grown in 2007 in two organic systems of the DOK long-term experiment in CH-Therwil. Eight of these cultivars were tested in 2008 at three organic farms in different pedo-climatic environments in Switzerland to observe genotype x environment interactions under different organic conditions. In contrast to the DOK site on a fertile loess soil, the on-farm sites were located on sandy or sandy-loamy sites with lower inherent yield potential. We assessed grain yield, yield components and parameters related to baking quality and NUE.

Grain yield at the on-farm sites ranged between 2.2 t ha<sup>-1</sup> and 2.8 t ha<sup>-1</sup> and was much lower than at the DOK sites ( 3.7 t ha<sup>-1</sup> to 4.2 t ha<sup>-1</sup>) (Hildermann et al., 2009). Cultivars and sites significantly affected grain yield. Genotype x environment interactions were significant across the three on-farm sites; however they were not significant across all five organically managed sites. Calculated across the on-farm sites, there was a tendency towards higher yields of the organically bred cultivars. This effect was statistically significant at the lowest yielding site, where yield of the organically bred cultivars was 14% higher than yields of the conventionally bred cultivars. However, at the DOK sites and averaged across all five organic sites, the organically bred cultivars could not outperform the conventionally bred cultivars.

Similar to the grain yield, the total gluten content increased from the on-farm sites to the DOK sites. Irrespective of the site, the gluten index of the cultivars Scaro, Antonius and Caphorn was stable. In contrast, the gluten index of the cultivars Sandomir, CCP and Titlis highly varied between the tested environments. Yield was significantly correlated with grain N yield ( $r = 0.93$ ) and nitrogen (N) utilization efficiency (NUtE) ( $r = 0.72$ ). Genotype x environment interactions were not significant for grain N yield, however strongly significant for NUtE ( $p < 0.01$ ). The conventionally bred cultivars strongly responded to environmental conditions and showed a low NUtE under at low N supply. In contrast, NUtE of the organically bred cultivars Scaro and Sandomir was stable across all test environments.

Beside high yields, cultivars suitable for organic farming should achieve high baking quality and nutrient use efficiency under nutrient limited conditions. Among the tested cultivars, the organically bred cultivar Scaro revealed such a performance across all tested sites.

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## 24. Comparison of interconnections between barley breeding material traits under organic and conventional growing conditions

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**Keywords :** breeding for organic farming, selection criteria, correlations, growing environments

While breeding for organic farming it is necessary to identify the most appropriate growing conditions in which to perform the selection process. Soil fertility, crop management, yield level and other factors may vary very much between each organic farm, and between organic farms and research institutions where the selection is usually performed. Since plant breeding requires considerable input of resources and the market for organic varieties is limited, it is essential to find the most appropriate selection conditions that will provide acceptable varieties for organic farms.

Spring barley breeding lines selected from two cross combinations (Primus/Idumeja and Anni/Dziugiai) at two distinctive organic and two conventional locations were used in the study. The organic location 1 was situated in a research field of the plant breeding institute (with green manure as fertilizer), the organic location 2 included an organic farmer's field (with stable manure as fertilizer), the conventional location 1 was located in a barley breeding field (with medium level of mineral fertilizer input) and the conventional location 2 in a seed production field of the institute (with high mineral fertilizer input). Selection of the breeding lines was done under the respective growing conditions starting from F<sub>3</sub> generation. Most appropriate lines for growing in organic farming were selected at all 4 environments. Selected breeding lines were evaluated in F<sub>5</sub> (n=20-23 per cross combination, without replications) and F<sub>6</sub> generations (n=10 per cross combination, 3 replications). Correlations between barley traits and the environments and cross combinations will be compared in the presentation.

The analysis of the correlation between grain yield and observed plant traits of F<sub>5</sub> lines indicated that tendencies between the lines obtained from both cross combinations as well as between the environments were dissimilar in most of the cases. Soil shading (evaluated at early stem elongation stage) correlated positively with grain yield in all cases; the correlation was always significant for lines from the cross Primus/Idumeja, but only at the conventional location 2 for Anni/Dziugiai lines (p<0.05). A significant positive correlation between grain yield and plant development speed at tillering stage as well as between yield and plant height at the beginning of stem elongation was found for Primus/Idumeja lines at both organic locations and at the conventional location 1 (p<0.05). Plant height before harvest did not correlate significantly with yield under organic conditions which is in contradiction with some other studies. There was a tendency for planophyle growth habit to correlate positively with yield under organic and conventional conditions for lines from both cross combinations. The analysis of correlation of the F<sub>6</sub> lines will be included in the presentation.

This study was performed with financial support of EEA grant EEZ08AP-27 and European Social Fund co-financed project 2009/0218/1DP/1.1.1.2.0/09/APIA/VIAA/099.

## 25. Triticale breeding for organic farming in Latvia

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**Keywords :** triticale, organic breeding

For creating winter triticale varieties suitable for organic farming a special breeding programme has been started in Priekuli Plant Breeding Institute. The evaluation of triticale genotypes in organic farming was done in Priekuli during 2005 - 2009. The aims of research are:

1. Estimating possibility for selecting genotypes desirable for organic farming in conventional fields.
2. to find desired traits for the organic triticale varieties breeding programme.

There were included different winter triticale (*xTriticosecale Wittm.*) breeding lines in our trials, selected from the conventional breeding programme. The different traits were tested for each genotype. The influence of different traits on yield and grain quality was analyzed and best triticale genotypes for organic and conventional conditions

The results showed that it is possible to select triticale genotypes suitable for organic conditions in the conventional field. However the testing of selected breeding lines have to be done in organic growing conditions. Genotypes with better stability traits must be selected (especially in the years with unfavorable weather conditions). For organic farming only

genotypes with good winterhardiness and resistance to snow mould should be selected. Triticale genotypes with different plant heights, growth habits, leaf size would be suitable for organic growing conditions.

## 26. Value for Cultivation and Use testing under organic conditions: experience in winter wheat in France

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**Keywords:** Breeding, registration, VCU, organic farming, winter wheat

In France the organic farming has increased since the late 1990's and is currently booming. Bread winter wheat, as one of the most important cash crop, is the most cultivated cereal in organic farming and covers more than 31 000 ha. Nevertheless, most of varieties used and available on the market were bred for conventional farming systems (with high inputs of mineral fertilizers and chemicals for crop protection). For the last ten years, the request has been rising for distinct breeding programmes and special adapted varieties for the organic conditions. Registration on national catalogue of such varieties is important to encourage breeding programmes for organic farming.

Before registration on the national catalogue and commercialisation of seeds, a new variety of wheat must submit official trials for DUS (Distinctness, Uniformity, Stability) and VCU (Value for Cultivation and Use). The evaluation criteria for the VCU-tests are based on the most important quantitative and qualitative characteristics, while new varieties are compared to standard varieties (the most important varieties on the market). Currently, VCU tests in France are run under two types of conditions: intensive conventional conditions and with no fungicide protection. To register a specific organic cultivar, the breeder has actually to ask for special experiments to show the added value. As these special experiments are technically and financially awkward to implement, an exploratory system has been set up for two years (yields 2010 and 2011) to assess the technical and economic feasibility of VCU testing under organic conditions.

In 2009-2010, height trials run under organic conditions form the official organic VCU-testing network. Two lines bred by INRA for organic farming and low input are assessed, compared to two standard organic varieties (Renan and Saturnus) and to two standard conventional varieties (Apache and Caphorn, in order to relate to conventional trials). Special attention is given to response to low level of nutrients and to the competitive ability against weeds. This official organic VCU testing, for which the costs are shared, results from an agreement between the breeder (INRA), the national coordinator of variety screening for organic cereals (ITAB), the official office for registration (GEVES), the height experimenters (mainly farm advisory services).

Traditional VCU-tests are run in parallel, as they are officially required for registration. Nevertheless, both data from traditional and organic VCU testing will be taken into account to evaluate the candidate lines. First results will be discussed in early 2011 (agronomic and technological data from yield 2010). Recommendation for registration (or no registration) will be known in October 2011, based upon results of the two-years experiments. Yet, most important results will be the conclusions about the implementation of official assessment for organic varieties of cereal for the first time in France.

## 27. Spring spelt varieties tested in organic farming system

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**Keywords:** Organic farming, spelt wheat, morphological and agronomical characters, grain quality

With the aim to compare spring spelt wheat with other wheat species, there were evaluated 7 spring spelt varieties on the fields certified for organic farming. Testing was arranged similarly (3 [exceptionally 2] replications, plots á 4.5 m<sup>2</sup>, row spacing 12.5 cm) in three localities (Prague-Ruzyně; Úříněves and České Budějovice). Evaluated characters were clustered into three groups (morphological, agronomical and product quality). Particular attention was paid to the characters valuable for organic growing system.

Nearly horizontal position of flag leaves was typical characteristic of tested spelt varieties. That corresponded with lower stand density measured by number of spikes per area unit. High plants of spelt wheat were slightly more susceptible to lodging in comparison to other wheat species. Two selected spelt varieties showed very good resistance to fusarium infection (*Fusarium culmorum*, *F. graminearum*) detected by PCR. Bread making quality parameters (wet gluten content,

gluten index and Zeleny sedimentation) were in certain wheat species mainly einkorn and emmer very low. On the other hand some spring spelt varieties had these parameters very similar to bread wheat check varieties.

## 28. Association of methods to control apple scab: Partial resistance, sanitation and soft chemical control

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**Keywords:** Partial resistance, apple scab, methods of disease control, sanitation

Today, the control of scab is mainly based on repeated sprayings of fungicides, due to the high level of susceptibility of current apple cultivars. Many negative impacts of these intensive treatments are, like the decrease of auxiliary fauna, environmental pollution and the atmospheric carbon emission. Moreover, in populations of *Venturia inaequalis* some resistances to fungicides have appeared. It is essential to improve the disease control with reducing the quantity of inputs. For this aim, partial resistant cultivars could be an efficient alternative. However to reduce the risk of control failure, which can have a great economic impact for the grower, a sufficient level of resistance must be associated with other methods of control. The strategy of control must be easy and reliable. The thresholds for fungicide application must be validated according to the level of resistance and the sanitation. To define this strategy, we studied the association of the high level of partial resistance of "Reine des Reinettes", with sanitation (removing of foliar litter) and a chemical control which prevents the most important risks of contamination. In this work carried out from 2006 to 2009, we observed a stability of the efficiency of disease control (less than 2% of scabbed fruits at harvest) despite the weather conditions, which were very favourable to scab. The number of sprayings was reduced by 50% in comparison with a susceptible orchard managed in Fruit Integrated Pest Management, in the same weather conditions.



## 29. Spring barley grain quality changes in conventional and organic growing conditions

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**Keywords:** breeding for organic farming, grain quality, correlations, growing environments

In Latvia traditionally barley grain is used for food in the preparation barley groats and peeled barley. The main quality parameters which processors take into account when determining the price of grain are volume weight and crude protein content. Processing enterprises have established the minimum volume weight at 640 g l<sup>-1</sup>. For the grain production for food and animal feed varieties with high protein content are more valuable; starch content is also essential for use in animal feed. Data summarized from 18 conventional and 6 organic farms in the project "Technological solutions in cereals production in Latvia" in 2008 showed that on conventional farms, the volume weight ranged from 578 to 712 g l<sup>-1</sup> and on organic farms from – 613 to 667 g l<sup>-1</sup>; the crude protein content on conventional farms varied between 9.7 – 15.2% and on organic farms between 10.4 - 14.0%, and the starch content from 59.65 to 63.3% on conventional and from 61.5% to 62.1% on organic farms. In organic farming it is important to choose varieties which produce high grain quality at reduced levels of nitrogen supply. The aim of our study was to investigate barley grain quality differences between conventional and organic farming systems and to clarify which conditions are more suitable for the selection of genotypes appropriate for organic farming. Ten diverse barley varieties were included in a three year trial at two organic and two conventional growing sites. The following grain quality parameters were evaluated: thousand grain weight (TGW), volume weight, protein content and starch content. Results of the investigation showed that grain quality significantly depended on both variety and growing conditions. The organic growing site O2 showed a significantly lower TGW and volume weight than the organic growing site O1 and both conventional sites. The highest protein content was obtained in the conventionally managed trial field C2 with high mineral fertilization level (120kg/N/ha). At the organic site O2 the protein content was higher compared to O1. This may be explained by the use of manure and soil differences. Assessing the correlations of grain quality parameters and the different environments, it was found that in order to improve the barley's grain quality genotypes should be selected for TGW, volume weight, and protein content under the organic growing conditions.

This study was performed with financial support of EEA grant EEZ08AP-27 and European Social Fund co-financed project 2009/0218/1DP/1.1.1.2.0/09/APIA/VIAA/099.

### 30. Assessment of some agri-products through organic farming management of crop cultivars evolved by 'A' and 'R' lines breeding programme

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*Key words* : CMS Breeding technology, yield components, water, heavy metal ions, AAS, metrical characters.

The discovery of Cytoplasmic Male Sterile (CMS) line in crop plant has no doubt indicated a new frontier for maximum and quality production from the crop cultivars. The CMS breeding research programme was undertaken in 1998-1999 for multi-location trial in 6 (six) locations for the studies of location effect for raising hybrid F<sub>1</sub> rice during winter-summer-boro season in this laboratory in collaboration with West Bengal State Seed Corporation Limited (WBSSCL). '25 A' and 'Ajaya' were allowed for the trial programme as CMS and restorer line respectively which were procured from the State Rice Research Station, Chinsurah, Hooghly, West Bengal, India.

'CR-1010', 'PUSA-221' and 'AJAYA' rice cultivars were selected as restorer lines considering their genetic characteristics of best production capabilities in low, high and medium condition of land respectively where the experiment was conducted prevailing various seasons and environment of two fertilizer regimes. The yield attributes were studied in each location and all recorded data were computerized for the analyses of G x E interaction following Comstock and Robinson's model (1952a and 1952b) and regression of stability following Finlay and Wilkinson's model (1963) for biometrical evaluation. It has been observed that the suitability of each combination for the desired agronomic factors. The main causes of getting comparatively better yield was nothing but due to the factors of the environmental and genetic characteristics of parental lines. More remarkable observations have been cited in this context.

Our observations were concentrated towards yield attributes of some agri-horticultural crops to find out the suitable environment based on its stability efficiency of newly evolved F<sub>1</sub> rice hybrid seeds by selected restorer line over specific 'A' line utilization for CMS technology. The similar effects were also expected from other vegetable crop plant cultivars. Random samples of soil and water were collected from each sub-division and a few metrical characters of various agri-horticultural crops were studied. The water samples were analyzed and estimated its mineral contents like sodium, potassium, copper, calcium, manganese, magnesium, chromium, iron, copper, zinc, lead, calcium, mercury and arsenic by atomic absorption spectrometry (AAS). In water samples though metals like sodium, calcium, copper, lead, potassium, mercury and cadmium are below their permissible limit but it has been found that the presence of magnesium, manganese, iron, zinc and arsenic have been exhibited above their maximum admissible concentration (OAC) or guideline value (GV) of WHO (1984) in a few samples.

The aims and objects of this experiment were to find out the effect of organic farming management of different crop cultivars involving CMS breeding technology different sources of agro-climate condition in the experimental field and to explore strategy of quality food production in this context.

## Session 5

### Regional participatory plant breeding

Friday, December 3 rd - 8:30 am

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#### Farmer-led participatory plant breeding. Methods and impacts. The MASIPAG farmers Network in the Philippines

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#### **Short history of MASIPAG**

The MASIPAG network is a partnership of people's organizations, NGOs, and scientists working towards the sustainable use and management of biodiversity through farmer's control of genetic and biological resources, agricultural production and associated knowledge.

MASIPAG was founded shortly after an international conference in 1985 that focused on the consequences of the green revolution for farmers. Analysing the worsening socio-economic conditions of smallholder farmers using high yielding rice varieties and high input packages, a researcher started up a breeding programme with traditional rice varieties for low-input farming. Work began with 4 farmer groups and a first collection of 47 traditional varieties. Today, 25 years later, the network has 635 farmer groups with about 35000 farmer members, a collection of 1090 traditional local rice varieties and a breeding output of 1085 MASIPAC new rice varieties or lines. The Network is supported by several NGO donors. A national secretariat and 3 three regional offices hold 40 staff that manage various support services for farmers: training, breeding, seed exchange and advocacy on many aspects of farmer's rights and food sovereignty.

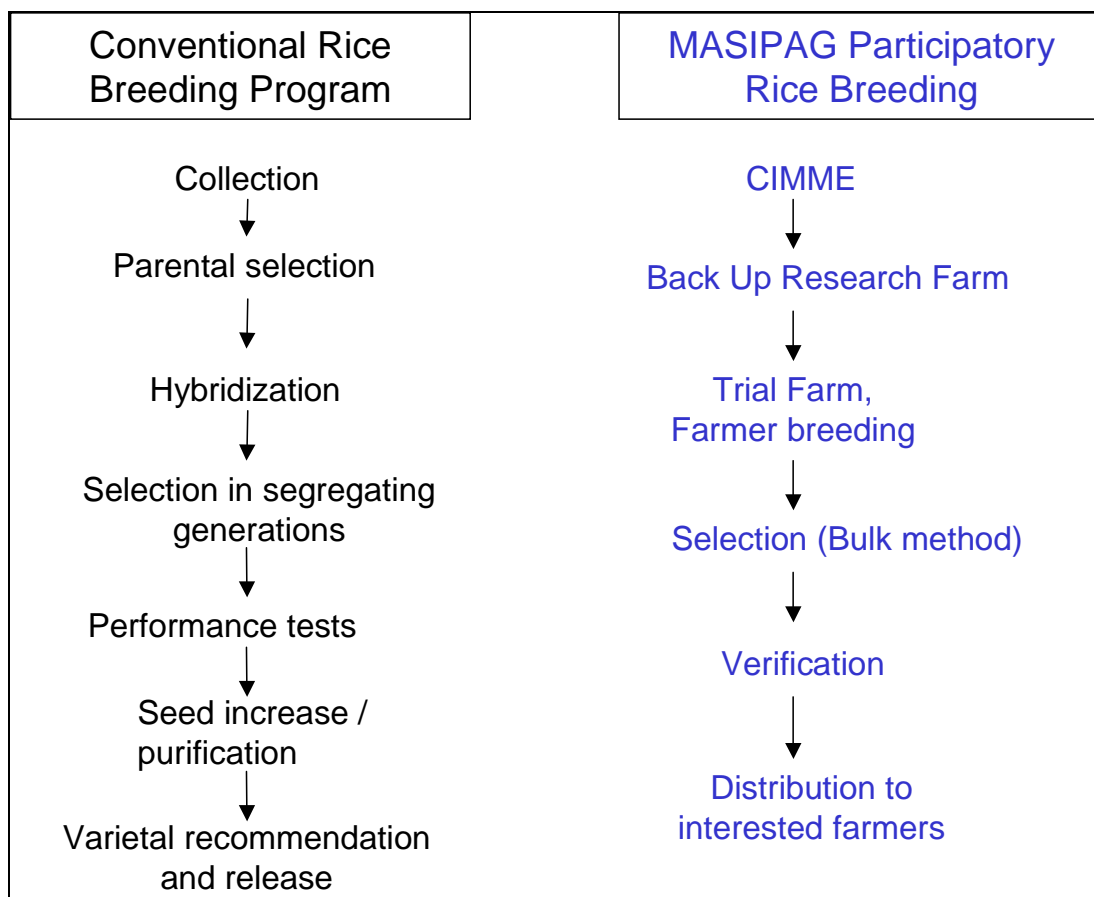
#### **Participatory plant breeding**

Breeding is generally done by specialized scientists working for large institutions or private companies. Within MASIPAG the actual breeding work is done by farmers. These breeders also provide initial training for young apprentice rice breeders to further expand the network. Currently there are 65 active farmer breeders including many women breeders all across the Philippines.

The breeding process starts with the discussion of breeding criteria. MASIPAG use many criteria for their seed. Farmers rank the criteria according to their needs and problems. That generates ideas for new innovative varieties. MASIPAG farmers actively use diversity on farms and do not always try to combine all criteria into one variety. They keep many varieties for many different purposes and different growing conditions. The following criteria are among the most frequently used: high yield without chemical fertilizer, early maturing, resistance to pest and diseases, resistance to drought, resistance to excessive flooding, medium height, strong stalk, non-lodging, high tillering capacity, long panicles, more grains per panicle, long grains, small seed embryo (=high milling recovery), no unfilled grains and good eating quality.

The different steps of the participatory breeding process are shown in comparison with conventional breeding in the following chart. For the selection of parental materials Masipag farmer breeders rely on the CIMME (collection, identification, maintenance, multiplication and evaluation programme. The organization maintains a big national backup farm with more that 2000 rice varieties, 2 regional farms with 1200 varieties and 9

provincial backup farms with about 300 varieties each. These backup farms hold MASIPAG’s own collections and the newly bred varieties and selections with documentation. The backup farms are in direct contact with currently 223 village based trial farms. These trial farms are the cornerstone of training and breeding work. Each trial farm contains at least 50 locally adapted varieties. Before becoming MASIPAG members, farmers go through a “farmers field school” in the trial farm. In an intensive 2 year training farmers learn all about organic farming and rice production. A strong focus lies on seed management, seed purification and breeding. Thus breeding work takes place in very diverse environments at many different sites.



For selection, MASIPAG breeders use the “modified bulk method”. For busy farmer breeders, this method is simpler, more convenient and practical. It tends to retain many variable traits within the a given population. Bulk samples may be considered as phenotypically uniform but genetically diverse. Promising selection are verified by the breeder and other local farmers. This process also provides the first yield information. After 2-3 planting seasons in verification, MASIPAG selections are released to more interested farmers locally. The parent material for most crosses so far have been 2 traditional varieties. The main purpose was the improvement of traditional varieties. But also an increasing number of crosses are made between traditional varieties and MASIPAG lines or elite lines from a local university (UPLB). Unlike conventional varieties, MASIPAG rice do not undergo seed increase and purification prior to distribution to farmers. This makes the process much faster compared to the conventional process. Due to the less strict selection process MASIPAG rice are not standardized and highly homogenized. More genetic diversity is intended. Looking at the system as a whole, the trial farm approach enables farmers to view a large number of rice varieties and selection under his/her local conditions. A farmer can take the most promising varieties from the collection and try out how they work under his/her very own farming conditions. This gives the farmer the chance for finding the best mix of varieties for his/her conditions. MASIPAG recommends for farmers to grow at least 3 different varieties under mass production on farms. The following table illustrates the performance of a number of selections and



the study in which geographic areas the selections proofed successful and thus became what MASIPAG calls locally adapted selections (LAS).

Table 1: Performance of cross M5 bulked lines

<b>Variety: ElonElon (F)</b> <i>(tall, prone to lodging, long panicles, drought tolerant)</i>	<b>Variety: Abrigo (M)</b> <i>(short, long panicles)</i>	<b>Obj: Reduce height of ElonElon; resistant to lodging; retain long, dense panicle</b>
<b>Selection</b>	<b>Feedback on Performance</b>	<b>Where it was Locally Adapted (LAS)</b>
M5-A-1	<i>Not selected</i>	
M5-A-2	<i>Not selected</i>	
M5-AS	80 cm tall, 11 productive tillers, resistant to lodging, resistant to tungro; tough grains	1) Negros Occidental; 2) Laguna; 3) Aklan; 4) Bohol; 5) Agusan del Norte; 6) Cotabato
M5-B-1	<i>Not selected</i>	
M5-B-2	75 cm tall, 11 productive tillers, erect flag leaf	1) Camarines Sur 2) Laguna
M5-B-3	<i>Not selected</i>	
M5-BD-1	90 cm tall, 15 productive tillers, elongate grain; resistant to black bug	1) Negros Occ.; 2) Agusan del Norte; 3) Lanao del Norte; 4) Cotabato

### Seed multiplication and marketing

It is a key principle of MASIPAG that rice seed is not sold commercially. MASIPAG rice can only be accessed by becoming a MASIPAG member. Among MASIPAG farmers, rice seed is shared for free. Again, the trial farm training plays a major role in the success of this system. As farmers are trained to select rice and maintain seed quality, very small amounts of seeds are sufficient (a few hundred grams) to share varieties. Each farmer is able to produce the necessary bigger seed volume in 1-2 growing seasons. The development of this “sharing culture”, as enabled farmers to become very diverse, not only in rice, but also for many other crops.

### Socio-economic impacts of MASIPAG

In 2007 and 2008 an evaluation study reviewed the socio-economic situation of MASIPAG farmers compared to conventional Philippino farmers. The study was conducted in three regions with a total sample of 840 households (Bachmann et al. 2009). More than 90 research questions were used in the study and for most of the parameters the MASIPAG system proofed superior or equal to the conventional farming system. A short selection of parameters is highlighted here.

Cropping yields were characterized by a considerable variation, however, average yields for organic MASIPAG farmers and the conventional group did not differ significantly. Yields varied according to regions from about 2,6 t/ha to 4.1 t/ha. The differences between the farming systems were below 200 kg/ha and not statistically significant. Reaching parity in yields can certainly seen as a remarkable achievement for the organic system in particular considering the fact that conventional farmers in the Philippines apply high levels of inputs (e.g. 100-150 kg N/ha). The results can probably be explained by the fact that MASIPAG farmers use a wide range of organic practices (composting, mulching, agroforestry) but the good result is probably also strongly influenced by the quality of the MASIPAG rice selections.

The MASIPAG farmers have a much more diverse farming system. On average, they cultivated 45 crops or tree crops, while the conventional farmers only cultivated 30. Thus, the organic group was 50% more diverse. This finding was in particular also true for diversity in the number of rice varieties grown on farms (3.1 organic versus 1.4 conventional). These findings confirm an important increase in biodiversity.

*Rice Paddy Yields in 2007/8 in kg/ha*

Region and yield in kg/ha	Full organic	Conversion	Reference
Mean Mindanao	3767 <sup>ns</sup>	3864 <sup>ns</sup>	4131 <sup>ns</sup>
STD Mindanao	1586	2016	1453
Mean Luzon	3743 <sup>ns</sup>	3436 <sup>ns</sup>	3828 <sup>ns</sup>
STD Luzon	1464	1216	1216
Mean Visayas	2683 <sup>ns</sup>	2470 <sup>ns</sup>	2626 <sup>ns</sup>
STD Visayas	1570	1406	1356

<sup>ns</sup> Differences not found statistically significant

Food security in the Philippines is a serious topic. Regarding the question of having enough food to eat 88% of MASIPAG farmers stated that food security improved in the past years and only a small group of 2% saw in worsening. For the conventional farmers only 39% rated it improving, while 18% considered it worsening.

Besides having more food for home consumption, the organic farmers also stated a better diet. 68% eat more vegetables, 56% more fruit, 55% more protein rich staples and 40% more meat compared to before. This increase is between 2 to 3.7 times higher than for the conventional farmer group. Probably as a consequence of more and better food, organic farmers also experienced better health. In the MASIPAG group, 85% of farmers rated that their health today is better than before in the year 2000. In the reference group only 32% rated health positively, while 56% saw no change and 13% reported worse health.

The farming systems were also compared by looking into total income, cropping income, subsistence income, household expenditure and investment in farming. The findings showed a big difference between the systems. In particular on rice, conventional farmers were earning more gross income, but less net income due to the high production cost. The study calculated that the net income from agriculture for the MASIPAG farmers was highly significant 17 % higher than that of the reference group.

*Net agricultural income and income per ha*

	Full organic	Conversion	Reference
Net agricultural income (Pesos)	36,093 <sup>ns</sup>	37,216 <sup>ns</sup>	30,819 <sup>ns</sup>
Net agricultural income per ha	23,599***	17,457***	15,643***

Particular noteworthy is the fact that MASIPAG managed to increase the incomes of the poorer farmers. Net agricultural income of the lowest 25% income group was 47% higher in the full organic group compared to the reference group.

These results indicate an outstanding contribution towards poverty reduction. Organic farming could achieve better incomes and reduce the risk of farmers for debts significantly.

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## On-farm conservation and farmer selection as a strategy for varietal development in organic agricultural systems

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**Keywords:** farmers' varieties, participatory plant breeding, on-farm conservation, bread wheat (*Triticum aestivum* L.), organic plant breeding

### Introduction

In recent years there has been increasing attention to on-farm management and informal plant breeding in developed countries as sustainable and organic farmers have been using and regenerating some of the genetic diversity that was historically present in plant varieties because they see the practical benefits of these types of varieties in their fields. On-farm breeding and conservation of crop populations gives value to genetic diversity by maintaining diversity within and among populations and by selecting for local adaptation and production stability due to the resilience of heterogeneous populations. In many cases, farmer selection has been shown to be just as effective as breeder selection at increasing agronomic traits such as yield in difficult environmental conditions, for example the program of barley selection at ICARDA (Ceccarelli et al. 2000), and of maize selection in Honduras (Smith et al 2001). Studies have also shown significant effects of natural selection on crop populations allowed to evolve in an agricultural context (Suneson 1956, Goldringer et al 2001). However, very few studies have analyzed the combined effects of natural selection and farmer management (including directed selection and exchanges) within heterogeneous crop populations in an agricultural context. This study seeks to address the need for more information on how on-farm selection and management affects the diversity of cultivated species and how these practices can be optimized for both the conservation of important genetic resources and the development of population varieties adapted to organic farming systems. The results presented in this paper look at the response of existing farmers' varieties to mass selection, and are in the context of a larger project of participatory breeding which includes selection in diverse segregating populations resulting from crosses of many different farmers' varieties.

### Materials and Methods

In a collaborative project with the farmers' network Réseau Semences Paysannes (RSP) in France and with farmers in Holland and Italy, eight farmers' varieties and two modern varieties of bread wheat (*Triticum aestivum* L.) were studied as part of the European Farm Seed Opportunities Project (FSO, 2007-2010). These varieties were grown at seven organic farms for three years to study on-farm evolution. In addition, four farmers selected individual spikes from plots of four farmers' varieties and Renan, a modern variety, on their farms (Figure 1). Morphological traits including plant height (PH), spike length (SL), the distance between the leaf and the spike (LLSD), the number of kernels per spike (KN), grain weight per spike (GW) and thousand kernel weight (TKW) were measured for individual selected and unselected plants of each variety. In 2009-2010, progeny head-rows of selected and non-selected spikes of all four farmers' varieties and Renan were grown at the research farm of INRA le Moulon. Plots of selected and non-selected progeny of Rouge de Bordeaux (RB), Blés de Redon (RD) and Renan (RN) were grown on one participating farm to analyze the response to selection.

**Results**

Significant selection differentials were found in 2009 for most farmers, varieties and traits. Farmers chose plants which were taller than average and had higher numbers of kernels per spike, grain weight per spike and thousand kernel weight.

Figure 2 shows selection differentials in 2009 (left panels) for RB, RD, and RN selected by FM and HF, and the selection response for the same populations grown in 2010 at FM (right panels). Selection differentials were positive for all three varieties for plant height, kernel number per spike and grain weight per spike, but only RB showed significant positive responses to selection, for selections by both HF and FM, except in the case of RD selected by HF for plant height, where height decreased despite a positive selection differential. This is in contrast to results at Moulon, where plant height always showed a positive selection response. Results from the experiment at le Moulon 2010 on selection in the 4 farmers varieties and Renan are still being processed, but an analysis of vegetative traits show significant differences due to selection (Table 1).

**Discussion**

The results of studying the response to selection in the generation following farmer selection show that a strong selection differential translated to a real response to selection in many cases. Plant height is one character where farmer selection increased mean values across all varieties when tested in a common garden experiment at le Moulon. Spike length and LLSD are more difficult to evaluate in the field, and may be only partially correlated with plant height, so a strong response to selection in plant height may affect the response to selection for these traits.

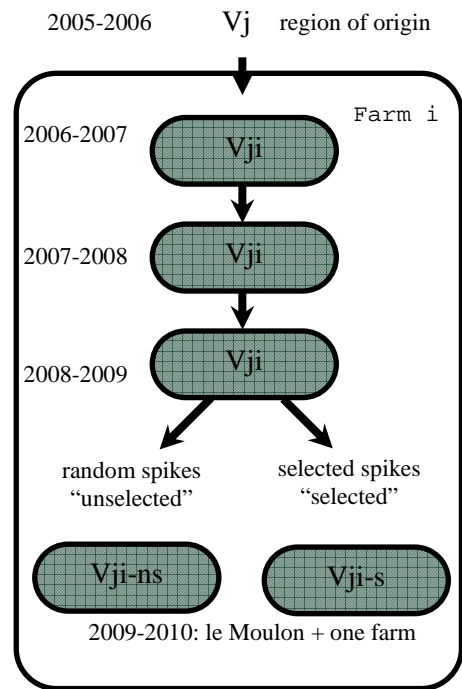


Figure 1: project design for each farm i, Vj=variety j

Table 1: Tests for significant differences due to version (selected vs unselected) for all farmers combined within each variety. PH = Plant height from the ground to the top of the spike not including the awns; LLSD = distance between the base of the spike and the flag leaf; SL = length of the spike from the base to the tip not including the awns; significance values given as: + : p<0.1, \* : p<0.05, \*\* : p<0.01, \*\*\* : p<0.001 direction of change: +: selected>unselected, -- selected<unselected

Variety	PH	direction	SL	direction	LLSD	direction
PI: Piave (Northern Italy)	*	+	+	+	**	--
RB: Rouge de Bordeaux (SW France)	*	+	ns		ns	
RD: Blés de Redon (Brittany)	***	+	ns		**	--
RN: Renan (modern variety)	***	+	ns		ns	
ZH:Zonnehoeve (Netherlands)	***	+	***	+	*	+

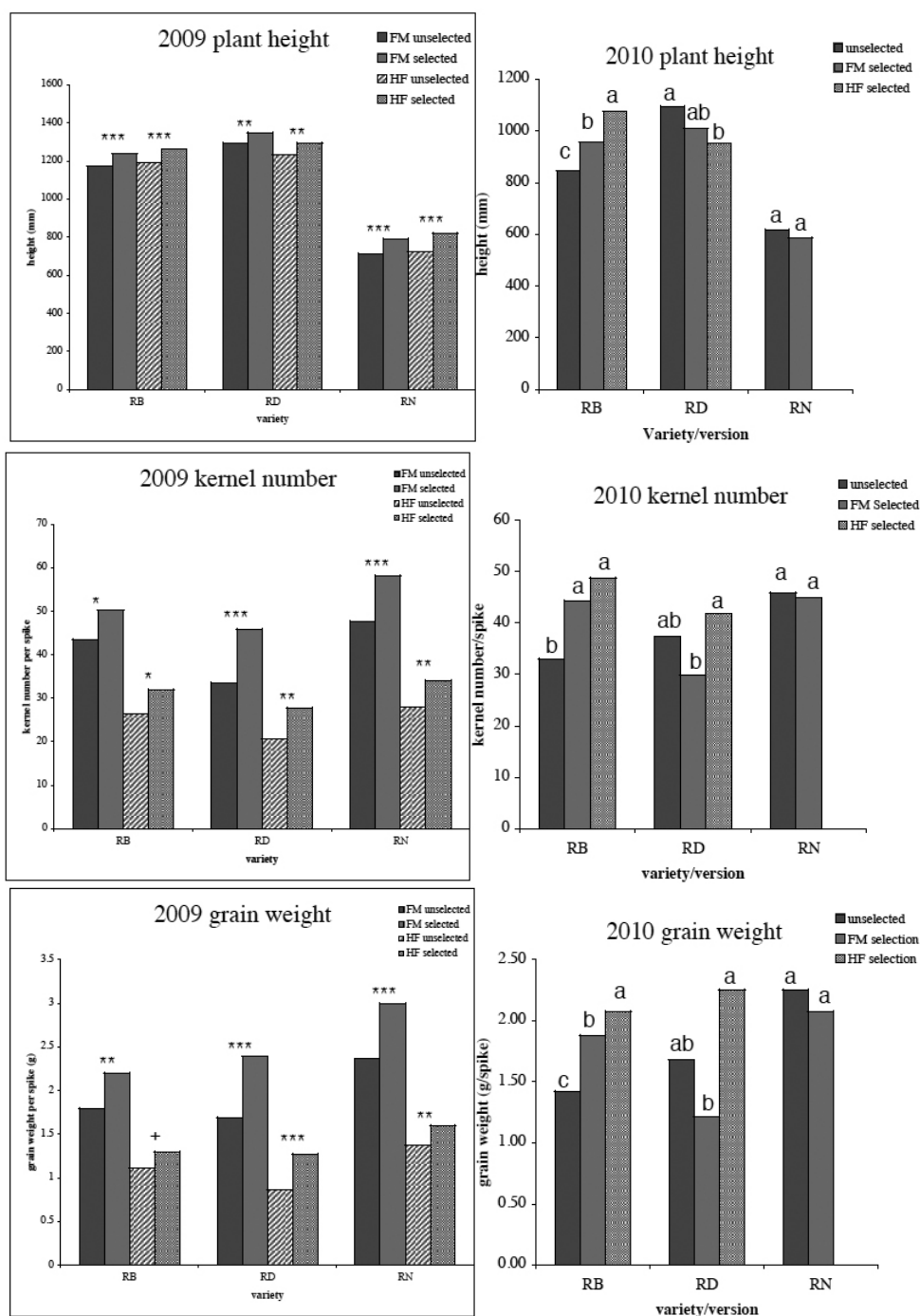


Figure 2: selection differentials (left panels) and response to selection (right panels) for Rouge de Bordeaux (RB), Blés de Redon (RD) and Renan (RN) grown in 2009 on the farms of FM and HF and in 2010 on the farm of FM. For tests of significant selection differentials, + :  $p < 0.1$ , \* :  $p < 0.05$ , \*\* :  $p < 0.01$ , \*\*\* :  $p < 0.001$ . For tests of significant response to selection, versions within the same variety with the same letter are not significantly different at  $\alpha = 0.05$ .

In the on-farm evaluation of the response to selection, one historic variety, Rouge de Bordeaux, responded positively to selection for almost all traits measured, while a landrace population, the Blés de Redon, and the modern variety Renan did not, despite significant selection differentials in 2009. This is in agreement with what farmers observed as Rouge de Bordeaux was one of the varieties that performed very well outside its region of origin, indicating greater adaptability than landraces such as the Blés de Redon. It also corresponds to molecular genetics findings of high levels of internal diversity of farmer-managed populations of Rouge de Bordeaux. In addition, the particular sample of the landrace Blés de Redon was composed by a farmer starting from a small

number of spikes, possibly inducing a genetic bottleneck and a reduction of adaptive potential. The significant decrease in PH for Redon selected by HF measured on-farm and significant increase for the same selection of RD on-station highlight the potential differences and problems of evaluating selection outside the target environment, even though precise observations and comparisons of many selections among different farmers are only possible on the research station. The difference in the comparison between Moulon and on-farm could also be because the selected versions are compared only to the unselected version of FM on-farm, while each selected version is compared to the unselected version of that farmer at le Moulon, however the two non-selected versions are very similar. The lack of response to selection in Renan is not surprising as we expected the observed selection differential to be due primarily to micro-environmental factors, however, there was a significant response in plant height at le Moulon, so there is likely some genetic variation even in this modern variety. Molecular genetic studies are in progress on the base populations to determine the levels of genetic variation and the on-farm evolution of both neutral variation and variation linked to hypothesized adaptive traits such as earliness.

### Conclusions

One of the main themes that emerges in discussions among farmers and scientists is the different approaches of farmers and scientists to the selection process. Scientists have a more analytical, quantitative approach, while farmers have an appreciation of the interactions of the plant with its environment and the overall performance of plant populations in their fields. In this case of participatory selection, the goal is to bring together the strengths of both these methods to improve the on-farm selection of populations that respond to the needs of organic farmers. Significant responses were found for many traits after only one cycle of selection in farmers' varieties, but this is dependent on the variety and sufficient genetic diversity needs to be present for selection to have an effect. Preliminary results from selection in segregating populations also support this conclusion. On-farm selection is thus complementary to on-farm conservation, using the diversity within an existing farmer network to maintain and enhance local adaptation and crop performance. While on-farm selection requires a significant commitment from farmers and researchers to be successful, a relatively small group of dedicated farmers and researchers can serve a broader public interest in conserving genetic diversity for important agricultural species through selecting diverse populations across a wide range of environments.

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## Breeding for specific bioregions: a genotype by environment study of horticultural and nutritional traits integrating breeder and farmer priorities for organic broccoli cultivar improvement

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*Keywords:* *Brassica oleracea*, farmer cultivar requirements, genotype by environment by management interaction, regional adaptation, glucosinolates

### Introduction

Organic agriculture now comprises 5% of overall agricultural production in the U.S. with an acreage growth rate of 15% in 2009. Consumer preference for more sustainably produced foods, combined with the perception that organically produced food is more nutritious, are the primary drivers behind the growth in demand. Broccoli is a significant crop for organic agriculture due to market demand as well as its nutritional contribution to the U.S. diet. The main broccoli production areas for conventional agriculture in the U.S. are in California, Oregon and Maine. However, organic broccoli production occurs across the country in diverse agro ecosystems and for diverse market segments. Organic growers need cultivars better adapted to organic and low external input growing conditions, but demands on specific characteristics can differ by crop. The commercial seed industry is challenged to satisfy the demands of organic agriculture, and often does not understand the special requirements of organic production systems that are characterized by diverse quality and adaptability criteria. Since organic farming is comprised of diverse types of organic growers, and since organic farmers have fewer tools at their disposal to influence their production environment to fit their crops, the assumption is that their seed cultivar needs and markets differ from their conventional production counterparts. Organic farmers want improved cultivars that display production stability under adverse environmental conditions, rather than cultivars that promise higher yields but largely lose that yield advantage because of inability to perform under conditions of organic production. An integrated study to understand organic growers' requirements and to evaluate performance of broccoli cultivars was conducted as part of the senior author's dissertation research. To define an organic broccoli cultivar ideotype, stakeholders' needs in the organic broccoli production chain were studied. In order to determine the adaptation of conventionally bred broccoli cultivars to organic systems, field trials were conducted to evaluate cultivars under conventionally and organically managed conditions in two main broccoli production regions in the U.S. Since broccoli is a relatively abundant source of phytochemicals, e.g. glucosinolates, tocopherols and carotenoids (Kurilich et al., 1999), that have been associated with human and plant health promotion, both horticultural traits (e.g. yield, head size and overall plant vigor) and nutritional composition have been evaluated. The results of horticultural trait and phytochemical content performance combined with farmer and breeder surveys and interviews will be used to generate strategies for the development of superior broccoli cultivars for the organic market.

### Materials & Methods

The extent to which organic agriculture can utilize germplasm generated from conventional breeding programmes was explored through surveys, semi-structured interviews and field day participation with broccoli breeders (n=5) and conventional (n=18) and organic (n=38) farmers. Field days were held in two consecutive years in Oregon (2007-2008), at which organic and conventional growers were asked through field trial observation to identify the top 5 performing cultivars for their production system. Organic and conventional

growers completed preliminary surveys identifying their priority broccoli traits and standard cultivars they used, while breeders ranked the primary trait objectives of their commercial breeding programmes. The horticultural and nutritional performance of 23 broccoli cultivars was compared between organic and conventional growing conditions through field trials with three replications conducted in the spring and fall between 2006 and 2008 in Oregon and Maine. Data was taken on thirteen different horticultural traits of value and phytochemical data on glucosinolates, carotenoids and tocopherols were evaluated from the broccoli samples derived from the field trials. The Oregon cultivar trial location was conducted at the same location that the farmer field days were held and the participatory data collected.

## Results and Discussion

### *Grower and Breeder Surveys for Broccoli Trait and Cultivar Preferences*

The surveys indicated that the size of the production acres and the market outlets of organic growers were more diversified, with a mix of fresh market and processed product growers, and conventional growers representing a higher percentage of processed product growers. Most of the conventional growers had an acreage ranging from 80 to 300 acres and aimed at mechanical harvesting. The acreage of organic growers ranged from 5 to 120 acres, and they tended to focus on fresh market production and sought broccoli cultivars that provided a primary harvest and a continued harvest with side-shoot development. Results of the survey are summarized in Table 1.

*Table 1: Grower and Breeder Broccoli Trait Ranking (1-5 scale)<sup>1</sup>*

Broccoli Trait	Org Ranking	Conv Ranking	Breeder Priorities	Breeder Ranking for Org
Head Shape & Size <sup>2</sup>	1	2	*	*
Yield	1	2		
Disease Resistance <sup>3</sup>	2	3		*
Insect Resistance <sup>4</sup>	2	5		*
Abiotic Stress Resistance <sup>5</sup>	2	2		*
Stem & Head Color	3	2		
Weed Suppression	3	5		*
Uniformity in Maturing	3	1	*	
Bead Size	4	2	*	
Flavor	4	5		
Plant Uniformity	4	3	*	
Leaf Mass Coverage	5	4		
Nutrition <sup>6</sup>	5	5		
Mechanical Harvesting	5	1	*	
Placement of Crown	5	1		
<sup>1</sup> Ranking Scale (1-5): 1 as highest; * indicates top priorities for breeders				
<sup>2</sup> Flat or domed: overall preference dome shaped				
<sup>3</sup> black rot; club root; downy mildew; head rot; other diseases				
<sup>4</sup> aphids; cabbage looper; symphylan; flea beetle; cabbage maggot				
<sup>5</sup> heat tolerance; drought tolerance				
<sup>6</sup> minerals; vitamins; phytochemicals (glucosinolates, carotenoids, tocopherols)				

While both groups prioritized ‘head size, weight and overall yield’, conventional growers ranked ‘uniformity in maturing’ and ‘capacity to harvest mechanically’ higher than their organic counterparts. The grower survey results indicated that the organic grower prioritized ‘abiotic stress resistance’ and ‘disease resistance’ higher than their conventional counterparts. Factors more important to organic producers than to conventional growers included broccoli cultivars with vigorous growth in soils with potentially low or fluctuating mineralization rates of nutrients, or the ability to tolerate weed competition.

The breeder interviews indicated that the priorities for conventional breeding were traits such as ‘mechanical harvesting capacity’, ‘head shape and size’, ‘bead size’, ‘plant uniformity’ and ‘uniformity in maturing’. These breeding objectives outlined by the breeders directly aligned with the traits prioritized with the conventional growers. When the breeders were posed the question, how would these priorities change if they were breeding for an organic system, the breeders reprioritized the breeding objectives to ‘nutrient use efficiency’, ‘abiotic



stress resistance', 'insect resistance', 'disease resistance' and 'weed competition'. These priorities aligned more closely with those priorities identified by the organic growers.

In the preliminary surveys both the organic and conventional growers identified 'Arcadia' as a standard cultivar that they use regularly. In addition to 'Arcadia' organic growers listed an assortment of cultivars used to support their production systems, whereas the conventional growers listed only one other cultivar ('Emerald Pride') suitable for broccoli production. When the organic and conventional growers selected their top 5 cultivars in the field trial, both chose 'Arcadia' and 'Gypsy', but the other three cultivars in the top 5 were not the same. Both the organic and conventional growers were '*surprised*' by the performance of select cultivars they hadn't seen or considered previously including 'Green Magic', 'Gypsy' and 'Maximo' and discussed adopting various cultivars into their production systems in the future. In the post-field evaluation discussions, the organic growers expressed interest in knowing the cultivars with higher levels of nutritional traits with the intent that they could translate this information to their customer base and incorporate these cultivars into their production systems.

#### *Genotype by Environment Interaction Field Studies:*

Results of the genotype by environment field trials in Oregon in the fall of 2006 and 2007 demonstrated top performance for head weight, size and overall plant performance in the cultivars 'Green Magic' (680g/head; overall ranking 7.6 out of 9) and 'Maximo' (655g/head; overall ranking 6.2) and Gypsy (overall ranking 7.1). All three cultivars performed significantly better in the organic trials than the conventionally paired trial, which could be explained by the higher levels of available nitrogen in organically managed soils during the warmer fall months and the longer growing period. These cultivars are classified as mid-late maturing cultivars with estimated maturity days ranging from 78-88 days. 'Arcadia' was not a top performer in yield or head size in Oregon over the seasons; it ranked 5 on a scale of 1 (lowest) and 9 (highest) in overall varietal performance and had an average yield of 399g/head. The results were contrary to the grower cultivar selections that identified 'Arcadia' as the number 1 ranked cultivar. In the Maine fall trials of 2006 and 2007 all cultivars under organic conditions outperformed those grown under conventional conditions in head weight except one cultivar, 'Everest'. Top ranking cultivars in the organic system for fall were 'Packman', 'Green Goliath' and 'Belstar' all cultivars categorized as early or mid-early cultivars with maturity days ranging from 55-70 days. Some varietal performance overlap occurs between Oregon and Maine organic and conventional in the fall trials, but the above mentioned cultivars distinctly outperformed all other cultivars. The open pollinated cultivar 'Green Goliath' demonstrated the most flexibility in overall performance for head weight, size and overall performance rating over all sites, all seasons and all treatments. The spring 2007 and 2008 field trials in both locations demonstrated few significant differences in cultivar performance under organic and conventional conditions. The best performing cultivars for spring trials in Oregon were 'Green Goliath' and 'B1 10', both early-to-mid-early season cultivars, while the best performing cultivars in Maine were 'Fiesta' and 'SBC 2519' both mid-to-late season cultivars. Early season cultivars performed poorly in the spring trials in Maine producing very low overall yields ('Early Green', 'OSU OP', 'Packman').

Overall yield performance by season was distinctly different with average fall yields across both locations ranging from 250 to 780g/head compared to average spring yields of 80 to 450g/head. Greater number of degree days was observed in Oregon compared to Maine (1,790 compared to 1,600). In contrast, Oregon had fewer degree days in the spring compared to Maine (1,585 compared to 2,600). It is apparent that mid-to-late season cultivars are more productive in Oregon in the fall, while early-to-mid season cultivars are better adapted to spring environments. Conversely, for greatest productivity, Maine requires early-to-mid season cultivars for the fall and mid-to-late season cultivars for the spring. The best performing early-to-mid season and mid-to-late season cultivars for Oregon were not necessarily the best performers for Maine.

#### *Genotype by Environment Interaction Glucosinolates:*

The literature has demonstrated that concentrations of health-promoting nutrients in Brassicas depends on the cultivar, the season and the management system in which they are grown, including organic versus conventional conditions (Farnham et al., 2003; Charron et al., 2005; Meyer & Adams, 2008). Broccoli florets have been found to be particularly abundant in the glucosinolates glucoraphanin, glucobrassicin, and neoglucobrassicin. Glucosinolate concentrations in broccoli floret tissue have shown dramatic variation among different genotypes (Kushad et al., 1999). Evaluation of 10 broccoli genotypes over two years at the University of Illinois indicated

that this variation in concentration for glucoraphanin was primarily due to genetic variation, while differences in neoglucobrassicin was due to environmental variation and genotype-by-environment interaction (Brown et al., 2002).

Glucoraphanin content in the broccoli cultivars derived from the field trials did not demonstrate season or location effect which is supported by the literature. However, genotypic variation was shown. The cultivars 'Arcadia', 'B1 10' and 'Gypsy' were the best performers consistently across locations, seasons and treatments. The glucobrassicin content showed a consistent year and season effect with higher levels in the fall compared to spring, and higher levels in fall 2006 compared to 2007. One aspect that characterized the season in 2006 was that the year was warmer and dryer. Best performing genotypes were 'Nutribud', 'USVL 093', 'USVL 048' and 'OSU OP'. For this trait, 'Arcadia' is in the bottom group. From the literature it is known that neoglucobrassicin is very responsive to biotic stress. Our data showed that there was a consistent treatment effect in which levels of neoglucobrassicin from broccoli cultivars derived from organic trials on average outperformed those grown in conventional trials. Neoglucobrassicin content levels, in contrast to glucobrassicin, showed an overall higher level in spring than broccoli cultivars from the fall trials. Best performing genotypes were 'USVL 093', 'USVL 048', 'Beaumont', 'Gypsy' and 'Diplomat'. For this trait, 'Arcadia' was a mid performer.

### Conclusions

The genotype by environment interaction study of broccoli, amongst others (e.g. Murphy et al., 2007 for wheat), demonstrates that traits of a cultivar are sometimes ranked differently when grown in an organic production system compared to a conventional system. This has strong implications for breeding strategies. The breeders interviewed acknowledged that more attention on abiotic and biotic stress resistance in a broccoli breeding programme is needed which is in accordance with the farmers' varietal requirements. The first findings of the field trials show that cultivar performance is influenced by season and region, and differences in treatment (organic versus conventional management). The field trials showed that there are cultivars with broad adaptation such as 'Green Goliath'. These cultivars performed across locations, seasons and treatments within the sub-top group, however, organic farmers would benefit more from cultivars specifically adapted to their region and season. The trial results showed a wide range of glucosinolate levels. Glucoraphanin was very genotype dependent, while glucobrassicin and neoglucobrassicin were more influenced by abiotic and biotic environmental factors. Therefore, there are opportunities for nutritional performance enhancement under organic conditions which would provide an added value to the product quality with respect to human and plant health. Further elaboration of the dataset will contribute to the design of regional breeding strategies for improved broccoli cultivars for the organic market.

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## On-farm conservation and participatory maize breeding in Portugal; lessons learnt and future perspectives

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### Introduction

Maize has more than five century's evolution in Portugal. Its influence is transversal to agriculture, landscape and socioeconomics (e.g. "Portuguese Agricultural Revolution" in the XVII/XVIII). During this period, farmer selection, within the diversified number of microclimates according to the sequence of valleys and mountains took place mainly oriented to human consumption (e.g. maize bread - "Broa").

After 2nd World War, American success in maize breeding had a tremendous impact in Europe due to the availability of the new hybrid seeds. Portugal was one of the first countries where American hybrids were introduced. The FAO programmes for hybrid production in Europe had excellent adaptation to Portugal and Breeding stations were established along the country from North to South, but only NUMI at Braga (NUMI – Maize breeding station) survived for long term. NUMI was succeed, creating hybrids for grain quality for human use as bread and early-maturing varieties adapted to highly intensified cropping systems until early 1990's.

The perception of genetic resources and participatory plant breeding importance for hybrids programme led Silas Pego at NUMI to praxis. First, he started in 1975 the collection missions for maize; which where the embryo of the first cold storages that where supported by FAO and the precursor of BPGV. Second, in 1984, Pêgo started, with the CIMMYT support, a participatory maize breeding (PMB) programme at Sousa Valley (VASO), based on: a) an integrant philosophy, i.e., intended to answer the problems of small farmers and increasing yield without losing the parameters defined by the farmer for bread making quality, potential for polycropping systems, and use in sustainable agriculture; b) the concepts of quantitative genetics in population improvement. Mass selection was applied both to landraces (e.g. 'Pigarro') and to 'Fandango', a synthetic population. Recurrent selection by S2 lines was also used for 'Pigarro'. On both, the yield component and pest and diseases evaluations were performed and implicitly the quality for Broa (maize bread) (Vaz Patto et al, 2009) was analysed in the case of 'Pigarro'. Seed samples were kept in cold storage from each selection cycle.

To start this program, three main choices had to be taken: 1) the location that better represented the region - a traditional maize area, where previous agro/sociologic/economics data existed. In addition, the support of a local elite farmers' association (CGAVS) that where committed to test the efficiency of an alternative project supposed to improve the local germplasm versus hybrids production, at least in certain specific circumstances; 2) the farmers to work with - side by side, to whom the decision power will be allowed, and which initial acceptance and enthusiasm were crucial; 3) the germplasm source to start from: 'Pigarro' (Moreira et al, 2008) and 'Fandango' (Pêgo & Antunes, 1997; Moreira et. al, 2006). These tacit choices imply a careful respect for the local traditional agriculture. While the breeder would apply his breeding methodologies, the farmers would continue a parallel programme with their own mass selection criteria. With this agreement, the breeder had to accept low input and intercropping characteristics, as well as accept and respect the local farmer as the decision maker. On the other hand, the farmer was able to compare the effectiveness of the two breeding systems (phenotypic recurrent selection and S2 lines recurrent selection). This allowed the farmer to base his decisions on

solid grounds. Finally, due to the choice of local adapted germplasm, diversity and quality were considered as the priority traits.

The aim of this work is to present the main conclusions obtained from the evaluation of the yield gain and plant performance after 20 years of a participatory plant breeding approach for: ‘Pigarro’ (two breeding methodologies) and ‘Fandango’ (different cycles of mass selection initiated by the breeder and continued by the farmer). Based on the collected data and applying MARS, a determination of the best explanatory variables for yield was performed. Future perspectives are outlined.

### **Material and Methods**

Depending on selection method and population, 6-7 cycles were chosen for mass selection on ‘Fandango’ and ‘Pigarro’ and 3 cycles of S2 recurrent selection cycles only on ‘Pigarro’. Trials started in 2005 and they were performed at four locations at Iowa, USA during one year and in Portugal, in 2 to 3 locations, in 3 to 4 years. Yield evaluation was done for Iowa and Portugal; hence for Portugal morphological data (e.g. ear length and fasciation level) were also obtained.

Data analyses included ANOVA and regression analyses. MARS was used instead of regression analyses when assumption of normality was not fulfilled.

Both regression analyses and MARS allowed the evaluating of response to selection of the traits studied.

To better understand what influences the yield, three methods for analysis have been used: MARS, Classification and Regression Trees (CART) and Random Forests (RF). More detailed information for ‘Pigarro’ and ‘Fandango’ can be found in Mendes-Moreira (2008, 2009b respectively).

### **Results and Discussion**

From these more than 20 years of on farm participatory plant breeding some results and considerations should be registered:

#### **I – Morphological analyses**

1 - For ‘Pigarro’ selection for bigger ears (larger and wider ears):

- After 20 cycles of mass selection: Ear length decreased and simultaneously, ear, cob, medulla and raquis diameters, kernel and cob weight and fasciation significantly increased. Thousand kernel weight decreased significantly and kernel weight increased.
- After 3 cycles of recurrent selection: convulsion decreased significantly, while row number decreased so as the fasciation.

2 – In the case of ‘Fandango’ during the mass selection process there were two phases: a) The breeder phase from cycle 1 to cycle 5 and b) The farmer phase, after cycle 5. Both have different goals, breeder for yield maximization and farmer for big ear size maximization. The results from response to mass selection in Portugal revealed that ear length significantly decreased and simultaneously plant and ear height, ear diameter, kernel row number and fasciation significantly increased so as convulsion. Nevertheless, for breeder cycles these traits were maintained. This selection also led to significant increases of days-to-silk and anthesis. Identical outcomes were observed in long-term divergent selection for ear length in maize (Hallauer, 1992). According with MARS ( $R^2=8.9\%$ ) conversely to the breeder (cycle 5), the farmer contributed to grain moisture increase, at Lousada ( $R^2=80.5\%$ ). This tendency occurred also with days-to-silk and days-to-anthesis. The lack of significant progress in yield for both ‘Pigarro’ and ‘Fandango’ (after C5) can be explained by low selection intensity due to the exclusion of stalk lodged plants in the basic units of selection. Specifically for ‘Fandango’ selection, plant and ear height significantly increased, which could mean less area available, *i.e.*, competition in trials was more severe in advanced cycles and some plants did not produce ears. Probably for this reason significant decrease in yield was observed at Iowa locations. In the case of recurrent selection by S2 lines, yield decrease could have been due to a decreasing of fasciation expression or even to the selection procedures for stalk and root lodging improvement. In order to better clarify this situation, more cycles of recurrent selection would be needed.

3 - The fasciation evaluation, indicates that the farmer explored fasciation for increase diameter and row number. This fact is significantly interesting at Lousada for: a) Pigarro ( $R^2=88.7$ ) with 3.32% of gain per cycle/year and b) Fandango ( $R^2=78.8$ ) with 4.66% of gain per cycle/year.

4 – The RF, CART and MARS analyses were used to determine the best variables that explain yield.

- For mass selection for ‘Pigarro’ and for ‘Fandango’, the most distinct traits for all methods in both populations were: kernel weight and ear weight. The secondarily distinct traits were: rows numbers, number of kernels per row, ear length and ear diameter;

- For recurrent selection in 'Pigarro', the most distinct traits for all methods were: plant and ear height, flowering date and kernel number.

5 - Both selection methods used in 'Pigarro' or differences in selection for 'Fandango', suggested that Mass Selection is better than S2 Recurrent Selection due to the following reasons: (a) mass selection is a cheaper methodology, technically more accessible to farmers, which is a great advantage in the establishment of on-farm conservation programs; (b) one cycle of selection can be completed each summer, and in situ/on-farm conservation of the genetic diversity is effective (Vaz Patto et al, 2008). This, highlight its role as a backup system (complementary with ex-situ) and a monitoring process for an effective on-farm conservation of diversity.

## II-Molecular analyses

Molecular data from Vaz Patto et al. (2008) using 16 SSR on 3 mass selection cycles (C0-84, C9-93, C20-04) of Pigarro revealed that no effective loss of genetic diversity had occurred during the selective adaptation to the farmer's needs and to the regional growing conditions. Variation among selection cycles represented only 7% of the total molecular variation indicating that a great proportion of the genetic diversity was maintained in each selection cycle. Genetic diversity has not been reduced from the 'Pigarro' breed before 1984 to those improved after 2004, but the genetic diversity maintained is not exactly the same. Mass selection seems to be an effective way to conserve diversity on farm.

## III-Socioeconomics and anthropology

The VASO project throughout its praxis:

- Allowed the farmer and the breeder to compare breeding methodologies in loco, i.e., decisions based on knowledge (Pêgo & Antunes, 1997).
- Allowed local initiatives, such as "Sousa Valley Best Ear Annual Contest" (selection for big ears) by the local Farmers' Cooperative Association (Cooperativa Agrícola de Paredes). Thereupon has contributed for the recognition of the farmer by the community, but also has attracted new farmers and new germplasm to this program that in this way could be identified and preserved on-farm by the same approach (Moreira, et al. 2008).
- The anthropological and sociological objective of a participatory plant breeding suggests that more attention should be given to: (a) learning more about how plant breeding itself has been influencing farm changes and agricultural systems; for example, is on-farm plant breeding simply conventional plant breeding on farms, or is it a whole different kind of plant breeding approach for the future? (Powell, 2000); (b) how on-farm conservation is managed to ensure genetic diversity and breeding success. (c) the definition of "yield" needs to be re-considered and broadened to include the total yield of the polycropping system and not just the yield of a single crop *per se* (Pêgo & Antunes, 1997; Powell, 2000); (d) it is important for breeders to work with other people involved in the food production "chain" like traditional grain millers and also bakers (Powell, 2002);
- Highlight the problem of successive agricultural policies pursued by the European Community, i.e., the disappearing of smallholder farming as a viable way of life in Portugal and the socio-economic "pull" factors that remove younger generations from the farm (Powell, 2000; Vaz Patto et al., 2007).

## Future perspectives

The success of on-farm conservation programmes needs a cluster. A cluster where farmers' activities, such as food production, genetic resources conservation, environmental sustainability (e.g. soil and water management), forest protection (e.g. cleaning the forest) and landscape management could be rewarded by stakeholders. Some of these stakeholders can come from industry (e.g. milling industry), plant breeding sector (e.g. supporting reserves conservation – that can support coevolutionary process and organic or low input niche markets), and tourism industry (e.g. rural tourism where specialties and traditional food are the major output). The multifunctional agriculture, so as green care and social agriculture could be also an interesting promoter of on-farm conservation, because in many of these farms yield is not the main goal.

Hybrid populations' development could also contribute to yield progress and to avoid the collapse of some interesting germplasm. This approach can be applicable in a rural development strategy if economic benefits

between associations for specialties (e.g., maize bread) and farmers could be achieved. This scenario is supported by hybrid populations' results from 2009 where yield was in average higher than 11 Mg/ha for two locations (data not publish). This could be of great importance to define heterotic groups that could enhance the breeding efforts (Tracy and Chandler, 2006).

The participatory plant breeding approach (PPB) can be associated with *in situ* conservation of landraces contributing to their economically sustained presence at the farmer's fields. Besides, it can also contribute to define *in situ/on-farm* strategies that could help to design better synthetic hybrid populations for a new generation of low input and organic farming adapted to environmental changes and marginal areas.

Current intellectual property rights do not protect farmers developing their own varieties, and this could also be a way to recognize the farmer conservation efforts.

Finally, lessons from the VASO project could help us design new on-farm conservation projects not only for the Portuguese reality but also for developing countries where adaptation to small farmers needs (e.g., maize quality for food, traditions) are out of the scope of multinational seed companies.

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## Participatory breeding in China in maize and its institutional challenges

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*Key words:* Participatory plant breeding (PPB), China, institutional challenges, access and benefit sharing (ABS)

### Introduction

The ongoing participatory plant breeding (PPB) project has been carried out since 2000 in Guangxi, southwest China as the first PPB project in China. This initiative built on an impact study carried out from 1994 to 1998 by the International Maize and Wheat Improvement Centre (CIMMYT) to assess the impact of CIMMYT's maize germplasm on poor farmers in Southwest China (Song et al., 2006). The key findings of the impact study included the systematic separation between the formal/national seed systems and local farmers' seed systems, and the lack of incentives for formal breeders to develop varieties adapted to the karst landscape and local livelihoods and seed markets. PPB has been adopted as a means for bridging the gap between professional maize breeders and farmers, in order to develop locally adapted varieties and enhance sustainable crop development and *in-situ/on-farm* management of plant genetic resources (PGRs). The project set out to identify technological and institutional options for PPB efforts. It is coordinated by the Centre for Chinese Agricultural Policy (CCAP) with the financial and technical support from the International Development Research Centre (IDRC) and the Ford Foundation.

### Methods

#### *Organizational arrangement*

The project is implemented by a group of people from various organizations/institutes, with different disciplinary and professional backgrounds and operating at different administrative levels.

- *Farmer-plant breeding villages* and *farmer groups* from these villages collaborate with formal-sector plant breeders, extensionists and other stakeholders in the research activities. The project started with six trial villages and expanded to thirteen in 2010.
- *Guangxi Maize Research Institute* (GMRI), a provincial breeding institute, collaborates in the formal plant breeding work, with direct involvement of selected villages and local extension stations;
- *The Institute of Crop Science* under the Chinese Academy of Agricultural Sciences (CAAS) works on technological and related policy issues on maize improvement and genetic biodiversity management;
- CCAP is the host institute of the project, coordinating project activities and providing policy analysis and suggestions.

#### *Genetic Resources and Trials*

The field experiments target on four types of genetic resources (mainly open pollinated varieties (OPVs) and landraces), i.e. 1) exotic populations and genetic resources, from CIMMYT, other Asian countries and other regions in China, 2) locally adapted varieties, which are originally delivered by formal breeders and later on improved and adapted by farmers, 3) farmer maintained landraces and 4) formally conserved landraces (Song et al., 2006).

With different genetic resources, the project covers a range of parallel activities both in farmers' field and on-station, to identify parental genetic resources, make improved populations and further selection to advance the populations into varieties.

Trials in the villages and on-station (at the GMRI) include both participatory plant breeding and participatory variety selection experiments. The trials allow for comparison in terms of locality, approach, objectives and the types of varieties tested (Song et al., 2006). Institute-led and farmer-led approaches are combined with different breeding objectives. Both the exotic populations and genetic resources and the formally conserved landraces are

usually introduced by the breeding institutes, and later on selected in farmers' field. The locally adapted varieties and farmer maintained landraces are continually tested in farmer-led PVS and PPB trials.

The project relies on farmers' experiences and expertise on maize breeding developed over many years, and involves the knowledge and expertise of formally trained plant breeders at the same time. Farmers' methods used in the experiments include use of detasseling, mass selection, and line selection by farmers with the support from breeders.

### Results of PPB breeding, 2000-2010

The participating farmers (most of whom are women) from the trial villages are encouraged to take part in a range of PPB research activities, including participatory seed selection, adaptation experiments and small-scale seed production. The research already accomplished has shown that, in addition to productivity and market price, farmers' preferences in seed selection also focus on taste, colour, short time to maturity, drought resistance and anti-lodging. These trait preferences are closely associated with the specific ecological and cultural context. The modern varieties selected by farmers have been shown to have adequate adaptation to the local environmental diversity, especially in relation to drought and other stresses. After ten years of collaboration, there are more than 100 newly bred genotypes tested for selection in the trials on-station and in farmers' fields. Five farmer-preferred maize varieties have been selected and released in the trial villages, i.e. *New Mexico 1*, *Guinuo 2006*, *Zhongmo 1*, *Zhongmo 2* and *Guisuzong*. Their breeding and registration information is shown in table 1.

Table 1. Breeding and registration information of PPB varieties in Guangxi

Variety	Year of release	Character	Genetic resources	Registration Status
<i>Xin Mo 1</i>	2002	OPV	locally adapted variety ( <i>Tuxpeño 1</i> from CIMMYT), and farmer maintained landraces	not registered, failed the regional VCU testing in 2003
<i>Zhong Mo 1</i>	2004	OPV	<i>Xin Mo 1</i> and exotic populations	not registered
<i>Zhong Mo 2</i>	2006	OPV	<i>Xin Mo 1</i> and exotic populations	not registered
<i>Guinuo 2006</i>	2001	Hybrid Waxy	waxy maize landraces collected from local villages 20 years ago	officially released and registered by GMRI breeders in 2003, farmers participate small scale on-farm seed production
<i>Guisuzong</i>	2007	OPV	farmer conserved landraces, exotic populations	not registered

Source: project database (2009).

The first PPB variety *Xinmo 1*, an open pollinated variety, failed at the VCU testing stage in 2003 because it did not perform well in all the six regions demanded by the VCU protocols. After *Xinmo 1* denied by formal seed release system, the OPVs are only tested and cultivated in local communities without official release. The hybrid variety *Guinuo 2006* has been officially released, registered and later on commercialized by GMRI breeders since 2003. Among the five PPB varieties, it is also the favourite among farmers and local communities, not only because of its exceptional taste, but also because of its market potential. To share the benefits with PPB farmers, in 2006 one of the trial villages started local seed production of *Guinuo 2006*. At first, the main difficulty for farmers was the lack of knowledge of hybrid seed production, but with the help and technical support from GMRI breeders, they have obtained the basic skills and knowledge and contributed to the local seed supply.



### **Institutional challenges**

However, there are several challenges for PPB activities within the existing institutions.

#### *Barriers from seed regulations*

The existing seed regulations can only recognize and release varieties passing the VCU and DUS testing. Varieties derived from PPB process are unlikely to comply with the variety release criteria (Louwaars, 2007) and can therefore not be marketed. Because varieties or landraces selected by farmers, as the result of the on-going genetic adaptation of the variety to the agro-ecosystem, generally have four distinct features: 1) they are adapted to the local circumstances; 2) they often exhibit a considerable degree of genetic heterogeneity and therefore are more flexible and reactive to changing natural conditions; 3) they are inherently not stable; and 4) they may or may not be regarded distinct from each other (Visser, 2002). Farmer selected variety from low-input conditions is difficult to be measured under favorable conditions, and cannot meet the VCU/DUS requirements that govern current seed regulations. After *Xinmo 1* failed the VCU testing in 2003, the OPVs improved through PPB experimentations are mainly produced and distributed within the trial villages without official release. In recent years, with the increasing reflections on the existing variety testing systems, experiences and practices from other countries have shown the options for PPB varieties.

Option 1: to get VCU out of the registration process and leave quality judgements as a localized process, as the fail of VCU testing always prevents their entry into market. VCU testing as a non-obligatory system can also be used for variety information, instead of restricted registration of new varieties. USA has developed this model for seed quality control, and the seed quality is monitored by market and consumers (Louwaars, 2007).

Option 2: to integrate environmental and other ecological conditions into the testing system, this has been practiced by European organic sectors. Some countries have experimented with different testing protocols, such like Austria has adopted a specific VCU system for organic, other countries, like Germany, are currently elaborating a new policy, while in some countries it is still difficult to put this issue on the agenda (Rey et al., 2008).

Such options have provided opportunities on further acceptance and recognition of varieties adapted to low-input agriculture.

#### *Addressing farmers' rights and their benefit sharing*

Since China entered into the WTO in 2001, it is under pressure to sign UPOV 1991, which restricts (essentially removes entirely) farmers' rights as seed entrepreneurs and breeders i.e. to sell their seeds and to set up seed businesses. There are increasing barriers to farmers' rights to use, exchange and sell their on-farm saved seeds, chiefly in the form of trade pressures and the expansion of the scope of plant breeders' rights. Meanwhile, at the national level, the farmers' rights-oriented legislation lags behind and there is no regulation or institutional arrangement in place to guide how farmers might develop their own seed markets and share the benefits derived from their varieties (such as PPB varieties) with other stakeholders, the breeding institutes take the 'breeders' rights' on the registration and commercialization of the protected variety for granted.

In the case of PPB, even if the variety passes the DUS and VCU testing, the issues on the 'ownership' and 'benefit sharing' will emerge afterward among farmers and breeders.

To address farmers' benefit on PPB products, the project is exploring the mechanisms on benefit sharing at the project level and undertaking an institutional experimentation on *Guinuo 2006*, through supporting small-scale seed production in the trial villages. The breeding institute and the seed company supply the commercial market of *Guinuo 2006*, while allowing PPB farmers supply the seed for local niche market. As local experimentation, such community-based seed production is protected from the plant breeders' right (PBR) and market related issues, in relation to the PBR transferring agreement and payment on protected varieties, the 'commercial line' restriction for non-commercial seed production, and the quality control of farmer produced seed.

The ownership and benefit sharing issues can also be addressed through access and benefit sharing (ABS) mechanisms (such like the contract). To support the fair access and benefit sharing of PPB varieties, an agreement has been established among farmers and GMRI breeders at the project level in 2010. The agreement recognizes the contribution of both PPB farmers and their genetic resources during breeding process, and regulates the way to share the benefit, in terms of the right on naming, sharing ownership (via joint PBR) and

royalties, obtaining subsidies on landrace conservation and experiment risks, and etc. This agreement has created a platform to discuss and negotiate ABS issues derived from PPB processes.

### **Conclusions**

In the past ten years, the PPB project in the southwest China has been dedicated to on-farm variety improvement and local seed provision, through bridging formal breeding institutes and farming communities. However, the conventional crop research system with the centrally defined seed regulations on variety release and market control, has limited the recognition of PPB varieties, and as a result restricted farmers' participation in crop improvement. The further development of PPB calls for the reflection and innovation on current seed regulations, and as well the institutional arrangements on ABS issues among its stakeholders.

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## Participation, utilization and development of genetic resources in the Organic Outdoor Tomato Project

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*Keywords:* Tomato, participation, organic breeding, ex situ, on farm

### Introduction

In Germany tomato is the most important vegetable. Like in many other regions of the world (Foolad et al. 2008) late blight caused by the fungus *Phytophthora infestans* severely impairs organic outdoor cropping as the most resource efficient production method. Since 2003 we have developed a participatory screening and breeding method on a national scale to improve the cooperation between safeguard, selection, and dissemination (Fig. 1). Market gardeners, seed savers, advisors, and scientists work together to ensure that the best use of genetic resources is made in a well targeted program to meet the demand in horticulture. Future genetic resources need to be developed.

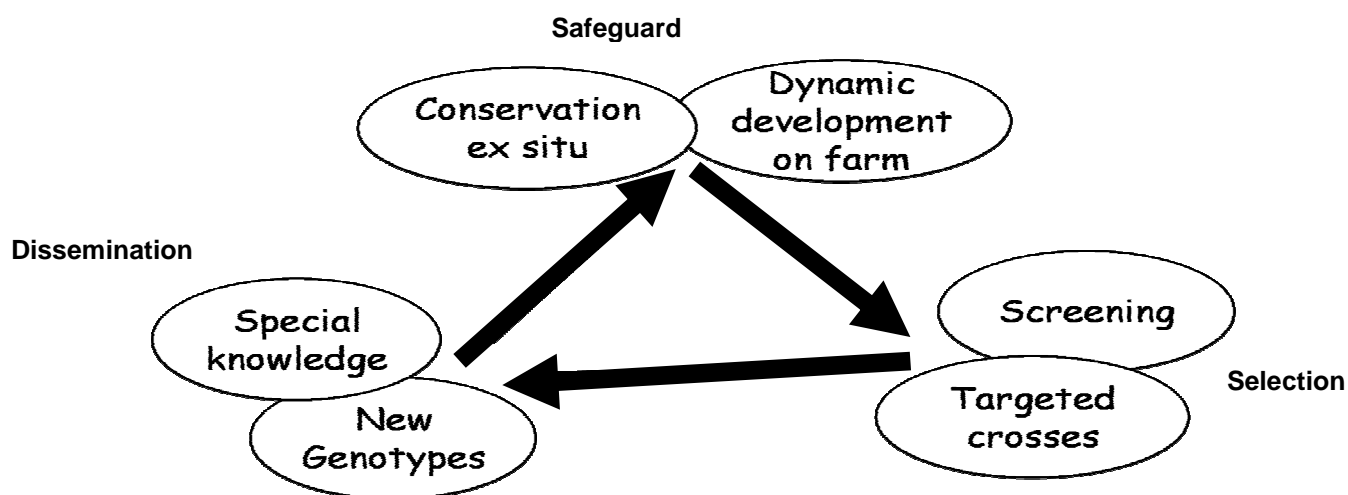


Figure 1: Cooperation between safeguard, selection, and dissemination

### Results and discussion

Interesting genotypes were donated from complementary sources ex situ (genebanks) and on farm / in garden. NGO and private seed savers contributed to the latter approach. Dynamic development in practical horticulture has the potential to select recombinant and adapted genotypes. The outcrossing rate in garden was determined (Fig. 2, Fischer 2008); depending on year and genotype the range covered 0 to more than 4%. Tomato production in Germany is based on app. 10 mio plants in commercial cropping and app. 30 mio plants in amateur gardening (Siebold 2006). An average outcrossing rate of 1% would lead to 400,000 recombinant plants p.a. if exclusively garden saved seeds were used. To avoid undesired genotypes, however, isolation distances or roguing need to be used.

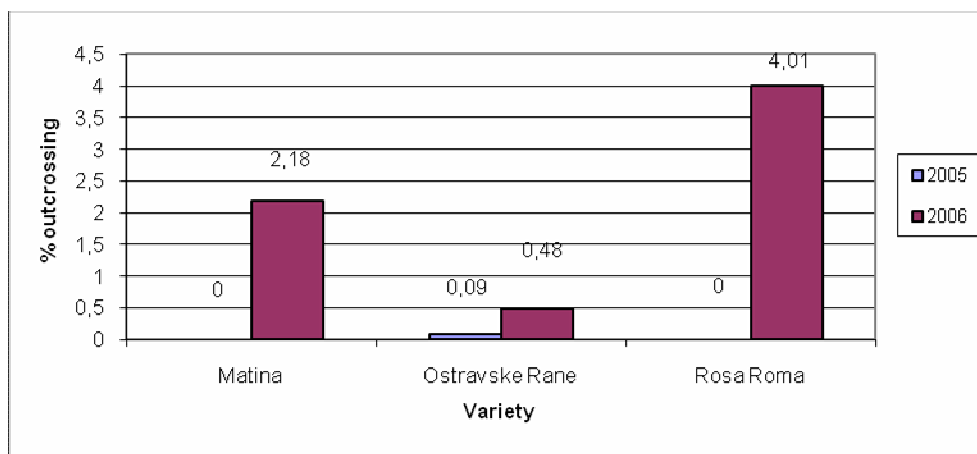


Figure 2: Outcrossing in outdoor tomatoes in Schönhagen 2005 and 2006

Screening and breeding program were carried out at three major locations in organic market gardens. Additionally a smaller number of genotypes was tested at up to 34 locations per year in amateur-, market-, and botanical gardens as well as in research institutions. After three years of evaluation, 88% of the best performing 33 varieties had been provided by non-commercial sources, i.e. genebanks, NGO and private seed savers. More than 60% were originally maintained and recommended by seed savers and NGO within organic horticulture (Fig. 3, Horneburg and Becker 2008). The best open pollinated varieties were made available to amateur gardeners.

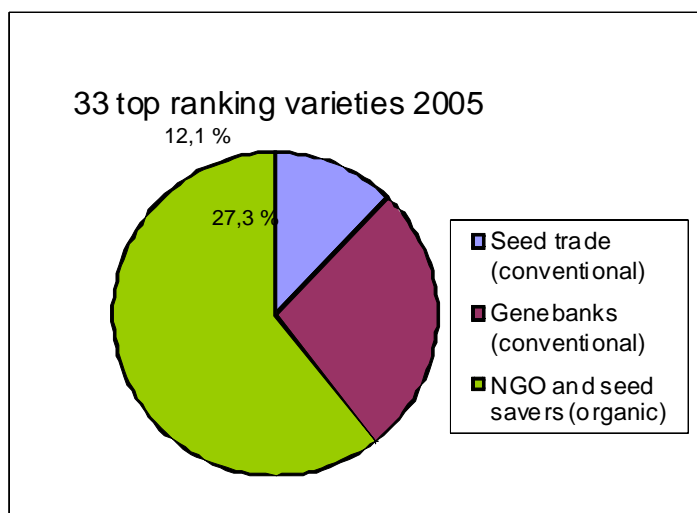


Figure 3 : Origin of the best performing genotypes based on 3,500 accessions after 3 years of screening at 3 locations

In the breeding program we could demonstrate the potential of regional selection. Site specific adaptation for yield was observed for one cross selected for two generations at three locations (Tab. 1, Horneburg and Becker 2008). The selections Schönhagen and Ellingerode yielded best at their site of selection. Relative performance of the selection Rhaudefehn was improved at Rhaudefehn, but was outyielded by the selection Ellingerode. Selection in the breeding program was a collaboration of market gardeners selling tomato fruits, professionals producing young plants, advisors, seed traders, and plant breeders. Important traits and their weighting was discussed. Traits include field resistance, earliness, positive sensory attributes, easy pruning, compact young plants, and other special customers demands. Selection of advanced breeding lines was carried out together. The results have been promising. Presently the first three open pollinated breeding lines are tested for registration and will probably be released in 2011.

Table 1: Influence of the selection site on the yield (g) of Golden Currant x Matina in three market gardens in the F<sub>5</sub> generation

Test site 2006	Selection site			Mean
	Schönhagen	Ellingerode	Rhauderfehn	
Schönhagen	1697	1571	1225	1498
Ellingerode	2475	2920	1884	2426
Rhauderfehn	1019	1095	1037	1050
Mean	1730	1862	1382	

Selected varieties of so called wild tomatoes, genotypes with very small fruits and many side shoots, combine a high level of field resistance with good fruit quality. Special production methods with multiple shoots were developed to increase yield. Compared to plants pruned to one shoot the content of soluble solids, mainly sugars, was increased, too (Fig. 4).

Dissemination of special knowledge for production and marketing, heritage varieties identified in the screening, and new varieties deriving from the breeding program is granted by the persons and organizations involved.

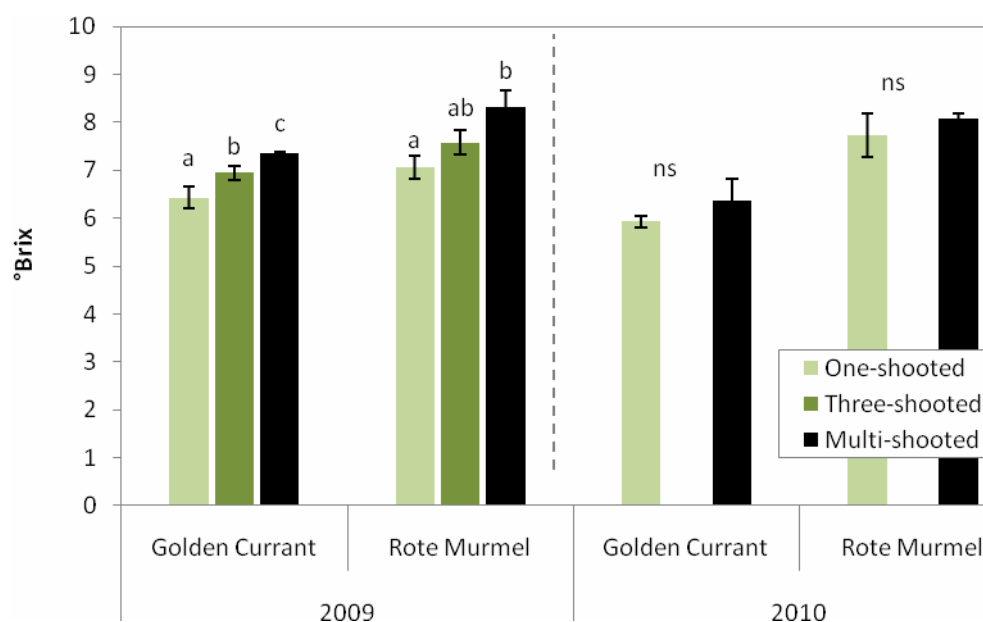


Figure 4: Influence of the production system on total soluble solids content (°Brix) of two varieties of “wild” tomatoes 2009 and 2010 at Göttingen.

Up to this point it has been demonstrated how genetic resources that were donated to the Organic Outdoor Tomato Project could benefit organic and low input horticulture. To serve future needs the continuous conservation and dynamic development of genetic resources needs to be fostered. Ideally advances in breeding are kept available by i) the *ex situ* conservation of varieties removed from the common catalog of registered varieties and ii) dynamic management on farm / in garden. Both processes are severely impaired today. With the advent of hybrid breeding the draining of the gene pool was accelerated. F<sub>1</sub>-varieties are only in exceptional cases stored in genebanks or taken into dynamic development on farm, because of the segregation in the F<sub>2</sub>-generation. The agreement Bundessortenamt – Genebank of the IPK Gatersleben states: “Once a year the Bundessortenamt passes the seed samples of varieties deleted from the national list on to the genebank. (...) This agreement covers all field crops and vegetables except potatoes, hybrid varieties, and inbred lines.” (Translation BH). The genebank of the Centre for Genetic Resources, the Netherlands, does not hold hybrid varieties. In the Netherlands all fruit vegetables produced by the breeding companies since the early eighties are hybrids. As a result we have to face a situation that in crops like tomato for two to three decades the publicly available

genepool has not been replenished. Advances in breeding are increasingly privatized and inbred lines are behind lock and key in breeding companies. Patenting further reduces the use of important traits in the long run. The relevance of genetic resources from non-commercial sources has been demonstrated above. To further illustrate the potential of garden saved seeds figure 5 gives the results of an inquiry among customers of a small organic seed trader in Germany who exclusively offers open pollinated varieties (Allmendinger 2001). 64,3% of the responding gardeners (758 out of 1179) did save seeds of one or more varieties of cultivated plants in their garden. Numbers were much higher than expected despite the fact that the target group was more committed than the average gardener.

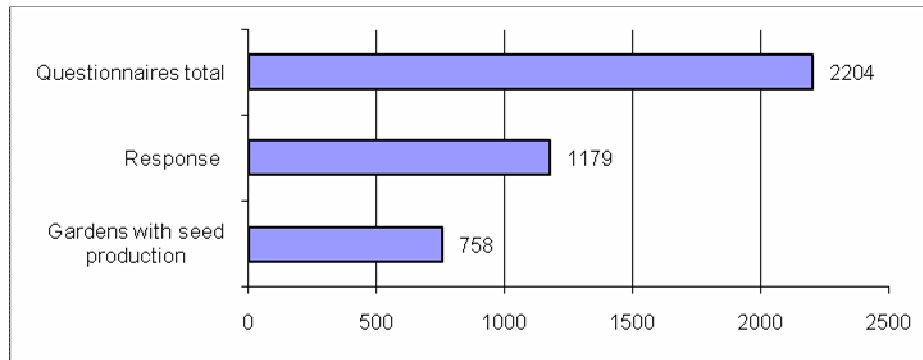


Figure 5: Inquiry among customers of a small organic seed trader

#### Thanks

to all colleagues involved in this long term project, especially I. Smit for quality analysis, and to A. Börner, A. Diederichsen, and W. van Dooijeweert who gave background information from *ex situ* collections.

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## Session 5 - posters

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### 31. Building a regional participatory research programme for wheat breeding and crop management in Brittany, France - example of the PaysBlé project

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*Key-words:* participatory research, organic agriculture, wheat, cultural practices, traditional baking  
Population varieties of local wheat, abandoned for several decades, may become of great value for their environmental, economic and cultural aspects. PaysBlé is a regional project which takes this into account with the overall objective of developing farmers' varieties of wheat from traditional landraces by breeding, crop management and baking experiments on-farm for local markets.

The project is primarily funded by the Regional council of Brittany, France. It extends over 3 years, involves science and society and aims to promote exchanges and dialogue between researchers, socio-economic actors and citizens for sustainable development in the region. The different partners are the seed associations Triptolème and Kaol kozh (from participatory plant breeding (PPB) activities), the organic sector (represented by IBB - Inter Bio Bretagne), INRA (The National Institute for Agricultural Research) and the University of Rennes. The project works with farmers practicing several types of organic farming in the region, associating participatory plant breeding with agronomical improvements to determine the best technical conditions for the cultivation and development of farmers' wheat varieties.

PaysBlé is divided in 3 steps, first building the experimental design to answer both the practical questions of the farmers and to stimulate scientific knowledge for the conservation of cultivated biodiversity. One year was needed to organize the participatory experiments which will:

- take into account the crop management innovations wished by the farmers
- enable the breeding activities of two kinds of species: wheat and associated species (legumes, false flax)
- evaluate breeding and cultural impacts on wheat quality through baking trials.

Three kinds of trials are organized in the framework of the farms, according to the ability of the farmers to manage trials on farm:

- Experimentation of diverse types of bread wheat varieties (one modern variety, one mixture of traditional population varieties, one variety from this mixture) and one mixture of durum wheat, in contrasted environments with various crop management practices (different tillage practices, with or without association with legumes). The aim of these trials is to explore the effects of diversity from grain to bread (plot sizes of about 200 m<sup>2</sup>)
- Participatory plant breeding experiments: selection among and within varieties, creation of mixtures (small plots)
- Co-breeding experimentation: small plots of wheat and different associated species in order to find interesting combinations.

This type of project aims to enhance the coherence of organic agriculture by increasing biodiversity, improving the technical performance of varieties on organic farms, supporting the evolution of the farming systems, promoting local marketing and the supply of high quality food, and increasing fairness in the seed supply system.



## 32. How to implement organoleptic quality assessment in participatory plant breeding programmes ?

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Sensory quality is a great expectation from consumers and more specifically from the new organic consumer (post food crisis), more interested in the nutritional aspect than the environmental one (Zanoli et al.). Yet, this criterion is rarely taken into account in the breeding process because of the inherent difficulty of the human perception analyses.

Whereas some objectives quality criteria can be measured by instrument (firmness, maturity, acidity...), sensory quality measurement need to analyze human perception or/and consumer's expectation. Those criteria are hardly quantifiable, linked to the consumer subjectivity in case of consumer study and in constant evolution (season effect) (Raynaud, E., 2010). In the context of the European project SOLIBAM, a new methodology is experimented to integrate gustatory criterion in the breeding process and particularly in participatory plant breeding programmes. This methodology called "ordering test" involves a panel of semi-naïve (consumer initiated to sensory analyses), who have to compare several products simultaneously by ordering them according to the perceived intensity of a given sensory characteristic (acidity, fibrousness...).

For example, knowing that the consumer shows two preferences profile on tomatoes' texture, those who preferred the meltiness and those who preferred the firmness (Causse et al., 2009), a panel composed of 7 judges had classified four tomatoes' variety (ABCD) on the "texture" attribute.

The results analyses using a Friedman test on rank allow the settle of varieties classification: the variety A is the more melting, then come the variety B. Nevertheless, we cannot conclude on the varieties C and D, the subject number is probably insufficient (the ISO 85 87 norm recommend 12 subjects). Then, it is possible to choose the varieties which best fit consumer's expectations.

varieties/subject	A	B	C	D
1	1	2	3	4
2	2	1	3	4
3	2	1	4	3
4	1	4	2	3
5	1	3	4	2
6	1	2	4	3
7	1	4	3	2
barycentre	1.3	2.4	3.3	3.0

At the end of the breeding process, once varieties have been selected, the methodology will be validated, on one hand by experts in order to highlight the existence of a breeding effect on organoleptic quality, on other hand, by consumers to define whether the varieties are better accepted by the consumer or not. Those steps are detailed in technical guides who will be soon published by ITAB and its Solibam Partners.

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### 33. Participatory Plant Breeding to Improve Sweet Corn for Organic Farmers

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**Keywords:** participatory breeding, organic, sweet corn, recurrent selection

This project is a collaboration among farmers, university researchers, and experts at non-profit organizations to develop high quality sweet corn germplasm for organic agricultural systems. Organic growers face unique challenges when raising sweet corn, and benefit from varieties that exhibit superior cold germination, early vigor, and disease and insect resistance. We are working with experienced organic farmers to breed supersweet and sugary-enhancer sweet corn populations that meet their particular needs. Using a recurrent selection program, the populations are being grown on two organic farms as single row plots. The selection of rows within each population is made based on the farmer's evaluation of the traits of interest. Remnant seed from the same ears that planted the selected rows are planted in a winter nursery and inter-mated to form the next cycle of selection. Selection began in 2008 for the sugary-enhancer population, and in 2010 for the supersweet population. The cycles of selection will continue for three years. We hypothesize that breeding with farmer input during every cycle of selection results in varieties that are specifically adapted to the farmer's needs. In addition, varieties that have been selected under organic conditions will contain traits that benefit organic systems, such as cold soil germination, early vigor and pest resistance. Our objective is to develop high quality sweet corn varieties that meet the specific needs of each participating farmer.



### 34. Conservation and development by participatory plant breeding and dynamic on-farm management of adapted resources of bread wheat for low-input and organic agriculture.

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**Key words:** bread wheat, participatory plant breeding, biodiversity conservation, organic agriculture

Development of varieties for organic agriculture and conservation of biodiversity are two major objectives in the context of environmental degradation due to intensive agricultural practices and predicted impact of climate change on agricultural systems. In this context, the research team works to understand how human selection and the other evolutionary forces shape population genetic variability. The desired goal is to understand these effects and to develop optimal strategies for preserving genetic diversity through on-farm dynamic management and plant breeding. Our team is working on bread wheat with participatory approaches in order to study those issues and look for a methodology to create, from the collaboration of farmers and researchers, varieties that are adapted to organic farming conditions and which conserve the biodiversity present in cultivated species. We aim to better understand: (i) variation of trait expression in contrasted environments under organic management, by taking into account the interactions with the environment; and (ii) impacts of exchange, selection, agronomic techniques related to evolutionary forces on important traits in order to select populations adapted to a specific local environment and stable over time.

Populations from crosses of landraces were created in 2005 on a farm in southwestern France. Selections within these new populations were made by a farmer in the F2 generation, i.e. while the segregation is not complete. We have shown that some traits responded to selection even in this early generation, including vegetative traits visible in the field such as plant height and spike traits such as grain weight per spike and thousand kernel weight for example. In collaboration with the farmers' network "Réseau Semences Paysannes", the F3 and F4 generations of these populations were also grown in several farms in France from 2008-2010, under unique agro-environmental conditions and farmer management. We aim to analyse the adaptation of those populations and the effects of farmer selection as well as the other evolutive forces (drift, migration, natural selection and mutation), taking into account intra-population variability and interactions between these populations and the specific on-farm environments.



## 35.PPBINGO: A user-friendly database for Participatory Plant Breeding programs

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*Keywords:* PPB • Database • Statistical analyses • Open source • GxE

In a decentralized and participatory plant breeding (PPB) program, in contrast to conventional plant breeding, farmers select from a large number of genotypes over a number of cycles in several locations. As selection is for location-specific adaptation a PPB program based on, for example, four stages of selection, generates large, unbalanced data sets. With common spreadsheet software it is not possible, or it is at least very cumbersome to extract all the information, particularly the one that is related to genotype x environment (GE) interactions. Therefore, there is a need for an information system that makes it as easy as possible to retrieve and compare data over all given

factors in the PPB-program. Due to a lack of education and financial resources in developing countries, the use of existing databases was not an option because of their complexity and/or price. We have built a simple, free and user-friendly database that allows to store, retrieve and analyze the plant breeding data. The database is implemented using the Java based HyperSQL Database Engine (HSQLDB) in the package of Open Office with links to the input formats of Genstat and GGEbiPlot. The proposed database is capable of managing the following data in a PPB program for barley: (I) trial data for any trial or field design (II) field data for any given trait (III) results from statistical analyses (IV) farmers selection individually or in groups.

## 36.Sustainable farming in cross-border region of Czech Republic and Austria (SUFA) - Growing and using of hulled wheats

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*Keywords:* Agronomy, genetic resources, quality, organic farming, Triticum dicoccum, T. monococcum, T. spelta

The Czech-Austrian project SUFA is coordinated by the University of South Bohemia, České Budějovice, that cooperates with the Association of Organic Farming Consultants (EPOS), Brno, and the Vocational School for Agriculture Edelhof, Zwettl. The project's aim is to support the development of sustainable farming on arable land on both sides of the Czech-Austrian border via a mutual exchange of information and experience in farming, processing and marketing of underutilized wheat species, i.e. einkorn wheat, emmer wheat and spelt wheat. The target groups of the project are farmers, consultants, processors and consumers of hulled wheat. Growing methods are elaborated and adapted genotypes are selected and multiplied. Moreover, seminars and field excursions are organised during the period of the project. All information and events concerning the project are published on the project's webpage (<http://sufa.zf.jcu.cz>).

Whilst some experience was gained in the last decade with autumn sown hulled wheats, almost no information is available on hulled wheats of spring growth habit. In Czech pre-trials genebank accessions of einkorn, emmer and spelt wheat with spring growth habit were selected for the project. These genotypes are grown along with old common wheat varieties of alternative growth habit and modern spring wheat varieties in organic field trials at four locations in the Czech Republic and Austria. A multitude of morphological, agronomic and quality traits will be determined and first results will be presented.

## 37. Participatory plant breeding in Denmark

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*Keywords:* participatory plant breeding

Plant breeding gets more and more concentrated on a couple of multinational companies, and financing plant breeding via the traditional royalty founded certification system exclusive for the specific needs in organic farming is not profitable in most field crops. The seed certification system only allows pure line varieties, and the royalty funded breeding system tend to focus on monogenic resistance with a short durability on the market. To develop new plant genetic material for organic farmers with durable stability and resistance, the Danish Organic Farmers Association has initiated a participatory plant breeding program with the aim to develop varieties and diverse populations for the organic farmers. The project is based within the advisory service in the organisation in cooperation with plant breeding research projects. In this way, it is the hope to overcome the economic and legal barrier for implementation of crop diversity and targeted selection for the different needs in the diverse organic sector.