



# **PROCEEDINGS OF INTERNATIONAL CONGRESS ON OIL AND PROTEIN CROPS**

**2-4 NOVEMBER, 2023**

**ANTALYA, TURKEY**

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INTERNATIONAL  
CONGRESS ON OIL AND  
PROTEIN CROPS**

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**Organized by  
Trakya University  
European Association for Research on Plant  
Breeding (EUCARPIA)  
International Researchers Association**

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## WELCOME NOTES

International Congress Oil and Protein Crops Section Conference of EUCARPIA which is organized by Trakya University and the International Researchers Association in cooperation with the European Association for Research on Plant Breeding (EUCARPIA). The congress is held in Megasaray Westbeach Hotel, Antalya, Turkey, on November 2-4, 2023 with supporting of several national and international partners.

The Congress topics covers Oil and Protein Crops: Plant Breeding and Genetics, Molecular Genetics and Biotechnology, Biology and Physiology, Genetic Resources, Plant Protection, Agronomy, Economy, Animal feeding, Food Science and Nutrients, Fats, lipids, and Protein studies.

Oil crops are rich sources of oils, proteins, minerals, vitamins, and dietary fibers for both human and animal feeding and provide the raw material for the production of biodiesel. Oil crops are soybean, cottonseed, sunflower, canola, rapeseed, peanut, safflower, flax, sesame, coconut, castor, copra, etc.

Almost 50% of the global food protein supply comes from cereal seeds. Soybean, peanut, common bean, pea, lupine, chickpea, faba bean, lentil, grass pea, cowpea, pigeon pea, etc. are currently the most important legumes for human consumption and animal feed. Because of the protein content of their seeds; grain legumes, cereals, and other minor crops such as amaranth, quinoa, hemp, caraway, etc. are protein crops growing for plant protein for food and feed.

The Congress is intended that the subjects to be kept broad in order to provide opportunity to the science and research community to present their works as oral or poster presentations. The Congress languages is in English. Researchers, breeders and others with an interest in the genetics and breeding of oil and protein crops are invited to participate. Among the topics to be discussed are directions of breeding for resistance to abiotic and biotic stresses, improved industrial use, and conventional versus organic production.

As there have been many different scientific meetings around the world, we aimed to bring three different communities together, namely science, research and private investment groups considering practical information sharing that is of value for breeders, seed enterprises, researchers and scientists, in a friendly environment of Antalya, Turkey to share their knowledge and experience and benefit from each other.

There are 38 orals and 63 poster presentation in the congress both joining and presenting normal and online with 141 participants from 20 different countries from the world.

The congress gathered scientists from around the world, and present their recent achievements. The organizers will also invite relevant stakeholders to provide a view on the current situation around the world as well as prospects to overcome the limitation for sustainable crop production to feed the world.

We would like to thank all of you for joining this conference and we would like to give also special thanks to our sponsors and collaborators for giving us a big support to organize this event.

Prof Dr Yalcin KAYA  
Head of the Organizing Committee

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## PREPARATION OF CuO-TiO<sub>2</sub> BINARY NANOCOMPOSITES FOR THE SUPERIOR PHOTOCATALYTIC DEGRADATION OF RHODAMINE B: MORPHOLOGICAL AND STRUCTURAL PROPERTIES

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### ABSTRACT

Rhodamine B (RhB) belongs to the xanthene dye family that is commonly used in textiles and various applications, including photosensitizers, paper dyeing, and laser dye production, as well as biological staining and a fluorescent marker. There are examples of toxic cationic dyes, which are carcinogenic and mutagenic with high potential antagonistic effects on all living organisms. Consequently, the elimination of hazardous RhB is paramount before discharging into water streams to protect the environment and ecosystem. Semiconductor photocatalysis as a wastewater treatment technology may be promising for RhB high-efficiency removal. The coupling of semiconductors between p-type CuO and n-type TiO<sub>2</sub> semiconductors is expected to produce effective photoactive particles with improved properties. The p-n heterojunctions formed can lead to enhanced degradation of organic pollutants that persist in the environment.

The study focused on the preparation, characterization, and photocatalytic evaluation of CuO-TiO<sub>2</sub> binary nanocomposites in the photo-assisted degradation of RhB under UV irradiation. CuO-TiO<sub>2</sub> binary nanocomposites were synthesized via a simple solid-state dispersion method. The structure and surface morphology of CuO-TiO<sub>2</sub> binary nanocomposites were determined using FTIR, XRD, SEM, and Raman spectroscopy. SEM images revealed the morphologies of both CuO and TiO<sub>2</sub> catalysts. XRD diffractogram of CuO-TiO<sub>2</sub> binary nanocomposites clarified the characteristic monoclinic structure of the CuO phase and the anatase and rutile phases of TiO<sub>2</sub>. The results could be beneficial for designing CuO-TiO<sub>2</sub> binary nanocomposites with superior photocatalytic degradation properties in wastewater management technologies.

**Keywords:** Binary nanocomposites, CuO-TiO<sub>2</sub>, decolorization, Rhodamine B, semiconductor photocatalysis.

### INTRODUCTION

RhB is a well-known member of the xanthene family dye, displaying a high molar absorptivity, tunable fluorescence, and high photostability. These properties make RhB usable for tracers in chemical and biological applications. Moreover, RhB is a cationic dye with a high water solubility that is widely used in the printing and textile industry, as well as in animal medicines, staining of biological trials, photosensitizers, and lasers (Al-Gheethi et al., 2022; Bar and Chowdhury, 2022; Li et al., 2023). This toxic cationic dye can be carcinogenic and mutagenic with high potential antagonistic results on all living organisms (Li et al., 2023). The untreated discharge of RhB can cause health problems to humans and animals, since it is classified as a carcinogenic and neurotoxic dye. Therefore, the efficient treatment of wastewater containing RhB is an environmental challenge facing the world today (Al-Buriahi et al., 2022; Al-Gheethi et al., 2022; Amalina et al., 2022).

Semiconductor photocatalysis is a promising water treatment process due to its easy handling, good reproducibility, simplicity, high efficiency, environmentally friendly, non-toxic, and cost-effective properties. In this technique, oxidizing species such as highly oxidized hydroxyl radicals are formed, and these radicals completely mineralize the hazardous and persistent pollutants into CO<sub>2</sub> and H<sub>2</sub>O (Durodola et al., 2023; Jabbar and Graimed, 2022; Solayman et al., 2023).

Several catalyst development studies have focused on using a variety of binary oxides to improve visible light absorption capability, charge separation, and transportation (Raizada et al., 2020; Turkten and Bekbolet, 2020). Recently, Hamad et al. synthesized a binary system of S-scheme CuO@TiO<sub>2</sub> heterojunction nanocomposite for the efficient degradation of acid red 8 dye (Hamad et al., 2022). Likewise, another binary TiO<sub>2</sub>-CuO system was reported as exhibiting a high photocatalytic activity in the degradation of tetracycline (Kubiak et al., 2020). Moreover, various organic pollutants and dyes such as acid yellow 36 (Wang et al., 2023), methylene blue (Li et al., 2008), methyl orange and cyanide (Koohestani and Sadrnezhaad, 2016) were used for the photocatalytic activity testing of CuO-TiO<sub>2</sub> binary nanocomposites.

In the present study, CuO-TiO<sub>2</sub> binary nanocomposite was prepared by using a facile mechanical mixing method. Fourier transform infrared spectrometer (FTIR) used with attenuated total reflection (ATR), Raman spectroscopy, X-ray diffraction (XRD), and Scanning electron microscopy (SEM) spectroscopic techniques were used to identify structural and morphological features of CuO-TiO<sub>2</sub> nanoparticles. RhB was employed as a representative dye to evaluate the photocatalytic activity of CuO-TiO<sub>2</sub> binary nanocomposite.

## MATERIAL AND METHOD

Rh B (C<sub>28</sub>H<sub>31</sub>ClN<sub>2</sub>O<sub>3</sub>) was obtained from Merck. TiO<sub>2</sub> P-25 (Evonik) and CuO (Thermo Scientific, ACS) were used to prepare CuO-TiO<sub>2</sub> binary nanocomposite. All aqueous solutions were prepared with distilled water. The chemical structure and properties of RhB cationic dye were given in Figure 1.

CuO-TiO<sub>2</sub> binary nanocomposite was prepared by mechanical mixing method with weight ratio of CuO/TiO<sub>2</sub>:1/1. Briefly, CuO (1 g) and TiO<sub>2</sub> (1 g) oxides were mixed in ethanol with mortar for 15 min and were sonicated in an ultrasonic bath for 30 min. Finally, CuO-TiO<sub>2</sub> binary oxide was dried in an air oven at 80 °C for 24 h, calcined in a muffle furnace at 500 °C for 3h, and grinded (Mohammadi et al., 2016).

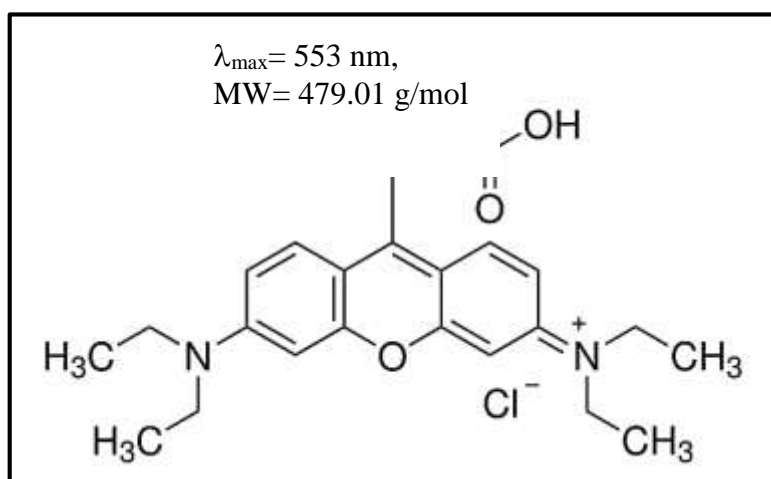


Figure 1. The chemical structure of RhB.

FTIR spectroscopy was acquired by using Thermo Scientific Nicolet 6700 spectrometer equipped with an attenuated total reflection accessory. Dispersive Raman spectroscopic measurement was performed on a Thermo Scientific DXR Raman Microscope with an application of an Ar<sup>+</sup> laser power (10 mW,  $\lambda = 532 \text{ nm}$ ). XRD diffractogram was carried out by a Rigaku-D/MAX-Ultima diffractometer with Cu K $\alpha$  radiation ( $\lambda = 1.54 \text{ \AA}$ ) as X-ray source. SEM analysis was performed on FEI-Philips XL30 Scanning Electron Microscope with an accelerating voltage of 10 kV.

The photocatalytic activity experiments were carried out in a cylindrical Pyrex reaction vessel irradiated from the top. A 125W black light fluorescent lamp ( $\lambda_{\max} = 365 \text{ nm}$ ) was used as the light source. The photocatalytic activity tests were performed without pH adjustment in 50 mL of RhB (10



mg/L) upon using a 0.25 g/L catalyst dose. The irradiated solution was immediately filtered through 0.22  $\mu\text{m}$  cellulose acetate filters. The absorbance values of the samples were monitored by a Thermo Scientific Genesys 10S double beam spectrophotometer using 1 cm quartz cells.

## RESULTS AND DISCUSSION

FTIR spectroscopy was used to verify the functional groups of the synthesized CuO-TiO<sub>2</sub> binary nanocomposite, and the spectrum was shown in Figure 2. The observed peaks at 420 cm<sup>-1</sup>, 482 cm<sup>-1</sup>, 523 cm<sup>-1</sup>, and 596 cm<sup>-1</sup> were attributed to the characteristic Cu-O stretching vibration modes (Islam et al., 2021). The peaks observed below 800 cm<sup>-1</sup> could be related to the stretching mode of Ti-O ( $\nu_{\text{Ti-O}}$ ) that was the envelope of the phonon peaks of a Ti-O-Ti bond of the TiO<sub>2</sub> network (Kanna and Wongnawa, 2008).

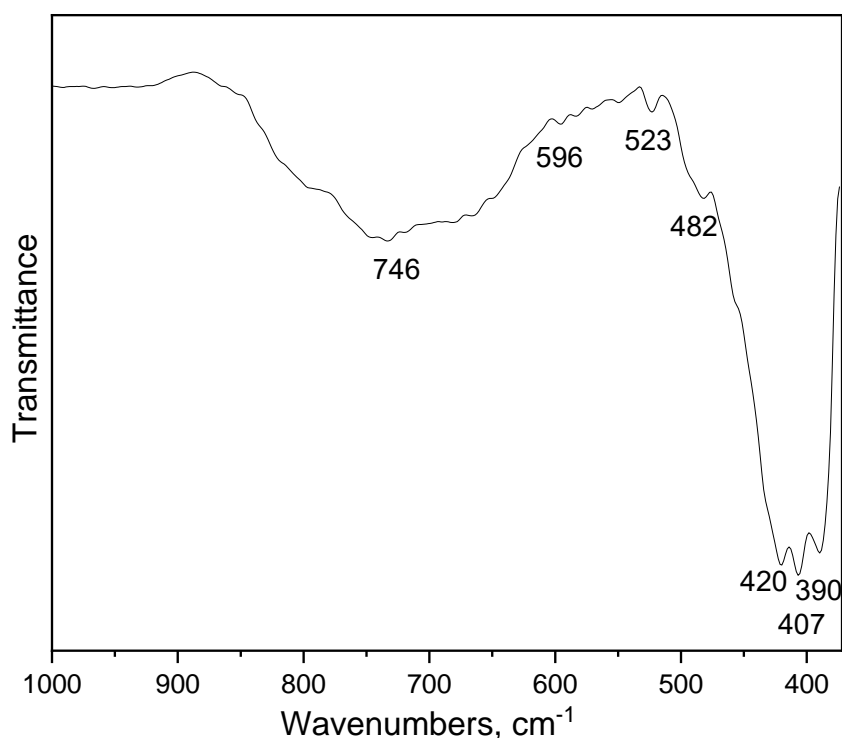


Figure 2. FTIR spectrum of CuO-TiO<sub>2</sub> binary nanocomposite.

The Raman spectrum of CuO-TiO<sub>2</sub> binary nanocomposite showed main bands as seen in Figure 3. The band centered at 637 cm<sup>-1</sup> corresponded to the B<sub>g2</sub> mode of CuO, while the bands located at 508 cm<sup>-1</sup> was attributed to the characteristic A<sub>1g</sub> anatase band of TiO<sub>2</sub> (Islam et al., 2021; Ohsaka et al., 1978).

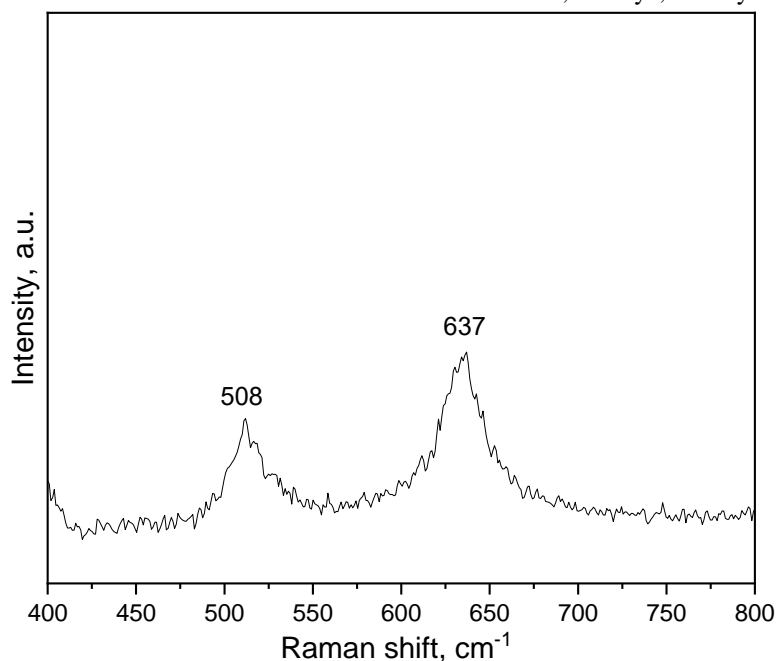


Figure 3. Raman spectra of CuO-TiO<sub>2</sub> binary nanocomposite.

The XRD spectrum of CuO-TiO<sub>2</sub> binary nanocomposite was presented in Figure 4. The diffractogram of CuO-TiO<sub>2</sub> exhibited the characteristic peaks at  $2\theta = 25.36^\circ, 37.89^\circ, 48.08^\circ, 53.98^\circ, 55.12^\circ, 62.82^\circ, 69.09^\circ,$  and  $70.38^\circ$  were attributed to (1 0 1), (0 0 4), (2 0 0), (1 0 5), (2 1 1), (0 0 2), (1 1 6), and (2 2 0) planes of anatase, while peaks at  $2\theta = 27.52^\circ, 41.34^\circ$  corresponded to (1 1 0), and (1 1 1) planes of rutile, respectively. The crystallite phases of TiO<sub>2</sub> were presented by anatase (JCPDS No. 73-1764) and rutile (JCPDS No. 99-0090) crystal structures.

A series of characteristic peaks of monoclinic CuO located at  $2\theta = 32.54^\circ, 35.56^\circ, 38.72^\circ, 48.80^\circ, 53.36^\circ, 58.32^\circ, 61.56^\circ, 65.84^\circ, 66.30^\circ, 68.12^\circ, 72.44^\circ,$  and  $75.02^\circ$  corresponded to the (1 1 0), (-1 1 1), (1 1 1) (-2 0 2), (0 2 0), (2 0 2), (-1 1 3), (0 2 2), (-3 1 1), (2 2 0), (3 1 1), and (0 0 4) planes. CuO data was in accordance with the standard (JCPDS card no. 89-5895).

SEM images of TiO<sub>2</sub>, CuO, and CuO-TiO<sub>2</sub> binary nanocomposite were presented in Figure 5. TiO<sub>2</sub> nanoparticles (Figure 5 (a)) consisted of almost spherical shapes with a slight agglomeration while SEM image of CuO revealed a variety polyhedral-shaped particles (Figure 5 (b)). The presence of spherical and polyhedral-shaped particles indicated that CuO-TiO<sub>2</sub> binary nanocomposite exhibited both morphologies of TiO<sub>2</sub> and CuO (Figure 5 (c)).

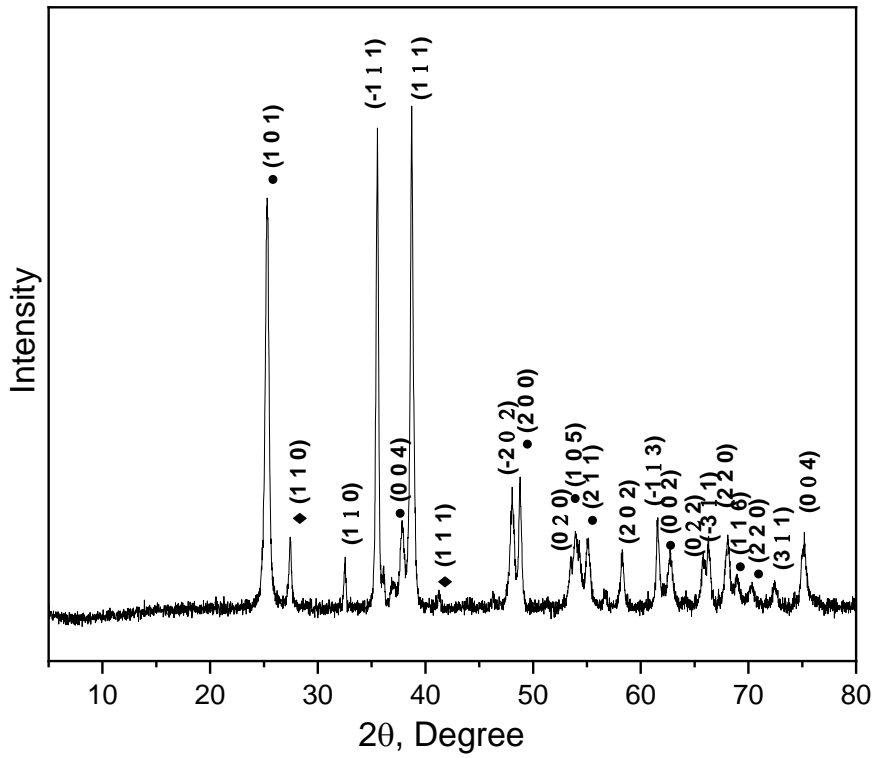


Figure 4. XRD spectrum of CuO-TiO<sub>2</sub> binary nanocomposite (• anatase, ◆ rutile).

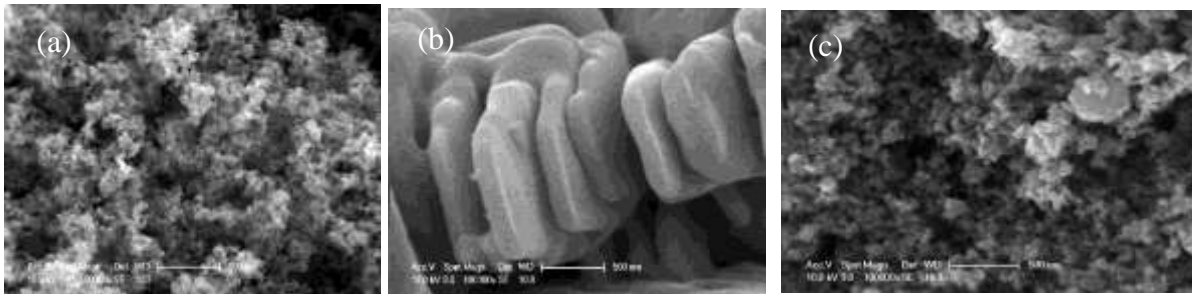


Figure 5. SEM images of (a) TiO<sub>2</sub>, (b) CuO, and (c) CuO-TiO<sub>2</sub>.

The degree of RhB decolorization by using CuO-TiO<sub>2</sub> nanoparticles (Figure 6) was calculated by the following equation (1).

$$\text{Decolorization, \%} = ((A_{0} - A)/A_{0}) \times 100 \quad (1)$$

where,

$A_{0}$  = initial absorbance of RhB and  $A$  = absorbance of RhB at irradiation time  $t$ .

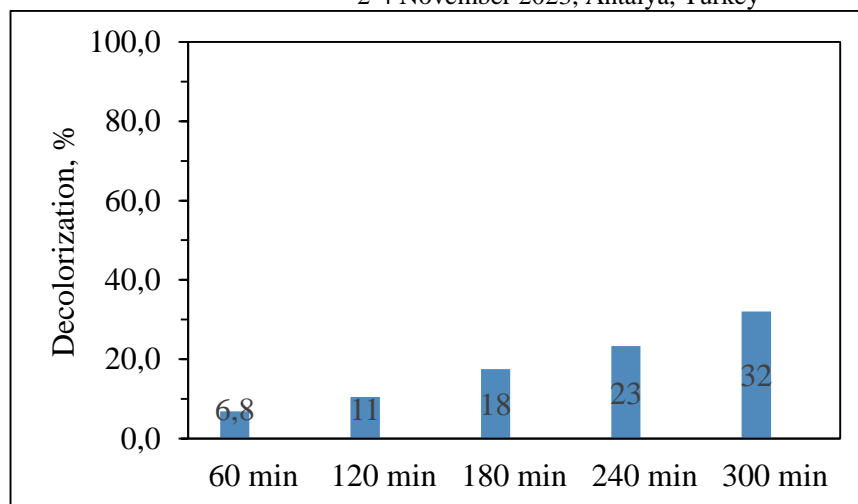


Figure 6. Removal efficiency of RhB upon using CuO-TiO<sub>2</sub> nanoparticles.

The removal efficiency of RhB in the presence of CuO-TiO<sub>2</sub> nanoparticles was found to be 32% after exposure light irradiation for 300 min.

## CONCLUSIONS

In summary, CuO-TiO<sub>2</sub> nanoparticles were successfully synthesized via a mechanical mixing method. FTIR spectrum of CuO-TiO<sub>2</sub> binary nanocomposite confirmed the presence of characteristic peaks of CuO and TiO<sub>2</sub> in the structure of the composite. XRD results indicated evidence of anatase and rutile planes of TiO<sub>2</sub> and the monoclinic structure of CuO. The surface morphology feature of CuO-TiO<sub>2</sub> binary nanocomposite consisted of both TiO<sub>2</sub> and CuO particles. The photocatalytic activity of CuO-TiO<sub>2</sub> binary nanocomposite was investigated on the degradation of RhB dye. The removal percentage of degradation of RhB in the presence of CuO-TiO<sub>2</sub> nanoparticles was 32% upon 300 min irradiation.

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## RECENT ADVANCES IN THE USE AGRICULTURAL-BASED MATERIALS FOR WASTEWATER TREATMENT

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### ABSTRACT

Nowadays, with the rapid growth of industrialization and agriculture, pollution of surface and ground water resources has become a global issue to address. Therefore, value-added and inexpensive materials using either agricultural or industrial wastes have become common and feasible alternatives in wastewater treatment. The application of adsorbents and biochar-based photocatalysts utilizing waste products of the agricultural sector, mostly sugarcane bagasse, rice husk, and coconut husk, has received considerable interest in eliminating recalcitrant pollutants from water. The preparation of biochar-based TiO<sub>2</sub> and ZnO photocatalysts from agricultural by-products not only promotes their higher degradation efficiency of organic compounds, but also promotes a high eco-efficiency. Moreover, these photocatalysts open a new path for further developments to overcome the limitations of TiO<sub>2</sub> and ZnO, which lower the application performance due to the reduction of the band energy gap and the recombination of electron/hole pairs.

**Keywords:** Agricultural-based materials, agricultural wastes, biochars, photocatalysis, water treatment.

### INTRODUCTION

The rapid increase in agricultural waste, population, and industrialization has led to severe water pollution worldwide (Noor and Khan, 2023). Traditional methods mostly used for the removal of pollutants from aqueous phase are biological treatment, flocculation, membrane separation processes, chemical precipitation, adsorption, and ion exchange. Among these numerous wastewater treatment techniques, adsorption is considered to be an effective and cost-effective method for the elimination of organic contaminants (Dai et al., 2018; Rosales et al., 2017). Recently, studies have focused on agricultural waste-based adsorbents because of their natural, renewable, environmentally friendly, and cost-effective properties (Yahya et al., 2018; Younas et al., 2023).

Vvarious organic waste peels of fruits and vegetables derived from agricultural or food industry may be promising as alternative biosorbents to commercial activated carbon due to their availability and efficiency (Akpomie and Conradie, 2020; Younas et al., 2023). To date, a wide variety of agricultural waste peels, such as grapefruit, ash gourd, banana, cucumber, and potato have been adopted as low-cost biosorbents for the treatment of pollutants (Saeed et al., 2010; Sreenivas et al., 2014; Stavrinou et al., 2018). Zou et al. have studied grapefruit peel as a biomass material on the adsorption of uranium(VI) from aqueous solution (Zou et al., 2012). Ash gourd peel as a vegetable-based waste has been used in biosorption of chromium (Sreenivas et al., 2014). The feasibility of using banana peel as an adsorbent in the removal of dyes (congo red) (Mondal and Kar, 2018), pesticides (atrazine and ametryne) (Silva et al., 2013), heavy metals (Pb(II), Cu, and Cd(II)) (Anwar et al., 2010; Vilaridi et al., 2018), fluorides, (Mondal, 2017), phenolic compounds (Achak et al., 2009; Mishra et al., 2022), oil spill (Alaa El-Din et al., 2018) from water has been reported.

Nowadays, numerous researchers have investigated the production of agricultural waste material, which mainly consists of cellulose, hemicellulose, lignin, and extractables (Othmani et al., 2022; Zhou et al., 2015). Coconut husk (Foo and Hameed, 2012), coconut shell (Zhu and Kolar,

2016), pistachio nut shells (Foo and Hameed, 2011), rice husk (Shamsollahi and Partovinia, 2019), sugarcane bagasse (Garg et al., 2008a; Garg et al., 2008b) are attractive agricultural waste materials used in wastewater treatment as the potential removal of various pollutants. However, the utilization of these materials as adsorbents for the elimination of recalcitrant pollutants is still not efficient. Therefore, photocatalysis is an effective method to achieve complete degradation or mineralization potential (Sutar et al., 2022).

### **BIOCHAR-BASED PHOTOCATALYSTS**

In wastewater treatment, photocatalysis can be applied under light irradiation at appropriate wavelengths and with proper catalysts. In a typical photocatalytic mechanism, light absorption by metal oxide semiconductors such as TiO<sub>2</sub>, ZnO, CeO<sub>2</sub>, and WO<sub>3</sub> leads to the generation of electron-hole pairs. Photogenerated electron hole pairs produce hydroxyl and superoxide anion radicals (Kumar et al., 2020; Ramalingam et al., 2022).

Agricultural residues are significant potential source of biochar-based catalysts (Sutar et al., 2022). TiO<sub>2</sub>-biochar composite has been prepared using coconut for the degradation of Reactive Brilliant Blue KN-R dye (Zhang and Lu, 2018). Lu et al. obtained biochar from pyrolysis of waste walnut shells to synthesize a series of TiO<sub>2</sub>-biochar composites. In another application, a high photocatalytic performance was reported for the photocatalytic degradation of methyl orange (Lu et al., 2019). Moreover, numerous studies have demonstrated the potential usage of ZnO-biochar in water treatment (Sutar et al., 2022). Leichtweis et al. prepared biochar derived from pecan nutshell with ZnO composites for the removal of acid red 97 from aqueous solution (Leichtweis et al., 2020). ZnO-biochar composites were produced with three different biomass (*Salvinia molesta*, sugarcane bagasse, and exhausted black wattle bark) and high photocatalytic degradation efficiency was reported on the degradation of sulfamethoxazole antibiotic and methyl orange dye (Gonçalves et al., 2020).

### **BIOTEMPLATE PHOTOCATALYSTS**

In recent years, TiO<sub>2</sub> materials using biotemplates such as butterfly wings (Yu et al., 2016), cellulosic cotton (Li et al., 2021), leaves (Gesesse et al., 2019; Li et al., 2009; Yan et al., 2017), leaf extracts (Goutam et al., 2018), pollens (Bu and Zhuang, 2013; Dou et al., 2012; He et al., 2013), rice husk (de Cordoba et al., 2019; Hui et al., 2015; Turkten et al., 2023), *Staphylococcus aureus* (He et al., 2014), starch (Tang et al., 2008), tobacco stem silk (Li et al., 2023), yeast (Chen et al., 2010) have been synthesized to improve the photocatalytic activity of TiO<sub>2</sub>. Thus, extraordinary morphologies and properties of these templates are often found in the designed materials. Additionally, the use of agricultural wastes such as rice husks can be an alternative route to reduce environmental pollution by producing a value-added product for water treatment (Turkten et al., 2023).

### **CONCLUSIONS**

The use of adsorbents mainly derived from agricultural waste or the food industry, in wastewater treatment is a promising green, low-cost and renewable resource. However, photocatalysis is more effective in mineralizing persistent pollutants than conventional methods. The use of biochar-based and biotemplate photocatalysts has opened a highly eco-efficient way to solve the environmental pollution problem. In the future, more studies are needed to develop green photocatalysts, and biochar-based and biotemplate photocatalysts can be considered as new promising catalysts in wastewater treatment.

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## OIL CONTENT OF SUNFLOWER HYBRID “DEVEDA” DEPENDING ON THE MAIN SOIL TILLAGE SYSTEM

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### ABSTRACT

The research was conducted in a stationary field experiment of the Dobrudzha Agricultural Institute - General Toshevo in the period 2016 to 2022. The impact of seven main soil tillage systems (MSTS) with and without turning the cultivated layer, No-till, as well as the alternative alternation between them in a 4-field crop rotation (beans-wheat-sunflower-corn) on the content and yield of oil. The MSTS are: 1. CP - conventional plowing (24-26 cm); 2. D – disking (10-12 cm) 3. C – cutting (chisel-plough); 4. NT - No-till (direct sowing); 5. Conventional plowing (for spring crops) – No-till (for wheat); 6. Cutting (for spring crops) - Disking (for wheat) and 7. Conventional plowing (for spring crops) - Disking (for wheat). The main objectives of the study were: (i) to investigate the seasonal variability in sunflower: (i) in the kernel/husk ratio; (ii) the oil content of the whole seed and its components; (iii) the obtained yields of oil per 1 area. Meteorological conditions during the years of study have an extremely strong influence on the proportion of the kernel and that of the husk in the whole seed. The share of the kernel varies from 74.91% (2016) to 80.20% (2018). This inevitably affects the share of the husk, whose share is higher in 2016, 2019 and 2020. The oil content in the kernel is also characterized by a well-defined dynamic - from 61.81% (2021) to 64.46% and 64.47% (2017 and 2022). The highest percentage of oil in the husk was found in 2018. In the whole seed, this high level of differentiation in oil content values depending on weather conditions over the years was preserved. The seed produced in 2019 is the highest oil (50.85%), and the least - in 2016 (46.68%). Yields of kernels, husks and their oil content, as well as whole seed, were more strongly affected by weather conditions during the study period. The tillage systems with or no deep turning treatment of the plow layer applied in crop rotation constantly or in combination with shallow tillage or No-till lead to obtaining seed highest oil content and, accordingly, oil yield compared to the others. The strict adherence to crop rotation, regardless of the diversity in the main tillage systems tested and the high level of selection work lead to a lack of observation of the parasite broomrape. An additional contribution to this fact is that the areas around General Toshevo are lightly infected with aggressive races of this parasite.

**Key words:** Main soil tillage systems (MSTS), Sunflower, Kernel/Husk components, Oil content.

### INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a major oilseed crop in almost the entire world. In recent years, the consumption of sunflower oil has increased significantly. In recent years, the consumption of sunflower oil has significantly increased. Application and combination of proper agrrotechnics and the fact that the sunflower has a very good root system it can actively draw water and nutrients from the 2-meter soil layer (Mc Michael and Quisenberry, 1993; Angadi and Entz, 2002; Balalić et al, 2012). This fact is a prerequisite for expanding the area of distribution in drier conditions. According to Miladinović et al (2019), due to its ability to grow under different agro-ecological conditions and its moderate drought tolerance, sunflower may become a preferred oilseed crop in the future, especially in light of global environmental changes. Significantly earlier, Part of

the research in Bulgaria on the problems of sunflower in relation to productivity and quality shows that these characteristics are dynamic and dependent not only on the genetic predisposition of the varieties/hybrids, but also on a number of important agrotechnical factors that fit into the different systems of agricultural production - the weather conditions, tillage, nutritional regime, care during the vegetation and others (Tonev and Nikolova, 1997; Tonev, 2006; Nankov, 2012; Koteva, 2014). Undoubtedly, the methods of soil cultivation under the relevant agro-climatic conditions play an essential role in obtaining maximally satisfactory yields and quality characteristics with the necessary respect for the environment (Botta et al, 2006; Celik et al, 2013). Dang et al (2015) and Peixoto et al (2020) no-till (no-till) practice is one of the three main principles of conservation agriculture. This practice has better protection against soil erosion and offers greater efficiency in plant nutrient uptake. Obtaining higher productive and quality characteristics is undoubtedly the ultimate goal of many researchers dealing with the agronomy and selection of this crop (Habib et al, 2007). In this connection our main objectives of the study were: (i) to investigate the seasonal variability in sunflower: (i) in the kernel/husk ratio; (ii) the oil content of the whole seed and its components; (iii) the obtained yields of oil per 1 area.

## MATERIAL AND METHOD

The research was conducted in a stationary field experiment of the Dobrudzha Agricultural Institute - General Toshevo in the period 2016 to 2022. The impact of seven main soil tillage systems (MSTS) with and without turning the cultivated layer, No-till, as well as the alternative alternation between them in a 4-field crop rotation (beans-wheat-sunflower-corn) on the content and yield of oil. The MSTS are: 1. CP - conventional plowing (24-26 cm); 2. D – disking (10-12 cm) 3. C – cutting (chisel-plough); 4. NT - No-till (direct sowing); 5. Conventional plowing (for spring crops) – No-till (for wheat); 6. Cutting (for spring crops) - Disking (for wheat) and 7. Conventional plowing (for spring crops) - Disking (for wheat). The mineral fertilization in the crop rotation was as follows: Common bean –  $N_{60}P_{60}K_{60}$ ; Wheat –  $N_{120}P_{120}K_{60}$ ; Sunflower -  $N_{60}P_{120}K_{120}$  and Maize –  $N_{120}P_{60}K_{60}$ .

For the indicated research period, an agrotechnical test of the Deveda hybrid was carried out. According to the main breeder of the team that created the hybrid, it is moderately injured. It is a single interline hybrid (Nenova, 2019). The seed oil content is 51.7% and the protein content- 27.1%. The hybrid is resistant to downy mildew /race 731 and 730/ and broomrape and middle resistance to phoma, phomopsis, althernaria and sclerotinia.

The oil content of the kernel and husk and the whole seeds was determined by the method of Rujkowski (1957).

## RESULTS AND DISCUSSION

Many factors influence the value of a number of sunflower indicators distinguishing crop productivity and oil quality. The abiotic factors of the environment, as well as the applied agricultural techniques, are the basis for the full development of the genetic potential when using the agricultural techniques under the specific weather conditions.

The response of the hybrid “Deveda” seed components in terms of their share in the seed formed, oil concentration and oil yield in kernels and hulls varied widely over the study period average (Table 1). The values of the coefficients of variation are the lowest for the index of the share of the kernel in the seed, the concentration of oil in the kernel and the seed - between 3 and 5%. Values below 30% were found for yields of kernels, oil from kernels and whole seeds, while for the index of oil content from husk it was 43.92%, which is an indication of a high degree of dispersion of the data.

Table 1. Degree of variation in the performance of the sunflower oil content and yields in seeds components according to MSTS by for the period 2016-2022.

Stat. Parameters	N	Minimum	Maximum	Mean	Std. Deviation	CV%
Kernal % in seed	98	73.50	86.40	77.25	2.45	3.17
Kernel Oil %	98	55.50	68.85	63.53	2.60	4.10
Yield Kernels	196	1239.6	5567.4	3456.5	996.5	28.83
Kernal Oil Yield	196	732.6	3507.5	2200.1	656.4	29.83
Husk %	98	13.60	26.35	22.76	2.45	10.76
Husk Oil %	98	3.10	12.10	7.16	2.08	29.06
Yield Husks	196	505.7	2059.9	1256.4	353.1	28.11
Husk Oil Yield	196	18.0	214.0	75.1	33.0	43.92
Seeds Oil %	98	42.90	56.20	49.20	2.46	5.01
Total Oil Yield	196	763.6	3721.4	2275.2	678.3	29.81

On the basis of the statistical analysis, it was also found that the tested factors in the experiment influence to the maximum extent both the oil content of the individual components of the seed and the obtained oil content from them (Table 2).

Weather conditions during the years of study have a strong influence on the proportion of kernel and husk in the whole seed. The strength of their influence on the values for the share of the components with the seed is over 50%. For their oil content, it decreases with values for oil concentration in the kernel and the whole seed. The interaction between the two factors has a stronger influence than the independent influence of MSTS, which is determined between 7-10%.

Table 2. Analysis of variances of sunflower oil content and productivity by seeds component according to main soil tillage systems (MSTS) averaged for the period 2016 – 2022.

Source	df	Content, %			Yields		
		Dependent Variable	F	Sig.	Dependent Variable	F	Sig.
Years (1)	6	Kernal, % in seed	5381.640	0.000	Kernal Oil Yield	257.341	0.000
	6	Husk, % in seed	15346.791	0.000	Husk Oil Yield	498.755	0.000
	6	Kernel Oil, %	6067.919	0.000	Total Oil Yield	254.409	0.000
	6	Husk Oil, %	11279.234	0.000	Yield Kernels	236.901	0.000
	6	Seeds Oil, %	4870.429	0.000	Yield Husks	211.444	0.000
MSTS (2)	6	Kernal, % in seed	1039.319	0.000	Kernal Oil Yield	129.396	0.000
	6	Husk, % in seed	2928.442	0.000	Husk Oil Yield	206.727	0.000
	6	Kernel Oil, %	1397.607	0.000	Total Oil Yield	130.504	0.000
	6	Husk Oil, %	1979.329	0.000	Yield Kernels	128.102	0.000
	6	Seeds Oil, %	1212.097	0.000	Yield Husks	124.837	0.000
1 x 2	36	Kernal, % in seed	607.218	0.000	Kernal Oil Yield	18.848	0.000
	36	Husk, % in seed	1702.090	0.000	Husk Oil Yield	79.220	0.000
	36	Kernel Oil, %	1712.275	0.000	Total Oil Yield	19.378	0.000
	36	Husk Oil, %	964.402	0.000	Yield Kernels	15.862	0.000
	36	Seeds Oil, %	1342.879	0.000	Yield Husks	13.305	0.000

The proportion of kernel in seed by year of study varied from 74.91% (2016) to 80.20% (2018) (Figure 2). These results have an impact on the values of the share of the husk in the seed accordingly. The same is the lowest in 2018 - 19.96%, when the kernel occupies the largest share in the seed. Both have the largest share in the seed in 2016 - 25.11%.

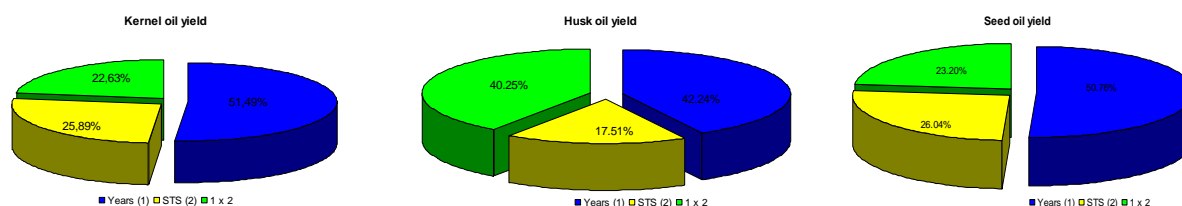


Figure 1. Strength of effect of the factors and their combinations average for the period 2016-2022 on the oil yields of sunflower seed according main STS, %

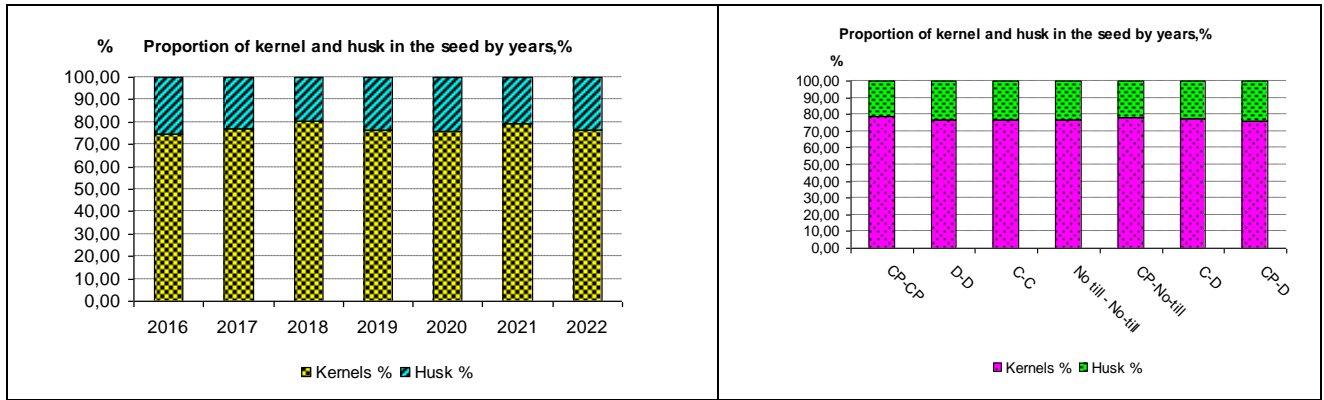


Figure 2. Proportion of seed components in seed according to years and MSTs, %

On average for the period 2016-2022, the share of the kernel in the seed in the tested MSTs was 77.25%. Only with the constant application of conventional plowing in the conventional plowing (CP-CP) and the alternative rotation CP-No-till, the share of kernels reaches 78.73% and 78.06%, respectively. The oil content of seed components and whole seed varied significantly between years (Figure 3). Kernels have the highest oil content in 2019 (66.38%). Against the background of the long-term experiment (2016-2022), the hybrid “Deveda” stands out with the highest oil content in 2017 (64.46%) - above the average for the experiment (63.53%). The kernels of the obtained product has the lowest oil content in 2021 - 61.81%. By years of study, oil content ranges from 4.50% (2022) to 9.66% (2018). Thus, the differentiation in the oil content of the whole seed is maximally expressed.

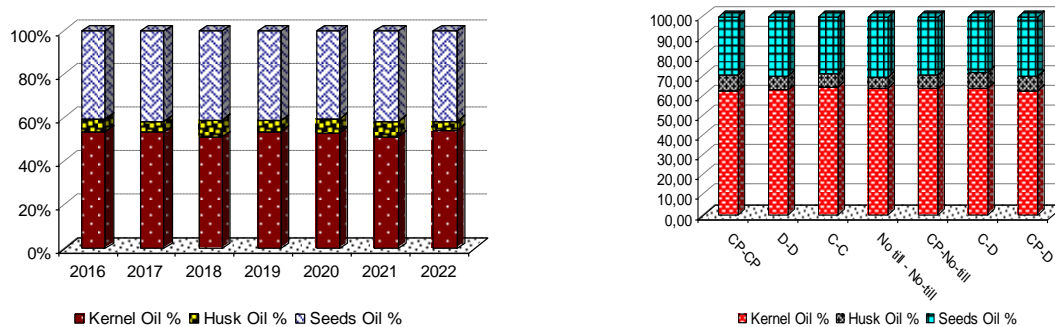


Figure 3. Oil content (%) in sunflower seed and its components, according to years and MSTs

The production of seeds in 2019 and 2018 is distinguished by the highest % oil, 50.85% and 50.45% respectively. The most unfavorable conditions for the processes related to the formation of oil in the seed were in 2016 (46.68%) and 2020 (47.48%). In the remaining years of the study, the oil content in the seeds was between 49% and 50%.

The tested tillage systems are also characterized by a well-expressed differentiation in the values of the investigated indices, and to a maximum extent. The percentage of oil in the kernel varied from 62.63% in (CP-D) to 64.83% in the deep non-rotating treatment (C-C).

On average for the period 2016-2022, the C-C systems (49.89%) are distinguished by the largest oil content in the seeds; constant No-till (49.70%); CP-No-till (49.66%); C-D (49.51%) and CP-CP (49.37%).

Discussing the obtained results for oil content from kernels by year we obtain values that distinguish each of the MSTs within the same year (Figure 4). This means that their place is not fixed in a strictly defined order. Almost every year there is a trend towards a lower oil content from kernel with permanent direct seeding. In two of the years (2017 and 2020), the three intermittent deep tillage systems (turned and no-turned tillage) in terms of kernel oil yield approached the constant application of conventional plowing in the crop rotation. In the variant with its 1-year break with (No till-No till)

in 2016, 2020 and 2022 an excess over CP-CP was found by 15.48%, 24.96% and 16.56%, respectively.

The influence of MSTs on the values of the obtained oil yields from the husks is expressed in a wide scale variation - from 19.49 kg/ha (2022) with the constant application of direct sowing in the crop rotation (No till-No-till) to 156.53 kg/ha (2016) the constant application of conventional plowing in the crop rotation (CP-CP).

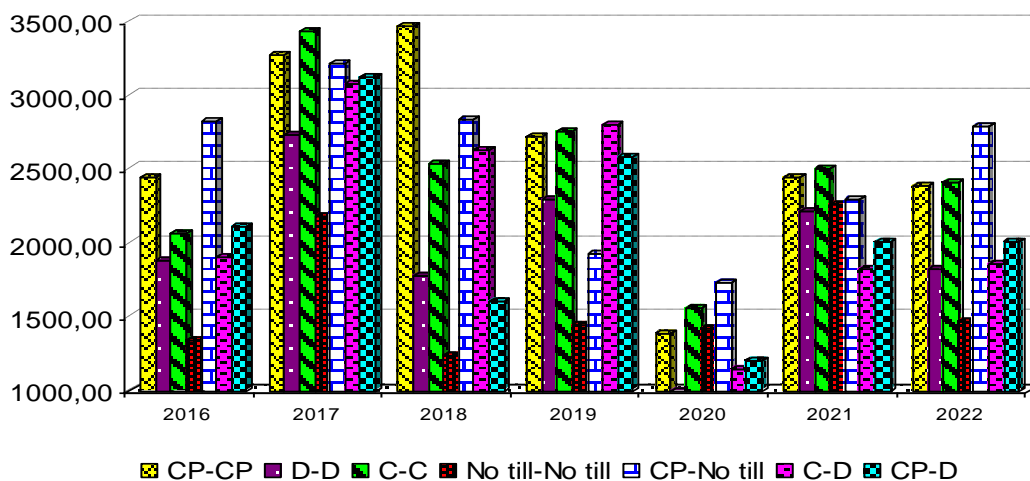
As a result of their share in the seed, the values of oil yields obtained from the husks are significantly lower than those obtained from the kernels. However, they are a valuable raw material in the preparation of animal feed. The indicated trends for the impact of MSTs on kernel oil yield are almost preserved for husk oil yield. Our results collaborates the view of Leon et al (2003) that the oil content and oil yield are complex quantitative traits, determined by genetic and environmental factors, along with interaction between them.

Final, whole seed oil yields as a composite measure also vary significantly by MSTs. The differentiation between the tillage options tested was enhanced in years with unfavorable weather conditions. An example of this is 2020, when seed oil yield varies from 874.13 kg/ha (D-D) to 1804.45 kg/ha (CP-No till). In the year with the most favorable conditions for developing the productive potential of the hybrid “Deveda” (2017), the variation was from 2222.48 kg/ha (No till - No till) to 3526.72 kg/ha (C-C) and 3388.61 kg/ha (CP- CP).

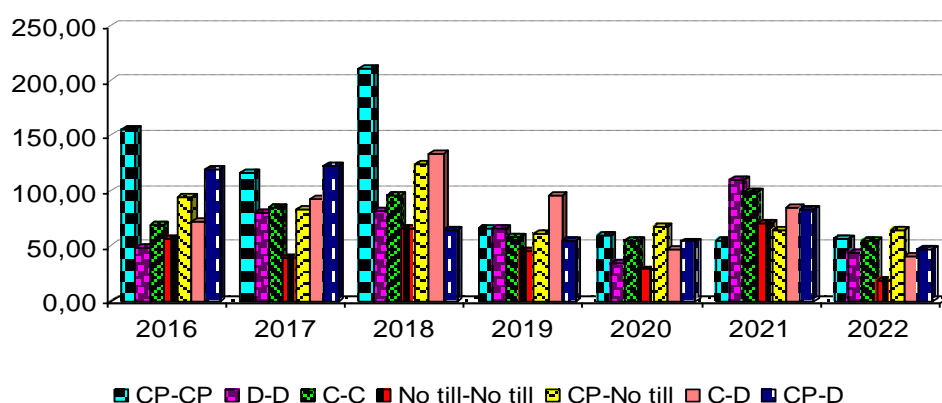
The factor with the greatest influence on the values of the tested indicators is meteorological dynamics in the amount of precipitation and temperature during the growing season. In our experiment, the interaction between these two main meteorological elements is the key factor determining the magnitude of oil yields (Table 3). The final oil yields formed are practically a complex of yields from the components of the seed and their oil content. On average for the long-term study period, the obtained oil yields from the seed ranged from 3095.85 kg/ha (2017) to 1376.62 kg/ha (2020) with an average yield in the experiment for the period 2016-2022 of 2275.15 kg/ha. This means an increase in oil yield by 36.07% on average for the tested MSTs under favorable conditions for culture development and, accordingly, with a combination of different types of stress, a drop to 60.51% of the average for this period of time.

A number of studies have been carried out in DAI-General Toshevo in relation to sunflower culture and the behavior and application of various agrotechnical practices on its productivity and quality, as well as the economic efficiency of its production in the country (Klochkov and Nankov, 1987; Nankov and Tonev, 1994; Nankov, 1996; Nankova et al, 1999; Nankov et al, 2002; Nenov et al, 2004). In this respect, Nankov's studies (1982a,b, Nankov and Dimitrov, 1985) related to the influence of autumn-winter moisture reserves in sunflower varieties and hybrids deserve special attention.

**Oil yields from the kernels**



### Oil yields from the husks



### Oil yields from whole seeds

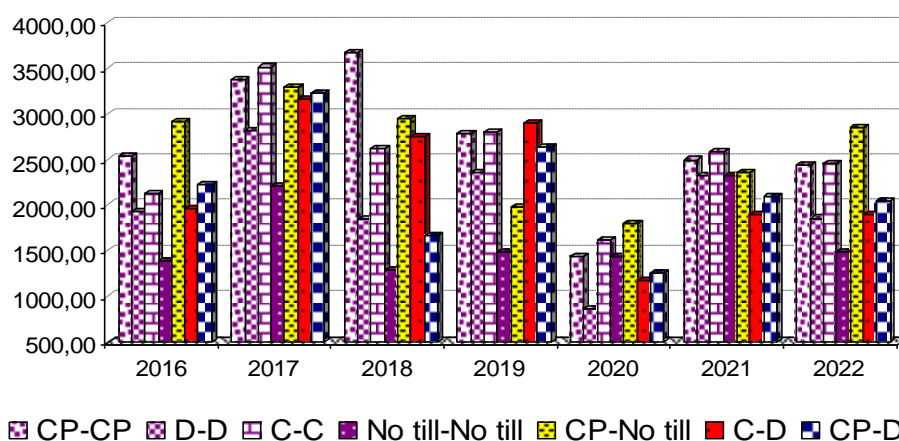


Figure 4. Final yields of sunflower seed oil and their components, kg/ha  
Table 3. Yields of kernels and husks and yields of oil in them and whole seed depending on weather conditions in the years studied, kg/ha

Years	Kernel Oil Yield	Husk Oil Yield	Total Oil Yield	Yield Kernels	Yield Husks
2016	2079.5 b	80.7 d	2160.2 b	3329.4 b	1249.9 c
2017	<b>3006.4 e</b>	89.5 e	<b>3095.9 e</b>	<b>4675.4 d</b>	<b>1669.0 e</b>
2018	2297.4 cd	<b>111.6 f</b>	2409.1 d	3639.5 c	1342.0 d
2019	2361.9 d	64.7 c	2426.6 d	3556.8 c	1194.9 b
2020	1326.5 a	50.1 b	1376.6 a	2122.4 a	795.9 a
2021	2222.2 c	81.7 d	2303.9 c	3600.5 c	1378.3 d
2022	2106.7 b	47.1 a	2153.8 b	3271.6 b	1164.9 b

The MSTs have a strong influence on the amount of yields obtained from the components of the seed and their oil yields (Table 4). The highest yields for the 7-year study period were obtained with the constant application of traditional plowing in the crop rotation. The total oil yield of 2685.68 kg/ha exceeds the average of all tested variants with MSTs by 18.04%. For the individual components of the seed, this increase is respectively for the husks by 25.92% and for the kernels - by 17.78%.



Table 4. Yields of kernels and husks and yields of oil in them and whole seed depending on the MSTs, kg/ha

MSTs	Kernel Oil Yield	Husk Oil Yield	Total Oil Yield	Yield Kernels	Yield Husks
CP-CP	2591.2 e	94.5 e	2685.7 e	4116.1 f	1525.1 f
D-D	1938.3 b	67.9 b	2006.2 b	3057.2 b	1118.7 b
C-C	2466.3 d	74.8 c	2541.0 d	3817.7 d	1351.4 d
No till - No-till	1621.0 a	47.1 a	1668.1 a	2547.1 a	925.9 a
CP-No-till	2517.0 de	80.7 d	2597.7 de	3956.6 e	1439.6 e
C-D	2175.5 c	81.5 d	2257.1 c	3390.6 c	1215.4 c
CP-D	2091.4 c	78.8 d	2170.3 c	3310.3 c	1218.9 c

Three of the tested MSTs in seed oil yield exceeded the experimental average yield (2275.15 kg/ha). These are the two deep (24-26 cm) and constantly applied soil treatments - conventional plowing (CP-CP) with turning the cultivated layer and deep loosening without turning it. The oil yield increase was 18.04% and 11.69%, respectively. Of the alternatively alternating treatments, only CP-No till stands out with an excess of 14.08%. The amount of seed oil yields obtained shows that over this long-term period of time, characterized by diversity in the combination of meteorological factors, none of the MSTs tested outperformed the continuous application of traditional/conventional plowing in the crop rotation. Significant correlations were found between the individual components of the seed, their productivity, oil content and the obtained oil yields from the whole seed. There were a number of negative correlations between the proportion of kernel in the seed with that of the husks and with the percentage of oil in the kernel (Table 5). The interrelationship between the proportion of the husk with the concentration of oil in it and the whole seed is also negative. The most strongly expressed positive correlation between the oil in the nut and that in the whole seed - 0.699\*\*. Kaya et al, (2007) through regression analysis results found a different relationship between seed yield of hybrids and oil content. The authors indicate that up to an oil content of 40%-45% the correlation with yield is negative.

Table 5. Pearson Correlation between sunflower seed components content and their oil concentration, 2016-2022

Indices	Kernal, %	Husk, %	Kernel Oil, %	Husk Oil, %	Seeds Oil, %
Kernal, %	1				
Husk, %	-0.995(**)	1			
Kernel Oil, %	-0.203(*)	0.221(*)	1		
Husk Oil, %	0.445(**)	-0.439(**)	-0.314(**)	1	
Seeds Oil, %	0.485(**)	-0.468(**)	0.699(**)	0.034	1

High levels of correlations were found between the oil yields of the seed components and their relative share in the seed (Table 6). The R-values between oil extraction of the kernel with the indicated indices is in the order of over 0.900, while in oil extraction in the husk the level of R is above 0.600. The correlation between the yield of oil in the kernel and the yield of oil in the whole seed stands out with the highest correlation value - 0.999.

Table 6. Pearson Correlation between sunflower seed components yields and their oil yields, 2016-2022

Indices	Kernal Oil Yield	Husk Oil Yield	Total Oil Yield	Yield Kernels	Yield Husks
Kernal Oil Yield	1				
Husk Oil Yield	0.649(**)	1			
Total Oil Yield	0.999(**)	0.677(**)	1		
Yield Kernels	0.993(**)	0.669(**)	0.994(**)	1	
Yield Husks	0.944(**)	0.680(**)	0.946(**)	0.976(**)	1

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

## CONCLUSIONS

Meteorological conditions during the years of study have an extremely strong influence on the proportion of the kernel and that of the husk in the whole seed. The share of the kernel varies from 74.91% (2016) to 80.20% (2018). This inevitably affects the share of the husk, whose share is higher in 2016, 2019 and 2020. The oil content in the kernel is also characterized by a well-defined dynamic - from 61.81% (2021) to 64.46% and 64.47% (2017 and 2022).

The highest percentage of oil in the husk was found in 2018. In the whole seed, this high level of differentiation in oil content values depending on weather conditions over the years was preserved. The seed produced in 2019 is the richest in oil (50.85%), and the least - in 2016 (46.68%).

Yields of kernels, husks and their oil yields, as well as whole seed, were more strongly affected by weather conditions during the study period compared to the effect of the MSTs. With the highest yields of the components of the seed, oil from the kernels and the whole seed, 2017 is noted, and unfavorable - in 2020.

The tillage systems with or no deep turning treatment of the plow layer applied in crop rotation constantly or in combination with shallow tillage or No-till lead to obtaining seed richer in oil content and, accordingly, oil yield compared to the others. Close to this systems are the results obtained when traditional plowing is interrupted with No-till when sowing wheat in the crop rotation.

The independent permanent application of deep cutting (chisel-plough) oil yield is less with 144.7 kg/ha compared to traditional plowing, while in the application of CP-No-till system this difference is only 88 kg/ha.

Shallow tillage alone and in combination, as well as long-term self-application of No-till lead to an increase in the share of husks and a lower yield of oil compared to the deep main tillage. The lowest yields of oil in the seed were obtained with the constant application of No-till - 1668.1 kg/ha, i.e. with 1017.6 kg/ha less compared to the constant application of traditional plowing.

The reliability of the obtained results is of the maximum degree of expression. The influence of meteorology as a factor is more pronounced than that of main STS. It has approximately the same values for kernel, %/husk, % in the seed, as well as for the percentage of oil in the kernel and the whole seed. However, it was found to have a much stronger influence on oil content in the husk compared to the kernel and whole seed.

The strict adherence to crop rotation, regardless of the diversity in the main tillage systems tested and the high level of selection work lead to a lack of observation of the broomrape parasite (*Orobanche* spp.). An additional contribution to this fact is that the areas around DZI- General Toshevo are lightly infected with aggressive races of this parasite.

The proportion of kernel in the seed is strongly negatively correlated (-0.995\*\*) with that of the husk. The proportion of kernel in the seed was also in a well-pronounced positive correlation with the percentage of oil in the seed (+0.485\*\*) and the oil content in the husk (+0.445\*\*). There is also a well-expressed correlation between the oil content of the kernel and that of the seed (+0.699\*\*). The correlation dependences between oil yields from the individual components of the seed are positive and with a very high level of statistical reliability. The highest correlation value was found between oil yields in kernel and seeds - 0.999.

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## CONTENT OF ESSENTIAL MACRONUTRIENTS IN THE ORGANS OF SUNFLOWER (*HELIANTUS ANNUS*) - HYBRID DEVEDA DEPENDING ON THE MAIN TILLAGE SYSTEM

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### ABSTRACT

During the period from 2016 to 2022, the changes in the concentration of main macroelements in the organs of hybrid Deveda depending on the application of main soil tillage (MSTS) were tracked. The research was conducted on slightly leached Chernozems (Haplic Chernozems) in the experimental field of the Dobrudzhan Agricultural Institute - General Toshevo. The tested systems for MSTS are: 1. CP - conventional plowing (24-26 cm); 2. D - disking (10-12 cm) 3. C – cutting (chisel-plough); 4. NT - No-till (direct sowing); 5. Conventional plowing (for spring crops) - No-till (for wheat) - CP-No-till; 6. Cutting (for spring crops) - Disking (for wheat) - C-D and 7. Conventional plowing (for spring crops) - Disking (for wheat) - CP-D. The first four were applied continuously and independently in the 4-crop rotation (beans-wheat-sunflower-maize). The other three involve alternating between them. The mineral fertilization in the crop rotation was as follows: Common bean –  $N_{60}P_{60}K_{60}$ ; Wheat –  $N_{120}P_{120}K_{60}$ ; Sunflower -  $N_{60}P_{120}K_{120}$  and Maize –  $N_{120}P_{60}K_{60}$ . The years and methods of soil cultivation are the basis of the highly varying values in the concentration of macroelements in the sunflower organs. Of all the organs, the defatted kernel of the sunflower seed has the highest concentration of nitrogen. It varies from 9.10 % N (2016) to 8.18 % N (2020). MSTS have significantly less influence, because the average variation is from 8.69% N and 8.68% N (in the 1. CP) to 8.40 % N (6. C-D). The content of phosphorus in the defatted kernel is also characterized by a strong dynamic in the values - from 1.70 %  $P_2O_5$  (2017) to 5.06 %  $P_2O_5$  (2020). Depending on the tillage systems, this variation is much weaker - from 3.96%  $P_2O_5$  (5. CP-No-till) to 5.06%  $P_2O_5$  (4.No-till). The indicated trends regarding the influence of the tested factors on the chemical composition of the defatted kernel were fully confirmed for the other organs as well. It was found that the head (without the seeds), followed by the defatted kernel and the vegetative mass, are distinguished by the maximum concentration of potassium. The concentration of nitrogen, phosphorus and potassium remained at the lowest values in defatted husks. Systems involving deep tillage lead to an increase in nitrogen concentration in the organs of the vegetative mass (leaves and stems).

**Key words:** main soil tillages systems, sunflower, NPK% concentration, organs

### INTRODUCTION

We are witnessing ever-increasing intensity and duration of changes in climatic conditions. Most often, moisture deficit turns out to be the critical factor that limits the course of a number of physiological processes, which in turn lowers the ability of plants to exhibit tolerance to adverse environmental conditions (Anavella et al. 2016; Leng and Hall 2019). Plants possess a self-defense regulatory mechanism by which they try to adapt to water scarcity (Waseem et al. 2021). Maintaining the work of this mechanism, as well as the overall cellular metabolism, so that plants can withstand adverse conditions for a longer time, it is necessary to ensure optimal concentrations of nutrients not only in the soil, but also in the plants (Ma et al. 2021; Barzana et al. 2021).

Undoubtedly, for identifying the maximum point of the overall appearance of the sunflower, quantitative and qualitative analyses, including the concentration of nutrients in individual organs,

are the exact tools for evaluating the peculiarities of development under different soil-climatic conditions (Peixoto et al. 2011). Puste et al.(2013) also indicated that the application of different nutrient packages in combination with water regimes significantly affected the concentration and uptake of nutrients in seeds and other organs after harvest.

Absorption of nutrients from the soil is one of the main nutritional characteristics of plants, through which they provide a specific mineral and biochemical composition, reflecting on the productivity and quality of sunflower production (Nankova and Tonev, 2004).

Our knowledge of nutritional management to increase sunflower tolerance to climate change stress conditions is particularly relevant. They must combine the efforts and knowledge of agrotechnicians and breeders to characterize especially the behavior of sunflower in order to obtain a sustainable and ecologically-economical production.

The aim of the present study is to characterize the production obtained from the Deveda hybrid at technical maturity in terms of the concentration of nitrogen, phosphorus and potassium in the sunflower organs when it is grown on different main tillage systems.

## MATERIAL AND METHODS

During the period from 2016 to 2022, the changes in the concentration of main macrolelements in the organs of hybrid Deveda depending on the application of main soil tillage (MSTS) were tracked. The research was conducted on slightly leached Chernozems (Haplic Chernozems) in the experimental field of the Dobrudzhan Agricultural Institute - General Toshevo.

The tested systems for MSTS are: 1. CP - conventional plowing (24-26 cm); 2. D - disking (10-12 cm) 3. C – cutting (chisel-plough); 4. NT - No-till (direct sowing); 5. Conventional plowing (for spring crops) - No-till (for wheat) - CP-No-till; 6. Cutting (for spring crops) - Disking (for wheat) - C-D and 7. Conventional plowing (for spring crops) - Discing (for wheat) - CP-D. The first four were applied continuously and independently in the 4-crop rotation (beans-wheat-sunflower-maize). The other three involve alternating between them. The mineral fertilization in the crop rotation was as follows: Common bean – N<sub>60</sub>P<sub>60</sub>K<sub>60</sub>; Wheat – N<sub>120</sub>P<sub>120</sub>K<sub>60</sub>; Sunflower - N<sub>60</sub>P<sub>120</sub>K<sub>120</sub> and Maize – N<sub>120</sub>P<sub>60</sub>K<sub>60</sub>. Mineral fertilization was done with common ammonium nitrate NH<sub>4</sub>NO<sub>3</sub> (34% N), triple superphosphate (46% P<sub>2</sub>O<sub>5</sub>) and potassium chloride (60 % K<sub>2</sub>O).

This field experiment is the successor of a stationary field experiment, including long-term application of 24 tillage systems in a 6-field crop rotation. The same was laid in 1987. After crop harvest in 2013, a reduction in the number of alternate tillage systems was carried out. Experience preserves the basic treatments constantly applied in crop rotation for both grain and spring crops and three of the alternate tillage systems have also been preserved that have been described above.

One day before harvest, plants of 10 linear meters were taken from each variant in three replicates. The same are divided by organs: vegetative mass (leaves + stems), heads (without seeds) and kernel and husks obtained after deoiling the seeds. The concentrations of nitrogen, phosphorus and potassium in the examined organs were determined in the Agrochemical Laboratory of DAI-General Toshevo. A classic method was used by burning the samples with sulfuric acid (Keldahl method). Total nitrogen was determined in aliquots by distillation of a Parnassus-Wagner apparatus, phosphorus - colorimetrically by the yellow molybdate-vanadate reaction, and potassium - flame-photometrically.

The resulted data were statistically processed using variance analysis, F test and LSD (Least Significant Difference) test, which are commonly utilized in the multi-criterial statistical analysis. We used the SPSS version 16.0 statistical package. Significance of the treatments' effect was considered at 0.05 probability level. After performing the analysis of variance, we compared the means for each treatments using the Waller-Duncan's Multiple Range Test. Finally, Pearson correlation coefficients ("R coefficients") were computed and tested for significance.

## RESULTS AND DISCUSSIONS

On average for the 7-year research period, the analysis of the variances of the concentration of the main macroelements, regardless of the variation in their values, show a maximum level of statistical reliability under the influence of the tested factors and their interaction (Table 1).

Table 1. Analysis of variances of NPK concentration in sunflower organs according to the main soil tillage systems for the period 2016-2022

Source	Dependent Variable	df	V.mass		Head		Kernels		Husks	
			F	Sig.	F	Sig.	F	Sig.	F	Sig.
Years (1)	N %	6	2110,89	,000	276,88	,000	129,21	,000	1139,53	,000
	P %	6	752,05	,000	3207,36	,000	5545,48	,000	790,22	,000
	K %	6	759,16	,000	2172,76	,000	2595,59	,000	4068,95	,000
STS (2)	N %	6	572,16	,000	48,15	,000	11,88	,000	70,68	,000
	P %	6	40,19	,000	84,73	,000	87,43	,000	23,62	,000
	K %	6	17,21	,000	9,43	,000	53,27	,000	66,28	,000
1 x 2	N %	36	117,95	,000	18,89	,000	16,65	,000	155,63	,000
	P %	36	34,79	,000	36,27	,000	26,93	,000	28,79	,000
	K %	36	11,75	,000	9,97	,000	43,62	,000	292,60	,000

The weakest variation in the values of the coefficients of variation for all three macronutrients was found in the analysis of heads (Figure 1). This fact is an indication of a high level of homogeneity of the sample, especially for their nitrogen and potassium content. The difference in the values of this statistical index regarding the phosphorus content is significant. The same is characterized by a large dispersion of the data, especially in the organs forming the non-profitable part of the production - vegetative mass (leaves + stems), heads and husks. This shows that for the study period these samples are heterogeneous. The variation of the coefficient is from 27.94% (kernels) to 63.05% (head without seed).

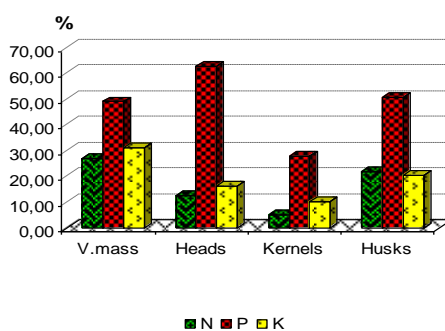


Figure 1. Dynamics in the values of the variation coefficients for the NPK concentrations by organs of the Deveda hybrid (2016-2022), %

The concentration of nitrogen in the above-ground vegetative mass (leaves+stems) varies widely depending on the MSTs during each of the years of the study (Figure 2).

With the exception of 2019 and 2020, the concentration of nitrogen in this part of the sunflower plant is the lowest in permanent direct seeding (No till-No till). At the end of the growing season, higher N% values were found in 2021, 2018 and partly in 2016. Systems whose treatments reach a depth of 24-26 cm, regardless of whether they are applied alone or included in combination with other treatments, make a significant contribution to this fact. Phosphorus concentration has a very pronounced differentiation depending on the weather conditions during the years of research. It was the lowest in 2017 (0.11% P<sub>2</sub>O<sub>5</sub>), while in 2016 and 2021 it reached 0.42% P<sub>2</sub>O<sub>5</sub> and 0.48% P<sub>2</sub>O<sub>5</sub>.

The methods of tillage significantly less influence the changes in phosphorus concentration within each year separately. The concentration of potassium is also subject to substantial differentiation depending on the meteorological factor. Under the conditions of 2018, the lowest values for the content of this element were obtained - between 1-2%  $K_2O$ , while in 2016, depending on the MSTs, the values varied between 3-5%  $K_2O$ .

The concentration of nitrogen in the heats (without seeds) almost equals that in the vegetative mass and even exceeds it in most years. For each of the years of study, MSTs differentiated N%, but the same was less pronounced compared to vegetative mass. The continuous application of direct seeding in crop rotations results in lower concentrations of nitrogen in the piles, compared to other tillage systems. Similar trends were also found for the content of phosphorus in the head. Its concentration was lowest in 2017 (0.12%  $P_2O_5$ ) and 2018 (0.13%  $P_2O_5$ ). Tillage methods differentiate the values, especially in 2020. The heats are the organ with the highest concentration of potassium in the sunflower plant. The highest concentrations were found in 2017 (the year with the highest yields - [Nankova and Nenova, 2023](#)) in the two deep treatments - 5.20%  $K_2O$  (CP-CP) and 5.26%  $K_2O$  (C-C).

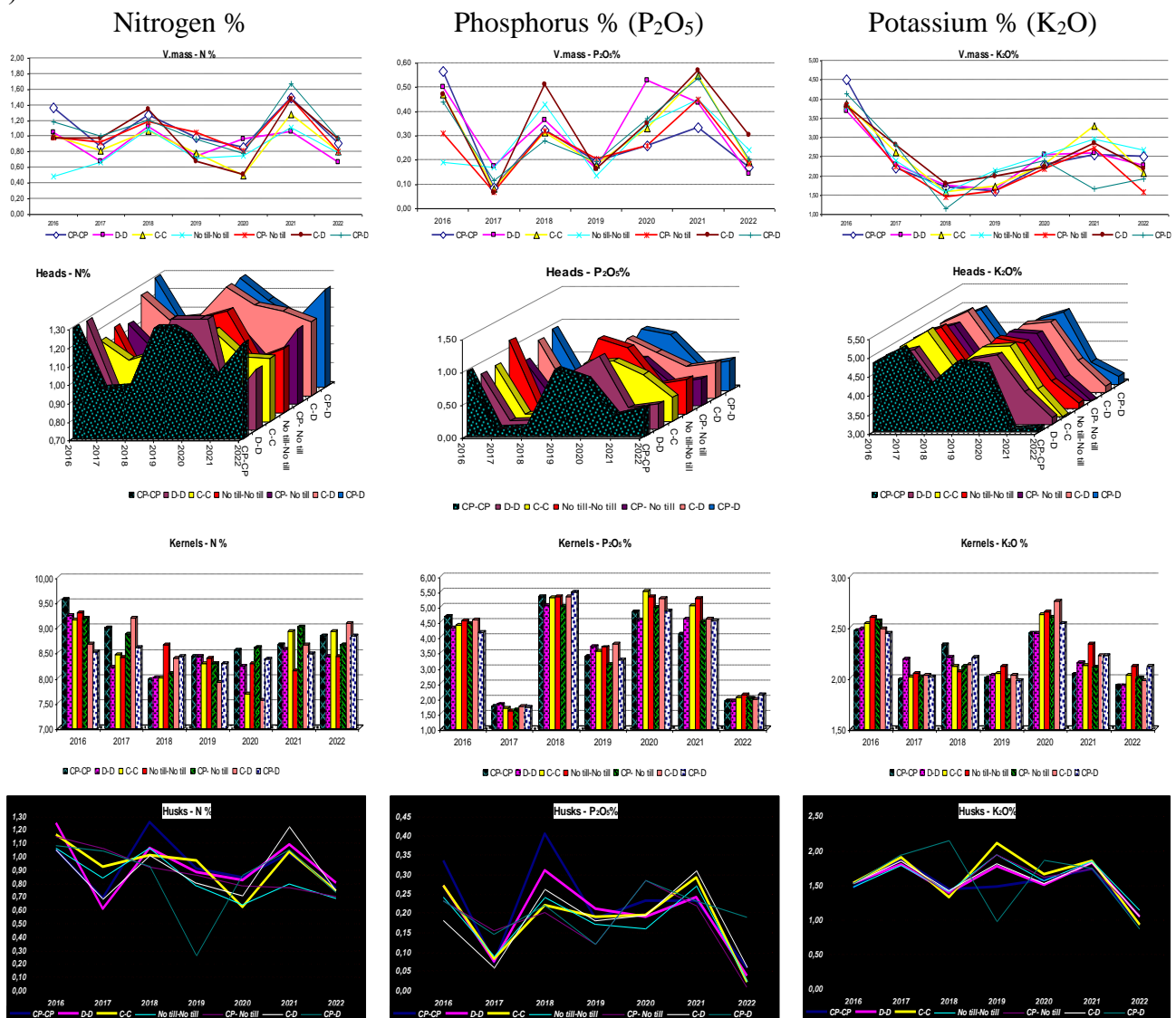


Figure 2. Concentration of nitrogen, phosphorus and potassium in sunflower organs by years of study depending on MSTs, %

Kernel are the most essential part of the commodity production of this crop. The content of basic macroelements in them are the basis, apart from the productivity, and the biochemical properties of the protein and oil. The content of nitrogen in the defatted kernel was the highest in 2016 in all



variants with independent and constant application of the treatments, regardless of the depth of their implementation. This component of the seed has the highest values for N% - of the order of 8-9 and over 9% nitrogen compared to the other organs. The same applies to the content of phosphorus and potassium, with the differentiation in phosphorus values being much more pronounced compared to that of nitrogen. The concentration of potassium in this organ is about 2% K<sub>2</sub>O and is distinguished by a lower variation in values both by years and by treatments.

The concentration of nitrogen in the defatted husk is similar to that of the vegetative mass. The values for nitrogen, phosphorus and potassium content are influenced by the weather conditions of the year, and the variations in concentration depending on the MSTs are less pronounced. The skims have the lowest N% are the harvests of 2022, 2020 and partly from 2017. Regarding the phosphorus content, it is the organ with its lowest concentration, especially in 2017 and 2022. The potassium content of the oilless husk is between 1 -2% K<sub>2</sub>O for the period 2016-2021. For 2022, the potassium content of the CP-CP, C-C and CP-D systems are respectively 0.92%, 0.92% and 0.86% K<sub>2</sub>O.

The content of nitrogen in the organs of the sunflower is strongly influenced by the weather conditions during the vegetation period of the crop during the years of research (Table 2).

Table 2. Nitrogen, phosphorus and potassium concentration in sunflower organs, by years of investigation, %

Years	V.mass	Heads	Kernels	Husks
N%				
2016	1,007 c	1,225 d	9,095 e	1,114 g
2017	,847 b	,987 a	8,681 cd	,834 d
2018	1,183 d	1,012 b	8,221 ab	1,035 f
2019	,846 b	1,256 e	8,290 b	,780 c
2020	,739 a	1,228 d	8,181 a	,753 b
2021	1,366 e	1,006 ab	8,635 c	,997 e
2022	,844 b	1,111 c	8,742 d	,736 a
P <sub>2</sub> O <sub>5</sub> %				
2016	,421 e	,896 d	4,459 c	,251 e
2017	,108 a	,119 a	1,704 a	,096 b
2018	,364 d	,133 a	5,269 f	,265 f
2019	,177 b	,919 e	3,502 b	,168 c
2020	,350 d	,876 c	5,058 e	,220 d
2021	,476 f	,430 b	4,675 d	,256 e
2022	,206 c	,446 b	4,421 c	,033 a
K <sub>2</sub> O %				
2016	3,947 g	4,808 d	2,514 d	1,505 b
2017	2,481 e	5,101 e	2,041 b	1,851 f
2018	1,575 a	4,101 c	2,171 c	1,498 b
2019	1,834 b	4,786 d	2,028 ab	1,716 d
2020	2,349 d	4,809 d	2,579 e	1,613 c
2021	2,880 f	3,634 b	2,174 c	1,793 e
2022	2,174 c	3,108 a	2,016 a	,996 a

The variation in the concentration of nitrogen is manifested to the fullest extent in the defatted husk - from 0.736 (a - Waller-Duncan test) in 2022 to 1.114 (g- Waller-Duncan test) % N in 2016. In the other organs, the differentiation in N% is also well expressed, but there is also uniformity or similarity in the reaction of the culture by years. The nitrogen concentration is highest in the defatted kernel.

The same applies to the concentration of phosphorus, its content being the lowest in the defatted husk. The differentiation in the values of this nutrient depending on the conditions of the year is also very well expressed and marked by uniformity in the reaction of the culture organs. A similar trend was found for the concentration of potassium in the sunflower organs. The head (without seeds) have the highest potassium content and the defatted husk has the least.

The tested tillage systems also lead to differentiation in the concentration of the main macronutrients in the sunflower organs (Table 3). The nitrogen content of the vegetative mass is subject to the greatest variations. It has been established that the treatments, including single or combined participation of conventional plowing, are distinguished by a more nitrogen-rich vegetative biomass. MSTs had the least effect on N% in the defatted kernel. The variation in values is between 8.494 N% in the deep no-turn tillage (Cutting-Cutting) to 8.713 N% in the permanent conventional ploughing.

Table 3. Nitrogen, phosphorus and potassium concentration in sunflower organs, by main soil tillage systems, %

STS	V.mass	Heads	Kernels	Husks
N%				
Plowing - Plowing	1,106 e	1,157 c	8,713 b	,932 c
Disking - Disking	,896 b	1,111 b	8,446 a	,929 c
Cutting - Cutting	,894 b	1,100 b	8,491 a	,922 c
No till – No till	,803 a	1,064 a	8,519 a	,838 a
Plowing – No till	1,034 d	1,053 a	8,673 b	,891 b
Cutting - Disking	,986 c	1,174 c	8,499 a	,887 b
Plowing - Disking	1,112 e	1,166 c	8,506 a	,849 a
P <sub>2</sub> O <sub>5</sub> %				
Plowing - Plowing	,277 b	,556 cd	4,033 a	,216 d
Disking - Disking	,330 d	,534 b	4,055 a	,190 c
Cutting - Cutting	,303 c	,561 d	4,277 b	,181 b
No till – No till	,281 b	,634 e	4,404 c	,170 a
Plowing – No till	,257 a	,439 a	4,021 a	,174 ab
Cutting - Disking	,348 e	,541 bc	4,251 b	,177 ab
Plowing - Disking	,306 c	,553 cd	4,046 a	,180 b
K <sub>2</sub> O%				
STS	V.mass	Heads	Kernels	Husks
Plowing - Plowing	2,478 c	4,281 a	2,170 a	1,496 a
Disking - Disking	2,396 b	4,330 bc	2,206 bc	1,549 b
Cutting - Cutting	2,500 cd	4,381 d	2,215 c	1,609 f
No till – No till	2,583 d	4,369 cd	2,279 e	1,594 e
Plowing – No till	2,230 a	4,279 a	2,199 b	1,581 de
Cutting - Disking	2,524 cd	4,400 d	2,236 d	1,567 c
Plowing - Disking	2,530 cd	4,307 ab	2,217 c	1,575 cd

The tested MSTs, although reliably influence the chemical composition of sunflower with a significantly less pronounced force compared to the meteorological conditions during the study period (Figure 3). Their impact on the concentration of nitrogen in the vegetative mass and heads is greater than that found for the defatted components of the seed. In the case of the latter, the strength of the interaction between the two factors exceeds 40%. The different MSTs differentiated to a greater extent the phosphorus concentration values in the components of the non-economic part of the production compared to those obtained for the defatted kernel.

The cultivation of sunflower under the Plowing-No till system is characterized by the lowest concentration of phosphorus compared to the other MSTs. It was found that the weather conditions of the year have a stronger influence on the concentration of phosphorus than that of nitrogen. This fact is especially strongly manifested in heads and defatted kernels and husks. Also, the strength of the interaction between the two factors (*Years x MSTs*), although statistically significant, is much less pronounced than that of the nitrogen concentration.

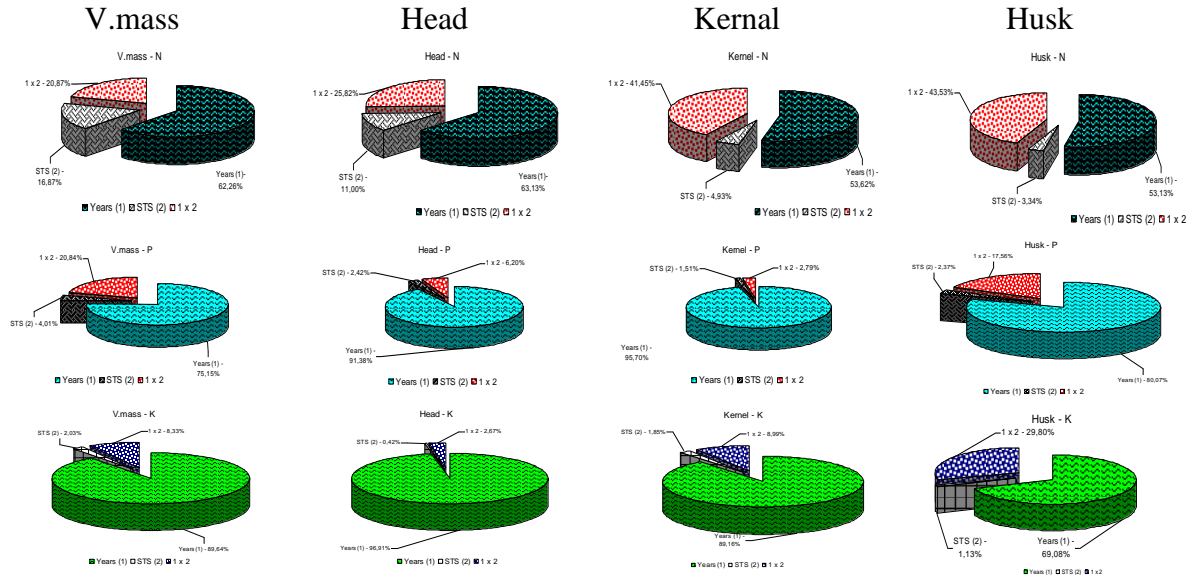


Figure 2. Strength of effect of the factors (independently and in combination) on the NPK concentration by sunflower organs, %

The concentration of potassium in the sunflower organs generally follows the indicated trends for their phosphorus content. The uptake of potassium, respectively its concentration, is mainly controlled by weather conditions during the growing season and significantly less by MSTs. The interaction between the two factors had a greater power of influence on the concentration of potassium in the defatted husks and kernels compared to that in the vegetative mass and heads.

The long period of research allows us to establish the presence of a high positive correlation dependence between the concentration of nitrogen and phosphorus in the organs of the non-economic part of the sunflower production (Figure 4).

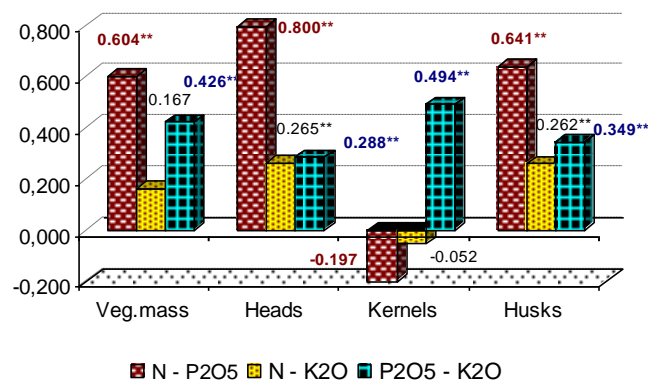


Figure 4. Values of Pearson correlation coefficients between macroelements by sunflower organs

There is a weak, but statistically significant, correlation between nitrogen and potassium in heads and defatted husks. For defatted kernels, the correlation between nitrogen and phosphorus and nitrogen and potassium were negative and unreliable. In all examined organs, the correlations

between phosphorus and potassium are positive, with different values of the correlation coefficients and statistically reliable.

In the final stage of sunflower development, the concentration of NPK% in the vegetative mass has no statistically significant relationship with that in the heat (Table 4). At a different level of credibility are the relationships between NPK% in the vegetative mass and their content in the kernel and NP in the husk. The correlation between the concentration of phosphorus in the vegetative mass and that of the defatted kernels and husks is most pronounced.

The content of nitrogen in the heat does not reliably correlate with the concentration of nitrogen in the other organs. However, the interaction of the content of potassium in this organ with that in the defatted components of the seed is reliable. Similar, but less pronounced, is that between the heat and the kernel in terms of phosphorus content.

Table 4. Values of Pearson correlation coefficients between sunflower organs for each of the macroelements separately

Organs	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
V.mass with			
Head	0.159	0.195	0.184
Kernel	0.230*	0.652**	0.479**
Husk	0.478**	0.656**	0.048
Head with			
V.mass	-0.159	0.195	0.184
Kernel	0.009	0.256**	0.349*
Husk	-0.109	0.191	0.544**
Kernel with			
V.mass	0.230*	0.652**	0.048
Head	0.009	0.256*	0.544**
Husk	0.251*	0.494**	0.080
Husk with			
V.mass	0.478**	0.656**	0.048
Head	-0.109	0.191	0.544**
Kernel	0.251*	0.494**	0.080

The concentration of phosphorus in the kernel is in a reliable relationship with the concentration of this element in all the investigated organs. In her research, Abbasova (2022) found a high correlation between the amount of total nitrogen, phosphorus and potassium (%) in the organs and obtained from sunflower during ripening.

These results show that the concentration of phosphorus in all organs of the above-ground biomass is extremely important for its interrelationships with the other two macronutrients and especially affects the components of the seed.

## CONCLUSIONS

The years and methods of soil cultivation are the basis of the highly varying values in the concentration of macroelements in the sunflower organs. Of all the organs, the defatted kernel of the sunflower seed has the highest concentration of nitrogen. It varies from 9.10 % N (2016) to 8.18 % N (2020). MSTs have significantly less influence, because the average variation is from 8.69% N and 8.68% N (in the 1. CP) to 8.40 % N (6. C-D).

The content of phosphorus in the defatted kernel is also characterized by a strong dynamic in the values - from 1.70 %P<sub>2</sub>O<sub>5</sub> (2017) to 5.06 %P<sub>2</sub>O<sub>5</sub> (2020). Depending on the tillage systems, this variation is much weaker - from 3.96% P<sub>2</sub>O<sub>5</sub> (5. CP-No-till) to 5.06% P<sub>2</sub>O<sub>5</sub> (4.No-till).

The indicated trends regarding the influence of the tested factors on the chemical composition of the defatted kernel were fully confirmed for the other organs as well. It was found that the head (without the seeds), followed by the defatted kernel and the vegetative mass, are distinguished by the maximum concentration of potassium.

The concentration of nitrogen, phosphorus and potassium remained at the lowest values in defatted husks. Systems involving deep tillage lead to an increase in nitrogen concentration in the organs of the vegetative mass (leaves and stems).

Meteorological conditions during the growing season have a decisive influence on the concentration of the main macroelements in the organs of the Deveda hybrid, especially for the content of potassium and phosphorus. The most significant correlations are between nitrogen and phosphorus in the non-economic part of production. No significant correlation was found between nitrogen and phosphorus and nitrogen and potassium in the defatted kernel.

The concentration of phosphorus in all organs of the above-ground biomass is extremely important for its interrelationships with the other two macronutrients and especially affects the components of the seed.

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## EFFECTS OF DIFFERENT SALT DOSES ON SEEDLING GROWTH AND RELATIVE WATER CONTENT OF SUNFLOWER (*Helianthus annuus* L.)

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### ABSTRACT

This study was conducted to determine the effects of different salt doses on seedling growth and relative water content of sunflower (*Helianthus annuus* L.) varieties. In the study, three sunflower varieties [Pioneer MM54 (C1), Pegaz (C2), Buleria (C3)] and three different salt doses [0 (control) (S1), 50 mM (S2), 100 mM (S3)] were used. In the study, seedling length (SL), root length (RL), seedling fresh weight (SFW), root fresh weight (RFW) and relative water content (RWC) characteristics were examined. As a result of the study, it was determined that there were generally decreases with increasing salt doses in all varieties in terms of the parameters examined. It was determined that the highest salt dose applied in the study (100 mM) had a greater negative effect on seedling, root development and relative water content. In terms of varieties, it was determined that the Pioneer MM54 variety was more tolerant to salt applications than other varieties.

**Keywords:** abiotic stress, relative water content, salt tolerance, sunflower

### INTRODUCTION

Sunflower is one of the most widely cultivated oilseed crops globally, known for its high oil content and quality (40-47%), making it a valuable vegetable oil source (Saleem et al., 2003; Monazzah et al., 2017). Its high oil content, along with the presence of a high proportion of unsaturated fatty acids and a low content of saturated fatty acids, makes it a valuable source of plant-based oil (Gürsoy, 2019, 2022; Rehman et al., 2019). Consequently, it holds significant importance in human nutrition (Tan, 2014). Given the challenges in achieving adequate and balanced nutrition and the difficulties in accessing reliable food sources, the production of plants like sunflower has become increasingly crucial (Tan, 2014). Moreover, due to its high adaptability, sunflower can be grown for oil production in nearly every region of our country (Kaya, 2016).

Salinity has emerged as one of the prominent stress factors increasing globally in recent years (Kireççi and Yürekli, 2019). Salt stress is an abiotic stress factor significantly affecting the development, yield, and quality of plants (Gürsoy, 2023a). In environments where various abiotic stress factors such as salinity and drought prevail, seeds of plants grown experience reduced vitality, suppressed germination, and weak seedling development (Demirbaş and Balkan, 2018). Consequently, plant yields decrease due to these effects (Ulukan et al., 2012). In high salinity conditions where plants are cultivated, several adverse conditions such as water stress, ion toxicity, membrane disorders, inhibited cell division, and growth retardation occur (Zhu, 2007; Gürsoy, 2023a). There are differences among plant species in response to salt stress (Ertekin et al., 2017). Therefore, it is crucial to identify these differences before cultivation.

The aim of this study was to determine the effects of different salt doses applied to various sunflower varieties on seedling development and relative water content.

## MATERIAL AND METHOD

This research was conducted at Aksaray University Scientific and Technological Research Laboratory (ASÜBTAM). Three different sunflower varieties [Pioneer MM54 (C1), Pegaz (C2), Buleria (C3)] and three different salt doses [0 (control) (S1), 50 mM (S2), 100 mM (S3)] were used in the experiment, arranged in a completely randomized design with three replications. Before the experiment, the seeds were soaked in 5% sodium hypochlorite solution for 10 minutes for surface sterilization. After soaking, the seeds were rinsed several times with distilled water and dried until they reached their initial weights. Ten seeds for each salt dose were placed on filter papers in Petri dishes, and 10 mL of solutions with appropriate concentrations of salt was added. Petri dishes designated as control received only distilled water. To prevent evaporation, the Petri dishes were sealed with parafilm and left for germination at room temperature. Filter papers were replaced every 2 days, and 10 mL of solutions with different concentrations of salt were added according to the applied doses. Seeds with a 2 mm radicle length were considered germinated (ISTA, 2003). Seedling height (cm), root length (cm), seedling fresh weight (g), root fresh weight (g), and relative water content (%) were measured in this study.

### Measurements:

**Seedling Height (cm):** The seedlings' heights were measured by separating the shoots from the roots and weighing them on a sensitive scale.

**Root Length (cm):** The roots of the separated seedlings were weighed on a sensitive scale to determine their length.

**Seedling and Root Fresh Weight (g):** The fresh weights of the seedlings and roots of plants subjected to both control and salt treatments were determined using a sensitive scale.

**Relative Water Content (%):** Leaf samples collected from plants subjected to both control and salt treatments were weighed to determine their fresh weights. They were then placed in glass tubes containing 5 mL of distilled water and left in light for 24 hours. After this period, the leaf samples were weighed again to determine their turgor weights. These leaf samples were then dried in an oven at 80°C for 48 hours to determine their dry weights. Finally, the relative water contents were calculated using the following formula (Ritchie et al., 1990):

$$\text{RWC (\%)} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

FW: fresh weight, TW: turgor weight, DW: dry weight

The results obtained from the experiment were analyzed using TARIST and MSTAT-C (MSTAT, 1989) statistical programs. Differences between means were determined using the LSD test ( $p < 0.05$ ).

## RESULTS AND DISCUSSION

Examination of the results of the analysis of variance showed significant differences at the 5% level for the traits of seedling height and root length concerning both varieties and doses. For the traits of seedling fresh weight and root fresh weight, significant differences were found only among the doses. As for relative water content, significant differences were observed among varieties, doses, and the interaction of varieties and doses (Table 1).

When the Table 1 was examined in terms of seedling height, it is observed that as the applied salt doses increase, the seedling height decreases in all varieties. The tallest seedling height was determined in variety C1, measuring 8.27 cm under control conditions. The shortest seedling height was 3.20 cm in variety C3 at the highest salt dose. In all applications, the longest seedling height was found in variety C1. In a study conducted by Çiçek et al. (2018) to determine the effects of salt doses on germination and seedling development of durum wheat genotypes, they reported that increasing salt concentrations resulted in reduced seedling height. Culpan and Gürsoy (2023) conducted a study with *Nigella* species and reported reductions in both species with increasing salt doses, with germination and consequently seedling development not occurring at the highest salt dose.

Table 1. Average values for the effect of salt doses applied to sunflower varieties on seedling characteristics and relative water content

Characters	Cultivars	Doses			Means
		Control (0)	50 mM	100 mM	
Seedling Length (cm)	C1	8,27	6,87	5,43	6,85 a
	C2	8,00	6,17	4,43	6,20 ab
	C3	7,10	6,10	3,20	5,47 b
	<b>Means</b>	7,79 a	6,38 b	4,35 c	
	LSD <sub>0,05</sub> :	Cultivar: 0,877		Dose: 0,861	
Root Length (cm)	C1	2,90	2,33	1,97	2,40 a
	C2	2,37	1,53	1,27	1,72 b
	C3	2,13	1,73	1,07	1,64 b
	<b>Means</b>	2,47 a	1,87 b	1,43 c	
	LSD <sub>0,05</sub> :	Cultivar: 0,353		Dose: 0,374	
Seedling Fresh weight (g)	C1	0,27	0,20	0,10	0,19
	C2	0,23	0,20	0,13	0,19
	C3	0,23	0,23	0,10	0,19
	<b>Means</b>	0,24a	0,21a	0,11 b	
	LSD <sub>0,05</sub> :	Dose: 0,068			
Root Freshweight(g)	C1	0,05	0,03	0,02	0,03
	C2	0,04	0,01	0,01	0,02
	C3	0,05	0,03	0,01	0,03
	<b>Means</b>	0,05a	0,02 b	0,01c	
	LSD <sub>0,05</sub> :	Dose: 0,010			
Relative Water Content (%)	C1	78,53 a	72,17 b	68,50 c	73,07 a
	C2	66,13 c	50,87 d	44,43 e	53,81 b
	C3	43,63 e	41,53 ef	38,87 e	41,34 c*
	<b>Means</b>	62,77 a	54,85 b	50,60 c	
	LSD <sub>0,05</sub> :	Cultivar: 2,810	Dose: 3,318	Cultivar x Dose: 3,147	

\*Different letters indicate different groups

In terms of root length (Table 1), it is determined that the root length decreases with increasing salt doses in all varieties, similar to seedling height. Variety C1 exhibited the longest root length at 2.90 cm, while variety C3 had the shortest root length at 1.07 cm. Doğan and Çarpıcı (2016) applied five different NaCl doses (0, 50, 100, 150, and 200 mM) to investigate the effects of different salt concentrations on germination of triticale lines. The study revealed that an increase in salt concentrations negatively affected radicle length, with the shortest radicle obtained from 200 mM salt concentration. Gürsoy (2023b) reported a decrease in root length with increasing salt doses in a study where priming applications were made to reduce the effects of salt stress in pea varieties.

Regarding seedling fresh weight, no statistical difference was found between 50 mM and 100 mM salt doses, and they were grouped together. However, the highest seedling fresh weight was 0.27 g in variety C1 under control conditions. The lowest seedling fresh weight was determined in varieties C1 and C3 at the 100 mM salt dose. According to these results, it is determined that seedling fresh weight is also affected by salt doses. Ertekin et al. (2017) investigated the effects of salt stress on germination parameters, root, and shoot length of some pea varieties in a study. They reported decreases in germination parameters and root and shoot length for all varieties as salt concentration increased. Kurtuluş and Boydak (2022) reported the lowest seedling fresh weight at the highest salt



dose (300 mM) in their study aimed at determining the effects of salt doses on the germination of pea varieties.

In terms of root fresh weight (Table 1), it is determined that the highest root fresh weight was under control conditions and the lowest was at the 50 mM and 100 mM salt doses. In terms of varieties, it was determined that the most tolerant varieties were C1 and C3, while the most sensitive one was C2. Türk and Alagöz (2020) reported significant decreases in root weight with salt stress in their study conducted with *Festuca arundinacea* seeds. Culpan and Gürsoy (2023) investigated the effects of salt doses on germination of *Nigella* species and reported that the highest value was under control conditions, and the lowest was at the 100 mM dose.

When examined in terms of relative water content (Table 1), it is determined that water content decreases as salt doses increase. The highest relative water content was under control conditions, while the lowest was at the 100 mM salt dose. The highest relative water content was determined as 78.53% in variety C1 under control conditions. The lowest was 38.87% in variety C3 at the 100 mM salt dose. Yakıt and Tuna (2006) investigated the effects of salt stress on maize plants and reported that relative water content decreased with salt doses, with the highest value observed under control conditions. Culpan and Gürsoy (2023) studied the effects of salt doses on germination of *Nigella* species and reported that the highest relative water content was under control conditions, and the lowest was at the 100 mM dose.

## CONCLUSIONS

In this study, the effects of salt applications on the seedling development and relative water content of sunflower varieties were investigated. The results showed that increasing salt doses had a negative impact on the examined characteristics of sunflower varieties. Significant reductions were observed, especially at the 100 mM dose. According to the results obtained from the study, it can be said that Pioneer MM54 variety is somewhat more tolerant to salinity compared to other varieties.

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## POLYMORPHISM OF GRAIN STORAGE PROTEINS IN TRITICALE LINES OF CIMMYT ORIGIN

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### ABSTRACT

A collection of seventy Mexican *Triticosecale* samples originating from CIMMYT was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The studied lines are spring forms with high resistance to yellow rust and with high productive potential under the conditions of Bulgaria. Electrophoretic analysis of grain storage proteins encoded by loci Glu-1 (Glu-A1, Glu-B1 and Glu-R1), Glu-3 (Glu-A3 and Glu-B3), Glu-B2 and Gli-R2 showed the presence of eleven alleles encoding the high molecular weight (HMW) subunits (seven for glutenins and four for secalins), six for the low molecular weight (LMW) glutenin subunits and four for 75K  $\gamma$ -secalins. The formed allelic configurations were characterized by higher polymorphism at the Glu-A1 and Glu-B1 loci, where seven alleles were identified. The number of triticale lines possessing subunits ,1' and ,2\*' at the Glu-A1 locus, coded by alleles ,a' and ,b' respectively, is the largest. These alleles are associated with good bread-making qualities of the flour. In the Glu-B1 locus, the fractional pair ,7+18' (allele 'r') was found with the highest frequency, and in the locus Glu-R1 the fractional pair ,6r+13r' (allele ,c') was most often expressed. In the area of low molecular weight glutenins with higher allelic diversity is the Glu-B3 locus. Alleles 'a' and 'b', encoding 75K  $\gamma$ -secalins 'd1' and 'd2', were identified with the highest frequency in the Gli-R2 locus. The obtained results for the allelic composition of the storage proteins of the Mexican triticale lines will find application in the selection program of the Dobrudzha Agricultural Institute (DAI) for the creation of spring forms combining high productive potential, resistance to abiotic and biotic stress and quality.

**Key words:** SDS-PAGE, Grain storage proteins, Genetic diversity, Polymorphism

### INTRODUCTION

Triticale (*x Triticosecale Wittmack*) is an artificially obtained amphiploid by hybridization between wheat (*Triticum turgidum* ssp. *durum* or *Triticum aestivum*) as a maternal component and rye (*Secale cereale*), which performs the function of pollinator. The resulting genotypes were hexaploid or octaploid depending on whether durum or bread wheat was used in the crosses with rye, respectively. The hybrids combine the good agrotechnical indicators of wheat with the high resistance to abiotic and biotic stress of rye (Salmanowicz et al., 2013; Sokol, 2014; Daskalova et al., 2021; Camerlengo and Kiszonas, 2023; Doneva et al., 2023; Sokol, 2014). The increased plasticity of triticale makes it suitable for cultivation under worse agro-meteorological conditions compared to wheat and other cereal crops (Kandrokov et al., 2019; Mergoum et al., 2019).

Triticale breeding, which began to develop very intensively in the second half of the 20th century, was aimed at creating varieties with higher yield, higher relative weight of the grain, reduced plant height and increased adaptability to changing climatic conditions. To date, the area sown with triticale

is increasing significantly, leading to a substantial increase in world production. The significant jump in the selection of triticale is due to its wide range of uses, from animal feed to various industrial applications. Although the technological and rheological properties of the dough obtained from triticale flour are of lower quality compared to those of wheat, in recent years it has been increasingly used in the modern food industry for the production of healthy foods. The reason for this is the high nutritional value of the flour. It is due to the increased protein content, the high proportion of soluble dietary fiber without starch, phenolic compounds with antioxidant activity, vitamins, etc. (Tohver et al., 2005; Jonnala et al., 2010b; Rakha et al., 2011; Dennett et al., 2013; Agil and Hosseinian, 2014; Pattison et al., 2014; Fraś et al., 2016; Langó et al., 2018b; Watanabe, 2019; Sirat et al., 2022). In addition, triticale flour is characterized by a higher level of the essential amino acid lysine, which increases the biological value of the protein, and this leads to a better balanced amino acid composition compared to wheat (Varugnese et al., 1996; Sokol, 2014; Doneva and Stoyanov, 2019; Doneva et al., 2023; Camerlengo and Kiszonas, 2023). The health benefits of consuming triticale foods have stimulated the selection of new varieties with the aim of making triticale more widely used in the human diet. Scientific research in recent years has shown that amphiploid has a high potential as an energy crop. In this regard, it is increasingly used as a source of biomass for the production of bioethanol (Bazhenov et al., 2015; Niedziela et al., 2016; Doneva and Stoyanov, 2019). Depending on the corresponding parental form of wheat, hybridization with rye produces triticale with different ploidy levels. Octaploid triticale genotypes ( $2n = 56 = AABBDDRR$ ) result from the cross between bread wheat ( $2n = 42 = AABBDD$ ) and rye ( $2n = 14 = RR$ ). Crossing between tetraploid species of wheat ( $2n = 28 = AABB$ ) and rye produces hexaploid amphiploids ( $2n = 42 = AABBRR$ ). There are also tetraploid hybrids ( $2n = 14 = AARR$ ), which are the result of the cross between hexaploid triticale and rye and subsequent self-fertilization. Breeding programs are aimed at obtaining hexaploid forms, which are more stable from a genetic point of view and realize high yields compared to octaploids, which in turn are characterized by better grain quality due to the presence of the D-genome (Lukaszewski, 2003, 2006; Daskalova et al., 2021; Camerlengo and Kiszonas, 2023). Triticale hybrids obtained by crossing wheat and rye are classified as "primary triticale". Octaploid genotypes are obtained only as primary triticale. The classification also includes "secondary triticale", obtained in one of the following ways: by crossing primary lines of triticale, by crossing primary or secondary forms with wheat and rye, or by crossing primary with secondary triticale. Hexaploid triticale are either primary or secondary depending on how the amphiploid was obtained. When tetraploid wheat is crossed with diploid rye, primary hexaploid genotypes are obtained, and when octaploid forms are hybridized, hexaploid secondary amphiploids are obtained (Oettler, 2005; Bellil et al., 2010).

Ripe triticale grains are often wrinkled, similar to rye, and more elongated than durum and bread wheat. They consist of bran, germ and endosperm. The protein content of the grain of the hybrid varies (9-16%) depending on the genotype, the environment and the applied agrotechnical practices (Rakha et al., 2011; Sirat et al., 2022). Three protein fractions have been identified in the grain endosperm: albumins/globulins, gliadins and glutenins. Albumins and globulins are known to be enzymes and physiologically active proteins that do not form gluten. In contrast, the other two groups of proteins - glutenins and gliadins - are gluten-forming (Todorov, 2006) and are the main reserve proteins that are stored in the endosperm of the triticale grain. Glutenins are high molecular weight (HMW-GS) and low molecular weight (LMW-GS). High molecular weight glutenins are x- and y-type depending on the number of cysteine residues. HMW-GS are encoded by genes that are localized to loci in the long arms of 1A (Glu-A1) (Lawrence and Shepherd, 1980), 1B (Glu-B1) (Bietz et al., 1975) and 1D (Glu -D1) wheat chromosomes. LMW-GS are encoded by genes in the wheat Glu-3 locus (Glu-A3, Glu-B3, Glu-D3), which is closely related to Gli-1 (Jackson et al., 1983). Triticale also contains glutenin-like secalin subunits (HMW-SS and LMW-SS) encoded by genes on the long arm of rye chromosome 1R (Glu-R1 or Sec-3) (Lawrence and Shepherd, 1981). Gliadins are classified as  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\omega$ -. They are encoded by genes located in the Gli-1 (Gli-A1, Gli-B1, Gli-D1) and

Gli-2 (Gli-A2, Gli-B2, Gli-D2) loci located in the short arms corresponding to wheat chromosomes 1 and 6 (Metakovsky, 1991). Triticale also possesses one  $\omega$ -secalin and two 40K  $\gamma$ -secalins encoded by genes at the Gli-R1(or Sec-1) locus (Shepherd, 1986), located on the short arm of chromosome 1R, two  $\omega$ -secalins that are encoded from genes in the Gli-R3 (or Sec-4) locus (Carrillo et al., 1992), located on chromosome 1RS and 75K  $\gamma$ -secalins, which are encoded by genes in the Gli-R2 (or Sec-2) locus, located in chromosome 2RS of rye (Shewry et al., 1984).

The aim of the present study was to identify the allelic composition of the reserve endosperm proteins in a collection of triticale lines of CIMMYT origin and, based on the obtained data, to calculate the allelic frequencies and genetic diversity in loci: Glu-1 (Glu-A1, Glu- B1, Glu-R1), Gli-2 (Gli-R2) and Glu-3 (Glu-A3, Glu-B3, Glu-B2). The obtained results will find application in the selection program of the Dobrudzha Agricultural Institute (DAI) for the creation of spring forms triticale combining high productive potential, resistance to abiotic and biotic stress and quality.

## MATERIAL AND METHOD

- Material

Seventy Mexican spring-type triticale samples originating from CIMMYT were studied. The collection is distinguished by high resistance to yellow rust and promising productive potential under the climatic conditions of Bulgaria.

- Method

A minimum of 50 grains from each accession were analyzed to determine the degree of its homogeneity. The single grains were ground to fine flour, having preliminary removed their embryos. The extraction was carried out in several consecutive stages according to Singh et al. (1991). Initially, 0.1 ml 50% (v/v) propanol, 0.08 M Tris – HCl, pH 8.0, containing 1% (w/v) fresh dithiothreitol (DTT) were added to the sample. After 1-hour incubation at 65°C, 0.1 ml 50% (v/v) propanol, containing 1.4% (v/v) fresh 4-vinylpyridine (VP) were added to each sample. Thus, the SH-groups in the probes were alkylated. This was followed by 1-hour incubation at 65°C and 10-minute centrifuge at 12000 g. 0.2 ml of each supernatant were transferred to a new Eppendorf tube and 0.2 ml solution, containing 2% SDS, 0.08 M Tris – HCl (pH 8.0), 40% glycerol and 0.02% bromophenol blue were added to it. The samples were shaken, incubated for 1 hour at 65 °C, centrifuged at 12000 g for 10 minutes, and then were ready for SDS-PAGE analysis. The additionally alkylated protein molecules prior to their treatment with SDS allowed obtaining even clearer electrophoregram. For precise identification of the allelic composition, the electrophoresis was carried out on a vertical electrophoresis system in two variants: conventional monomeric polyacrylamide gel electrophoresis by the method of Laemmli (1970) on 10% separation gel, and monomeric polyacrylamide gel electrophoresis on 17% separation gel by the method of Payne et al. (1980). By the method of Laemmli (1970), the electrophoresis occurred at constant current of 20mA on a plate at room temperature for 18-20 hours. The duration of the electrophoresis by the method of Payne et al. (1980) was 3-4 hours at 60 mA. After running the electrophoresis, the gel plates were stained with 1 % solution of coomassie brilliant blue (CBB), R250, acetic acid, methanol and water at ratio (1:5:4) overnight. De-staining was done with solution containing acetic acid, methanol, distilled water (1:2:7) until clearing of the background.

The nomenclatures of Payne and Lawrence (1983) and Vallega and Waines (1978) were used for identification of HMW of triticale. The allelic composition of LMW was determined by using the bread wheat nomenclatures of Gupta and Shepherd (1990) and Jackson et al. (1996). The allelic forms in Glu-R1, Gli-R2 and in Glu-2 were identified according to the nomenclature suggested by Amour et al. (2002a).

- Statistical analysis

The genetic variation (H) in the loci was calculated through the index of Nei (1973), where Pi was the frequency of alleles in the respective locus:  $H = 1 - \sum P_i^2$ .

## RESULTS AND DISCUSSION

Genetic variation of the glutenin and secalin subunits was found in loci *Glu-A1*, *Glu-B1*, *Glu-R1*, *Gli-R2*, *Glu-A3*, *Glu-B3* and *Glu-B2*, which were localized on the arms of chromosomes 1AL, 1BL, 1RL, 2RS, 1AS, 1BS (Table 1).

SDS-PAGE electrophoretic spectra showed that the analyzed samples were homogeneous. This result is an indicator of the presence of electrophoretic control already in the initial stages of the selection process. They are identified eleven alleles encoding the high molecular weight (HMW) subunits (seven for glutenins and four for secalins), six for the low molecular weight (LMW) glutenin subunits and four for 75K  $\gamma$ -secalins.

In locus *Glu-A1*, alleles 'c', 'a' and 'b' were identified, which coded for the high-molecular weight glutenin subunits 'N', '2\*' and '1', respectively. Allele 'a' was with the highest frequency – 52.9 %, followed by allele 'b' (28.6 %). Allele 'b' and especially allele 'a' were markers of good and high gluten quality in common winter wheat (Todorov, 2006). The lowest frequency was that of allele 'c' – 16.7 %. Allele 'c' was related to null synthesis of protein, which determined low bread-making properties. The genetic variability in this locus was above the average –  $H = 0.60$  (Table 1).

Table. 1 Frequency of alleles and genetic variation in triticale lines of CIMMYT origin

Locus	Subunit/Allele	Number of biotypes	Frequency,%
<i>Glu-A1</i> H = 0.60	1 / a	37	52.9
	2* / b	20	28.6
	null / c	13	18.6
<i>Glu-B1</i> H = 0.34	7+18 / r	56	80.0
	7+9 / c	9	12.8
	7+8 / b	2	2.9
	23+18 / p	2	2.9
	6.8+20y / s	1	1.4
<i>Glu-R1</i> H = 0.23	6r+13r / c	61	87.1
	6.5r / e	6	8.6
	2r+6r / ni	2	2.9
	5.8r/g	1	1.4
<i>Glu-A3</i> H = 0.42	d	52	74.3
	d'	10	14.3
	e	6	8.6
	a	2	2.8
<i>Glu-B3</i> H = 0.66	h	38	54.3
	b	9	12.9
	k	8	11.4
	b'	6	8.6
	h'	5	7.1
	i	4	5.7
<i>Glu-B2</i> H = 0.00	B	70	100.0
<i>Gli-R2</i> H = 0.47	d1 / a	49	70.0
	d2 / b	11	15.7
	t1 / c	8	11.4
	null / d	2	2.9

ni - unidentified allele

A significant polymorphism of the storage endosperm proteins was found in locus Glu-B1, where five allelic forms were identified. A similar trend has been found in other studies (Amiur et al., 2002b; Bellil et al., 2010). Alleles 'b' (2.9 %), 'p' (2.9 %) and 's' (1.4 %), coding for subunit pairs '7+8', '23+18' and '6.8+20y' were comparatively rare. Allele 'c' was identified more frequently (12.8 %). The heritability potential of locus Glu-B1 was concentrated in allele 'r', coding for subunit pair '7+18' to the highest degree (80.0 %). This pair of subunits was first identified in the electrophoretic spectra of Portuguese triticale cultivars (Igrejas, 1999) and occurs only in the Glu-B1 locus of this cereal (Amiur et al., 2002b). The genetic variability in locus Glu-B1 was a under the average –  $H = 0.34$  (Table 1).

In locus Glu-R1 subunit pair '6r+13r' coded for by allele Glu-R1c, was dominant (87.1 %). Allele Glu-R1e (6.5r), identified in six lines, was with lower frequency (8.6 %), followed by unidentified allele encoding fractional pair 2r+6r, which was found in two accessions (2.9 %). The Glu-R1g allele occurs with the lowest frequency (1.4 %). In this allelic composition, the calculated genetic variability in locus Glu-R1 was below the average - 0.23 (Table 1). The low value of the indicator in locus Glu-B1 and locus Glu-R1 is due to the concentration of the hereditary potential of the loci mainly in one allele - 'r' in Glu-R1 and 'c' in Glu-B1, which have been found to occur with a very high frequency in many European triticale cultivars (Amiur et al., 2002a; Amiur et al., 2002b; Bellil et al., 2010; Doneva and Stoyanov, 2019).

75K  $\gamma$ -secalins coded for by locus Gli-R2 were represented by four allelic variants – 'a', 'b', 'c' and 'd'. The most frequent allele Gli-R2a (d1) was found in 49 genotypes (70 %), and alleles Gli-R2b (d2) and Gli-R2c (t1) were identified in 11 (15.7 %) and 8 (11.4 %) accessions, respectively. A 'null' allelic variant (Gli-R2d) of 75K  $\gamma$ -secalins was identified in the electrophoretic spectra of two lines. The genetic variability in locus Gli-R2 was 0.47 (Table 1).

In the analyzed triticale collection, eleven low-molecular weight glutenin subunits (LMW-GS) were identified. The alleles GluA3d (74.3 %) and GluA3d' (14.3 %) were found in the Glu-A1 locus with the highest frequency in the analyzed genotypes. GluA3e allele occurs with a lower frequency (8.6 %), while allele GluA3a was registered in the spectrum of only one accession. In this allelic composition the index of genetic variability was 0.42 (Table 1).

In locus Glu-B3 the main part of the heritability potential of the analyzed collection was concentrated in allele Glu-B3h (54.3 %). Next in frequency were alleles Glu-B3b (12.9 %), Glu-B3k (11.4%). Alleles Glu-B3b', Glu-B3h' and Glu-B3i were with the lowest frequency. They were identified in six, five and four genotypes, respectively. In this locus the genetic variability was comparatively high –  $H = 0.66$  (Table 1).

Locus Glu-B2 was characterized by extremely low polymorphism represented by one allelic variant - Glu-B2b. This determined the nil value of the parameter genetic variability (Table 1).

The identified alleles of the reserve endosperm proteins in loci Glu-1 (Glu-A1, Glu-B1), Glu-3 (Glu-A3, Glu-B3), Glu-B2 and Gli-R2 form 24 allelic configurations, which are presented in Table 2.

The allelic composition of glutenins and gliadins is a determining factor for triticale quality. Glutenins encoded by chromosomes 1A and 1B have been shown to influence gluten quality characteristics much more than glutenins encoded by chromosome 1R. The alleles 'a' (subunit 1) and 'b' (subunit 2\*) at the Glu-A1 locus found with high frequency in the present study have a high quality score and increase gluten quality (Todorov, 2006). The high molecular weight glutenin alleles encoded by the Glu-B1 locus also have a very strong effect. In our study, the allele 'r', encoding the fractional pair '7+18', was found with the highest frequency, followed by the allele 'c', encoding the pair of subunits '7+9'. The 'b' (7+8) allele is less common. This allele, as well as the 'd' allele (6+8), has been shown to have the strongest effect on gluten quality in triticale (Salmanowisz et al., 2013).

Tohver et al. (2005) found that the high molecular weight glutenin subunit 2\* (chromosome 1A) and the fractional pairs 7+26 and 7+19 (chromosome 1B) represented the best combination of alleles in the amphiploid with a positive effect on gluten quality. Several authors prove that glutenins encoded by the *Glu-R1* locus, inherited from rye, negatively affect the quality parameters, and the different combinations between HMW-GS/SS and LMW-GS/SS can significantly change the baking qualities of the different genotypes (Makarska et al., 2008; Belill et al., 2010; Camerlengo & Kiszonas, 2023). In triticale grain, the proportion of  $\omega$ -gliadins is greater compared to  $\alpha$ -,  $\beta$ - and  $\gamma$ -gliadins (Pruska-Kędzior et al., 2017).  $\alpha$ - and  $\beta$ -gliadins are not involved in the formation of gluten (Salmanowicz and Novak, 2009) and, together with low molecular weight glutenins, have a weaker effect on the viscoelastic properties of triticale dough. The most significant influence on the formation of strong gluten in triticale is the absence of the D-genome. It has been proven that through introgression of high- and low-molecular glutenin alleles encoded by the 1D-chromosome, the quality of triticale lines and varieties is significantly increased (Lukaszewski, 2006; Martinek et al., 2008; Jonnala et al., 2010; Daskalova et al., 2021).

Table 2 Allelic configuration of storage proteins of triticale lines of CIMMYT origin

№	HMW			LMW			75K $\gamma$ -sec
	<i>Glu-A1</i> subunit/ allele	<i>Glu-B1</i> subunit/ allele	<i>Glu-R1</i> subunit/ allele	<i>Glu-A3</i> allele	<i>Glu-B3</i> allele	<i>Glu-B2</i> allele	
1	2*/b	7+18/r	5.8 <sup>f</sup> /g	a	h	b	d1/a
2	2*/b	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	d	h	b	t1/c
3	2*/b	7+18/r	2 <sup>f</sup> +6 <sup>f</sup> /c	d	b	b	d1/a
4	Null/c	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	d	h	b	d1/c
5	Null/c	7+9/c	6 <sup>f</sup> +13 <sup>f</sup> /c	a	h	b	d1/c
6	1/a	23+18/p	6.5 <sup>f</sup> /e	e	i	b	d2/b
7	2*/b	7+18/r	6r+13r/c	d	i	b	d1/a
8	1/a	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	d	k	b	d1/a
9	1/a	7+18/r	6.5r/e	d'	i	b	d1/a
10	Null/c	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	d'	b	b	t1/c
11	1/a	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	e	i	b	t1/c
12	N/c	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	d	h'	b	t1/c
13	Null/c	7+8/b	6 <sup>f</sup> +13 <sup>f</sup> /c	d	h	b	t1/c
14	1/a	7+9/c	6.5r/e	d'	b	b	d1/a
15	1/a	7+18/r	6.5r/e	d	h'	b	t1/c
16	1/a	7+18/r	6.5 <sup>f</sup> /e	d'	b	b	d2/b
17	2*/b	7+9/c	6 <sup>f</sup> +13 <sup>f</sup> /c	d'	k	b	d2/b
18	1/a	7+9/c	6r+13r/c	d'	b	b	d2/b
19	1/a	7+9/c	6 <sup>f</sup> +13 <sup>f</sup> /c	d	h	b	d1/a
20	Null/c	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	e	k	b	t1/c
21	1/a	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	d	h	b	d2/b
22	Null/c	7+18/r	6 <sup>f</sup> +13 <sup>f</sup> /c	d	h	b	d2/b
23	2*/b	7+8/b	6 <sup>f</sup> +13 <sup>f</sup> /c	d	h'	b	Null/d
24	Null/c	7+9/c	2 <sup>f</sup> +6.5 <sup>f</sup> /b	e	k	b	Null/d

The identified allelic combinations in the Mexican triticale lines, which are not found in the Bulgarian varieties selected in Dobrudzha Agricultural Institute – General Toshevo (Doneva and Stoyanov, 2019), combined with the high resistance to yellow rust and the promising productive potential under



the climatic conditions of Bulgaria, show that these samples can find application as new sources of genes in our selection program with the aim of improving the quality and expanding the possibilities of application of the cereals.

## CONCLUSIONS

Based on the study, the established alleles at loci Glu-A1, Glu-B1, Glu-R1, Glu-A3, Glu-B3, Glu-B2, Gli-R2 form twenty-four configurations. The frequencies of Glu-A1*a* and Glu-A1*b* alleles, which are quality indicators, are relatively high. In the Glu-B1 locus, the proportion of Glu-B1*c* and Glu-B1*b* alleles that increase baking qualities is relatively low. A fractional pair 7+18, which is encoded by the Glu-B1*r* allele, was found in 56 % of the studied lines. Its influence on the final quality indicators has not yet been clarified. The identified alleles at loci Glu-R1 and Gli-R2 were inherited from rye. They have a negative effect on quality, but increase resistance to abiotic and biotic stress. The highest values of the index of genetic variation (H) were calculated at loci Glu-A1 and Glu-B3. At the Glu-B2 locus, the value of this indicator is zero.

The results obtained in the present study show that the triticale lines from CIMMYT have a high potential for quality and sustainability and are included in the triticale breeding program of the Dobrudzha Agricultural Institute.

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## **ROLE OF POST-HARVEST RESIDUE TREATMENT ON THE WHEAT PRODUCTIVITY, FLOUR PROPERTIES AND BREAD-MAKING QUALITIES**

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### **ABSTRACT**

The effect of the soil tillage and sowing machines (Traditional system - TS and Combination - CS) and of the type of the previous crop post-harvest residue treatment (common bean, maize, sunflower) on the wheat flour properties and bread-making qualities were investigated in six-field crop rotation. The wheat post-harvest residues (PHR) were utilized in three different ways (removed from the field -RF; chopped and subsequently incorporated into the soil - I; and burned - B). The trial was carried out at Dobrudzha Agricultural Institute on Haplic Chernozems.

It is characteristic for the research period that the bean predecessor in both systems increases the farinographic evaluations of stretchability and stability of the dough, degree of softening and the number of the quality coefficient of the flour. Except for the softening degree data, the TS has contributed higher data values for the listed indicators compared to the CS one. Sedimentation and wet/dry gluten content highest after maize predecessor and again with TS.

The removal of plant residues from the field leads to an increase in the values of sedimentation (TS-CS), wet gluten (TS), dough stability (TS-CS) and especially the rheological properties. Contrary, incorporation of plant residue into the soil leads to a noticeable decrease in the values of these indicators. However, in the case of CS, the same leads to an increase in the values of the degree of softening. It was also found that the burning of post-harvest residues and CS leads to higher values of wet gluten and increases the extensibility of the dough.

The years with an optimal combination of the main meteorological elements (2017) have a significant contribution to obtain higher values for sedimentation, wet/dry gluten and farinographic indices for quality. In years with an insufficient amount of precipitation combined with higher temperatures in critical phases of the permanent wheat vegetation (2018), the dough extensibility and degrees of softening are higher compared to the other years.

Bread volume is the quality characteristic that is practically unaffected by the way of using the post-harvest residues and by its interactions with other factors in both tested systems. Its values in both systems are mainly influenced by the meteorological factor, and this fact is to a much greater extent valid for TS compared to CS. Multiple correlations were established between the tested qualitative characteristics of the tested indicators by years of research and average for the period. They differ both in the direction of interaction and in the strength of the correlation dependences by sowing systems.

**KEY WORDS:** Ways of utilization of post harvest residue, wheat, flour properties, bread-making qualities

### **INTRODUCTION**

The qualitative characteristics of the wheat grain are a complex of chemical composition of the grain, biochemical, technological and bakery properties. They are highly dependent both on the genetics of the variety and, in general, on the growing conditions. The latter, in turn, include the system of agriculture, the characteristics of the soil type and care for its fertility, the type and method of introducing nutrients, the soil tillage systems in the crop rotation.

The current stage of agricultural production in the world requires expanding knowledge about the impact of plant residues from the previous crop on soil properties and the development, productivity and quality of the next crop in the crop rotation. The processes of desertification, reduction of the area of agricultural lands worldwide and a number of crises accompanying the modern human world further strengthen the need to expand our knowledge of post-harvest residues so that they can be maximally effective. Their amount as total biomass, nutrient content, C/N ratio and others depend on the type of crop and its cultivation technology (Singh et al. 2005, Shi 2013, Torma et al., 2018). According to Matumba et al. (2021) cereal crops are considered highly susceptible to various types of infections during the pre- and post-harvest stages. A number of studies show that there is undoubtedly a connection with what plant residues contain, including the possibility of provoking the development of diseases, multiplication of enemies, decomposition time and others (Medina et al., Abou Dib et al., 2022; 2017, Gómez et al., 2022).

We are witnessing real climate change that seems inevitable. This fact threatens terrestrial ecosystems and the production of agricultural products. According to Maity et al. (2023) climatic anomalies, may not always seriously affect the yield, but may reduce the morphological, physiological and biochemical quality of the produce. A special imprint on the biological value of the quality indicators is left by the climatic conditions, which together with the overall agrotechnics put every variety to a serious test (Nankova et al, 2020).

The aim of the research is to evaluate the changes in a number of quality indices characterizing the bread-making properties of the Enola variety, depending on the type of plant residues of the predecessors and the ways of their use.

## **MATERIAL AND METHODS**

The research was conducted during the period 2017-2019 in a long-term stationary field experiment in the Experimental Field of at Dobrudzha Agricultural Institute on Haplic Chernozems. The same is the 6-field six-field crop rotation (grain maize–wheat–sunflower–wheat–bean–wheat). Plant post-harvest residues (PHR) from the 3 predecessors of wheat (beans-sunflower-corn) are utilized in three different ways: they were removed from the field (RF); they were burned (B) and they were chopped and subsequently incorporated into soil (CSIS).

The trial was performed on 10 ha area in 4 replicates. The mineral fertilization of the spring crops in the crop rotation were applied as follows: bean –  $N_{60}P_{100}K_0$ ; sunflower –  $N_{60}P_{100}K_0$ ; and maize –  $N_{120}P_{100}K_0$ . Two systems of machines - traditional and combined - were used for tillage and sowing. Soil preparation in the traditional system (TS) includes 2-fold discing and sowing with SZU-3,6. The combined system is the aggregation of a Fendt 820 Vario tractor and a Horsch 6.0 planter with a preliminary single discing, and the second is together with sowing. In both systems, sowing was carried out at a rate of 500 g.s/m<sup>2</sup>.

During the study, the changes in the values of the following indicators, characterizing the bakery properties of the Enola variety, were investigated: sedimentation value of flour, ml (SED) (Pumpyanskii, 1971); wet gluten content in grain, % (WGC) (BSS 13375-88); dough development, min (DD); stability of the dough, min (SD); degree of softening, f.u. (DS); number of the quality coefficient of the flour (NQCF); bread loaf volume, ml (LVol). The preparation of the samples and their grinding was carried out on a mill MLU-202 up to 70% flour.

The results were processed statistically using analysis of variance (Anova), while the significance of differences between mean values was evaluated with the Waller-Duncan's HSD test,  $P < 0.05$ . The value of variability for traits was determined and expressed by coefficient of variation of traits. Pearson correlation coefficients (“R coefficients”) were computed and tested for significance.

## RESULTS AND DISCUSSIONS

At the Dobrudja Agricultural Institute, research on the use of plant residues dates back to the beginning of the 21<sup>st</sup> century and covers the ways of using different types of plant post-harvest residues (PHR) on the productivity and quality of the crops included in the crop rotation (Nankova et al., 2010; Iliev et al., 2018; Iliev, 2021), as well as the use of various products to accelerate their decomposition (Milev et al., 2014). Some of the obtained results have been published in various international forums and scientific journals. The present publication is the first to treat the influence of plant residues from different predecessors and their utilization methods on the baking qualities of the Enola variety.

The reaction of this wheat cultivar to the tested factors in the experiment was highly expressed both by year and depending on the tested sowing systems (Table 1). In 2017, all quality indices have higher values for TS of sowing compared to CS with a pronounced variation between min and max values. The LVol and SED indices are characterized by the lowest variation, and the difference between the systems is insignificant. The yield of wet gluten (WGC) in the grain is characterized by an increase in CV% values, but they are still below 30%. The number for the quality coefficient of flour (NQCF) shows the largest difference in CV% values - 11.90 % for TS and 54.52% for CS. The latter is an indication of high scatter in the data and, accordingly, non-uniformity of the sample. The variation in the data characterizing the rheological properties of the dough depending on the used system of seeding machines is also characterized by a strong dynamic in the indicated statistical parameters.

Table 1. Degree of variation in the performance of the quality parameters according to sowing systems by years of investigation

Stat. Parameters	SED, ml		WGC, %		DD, min		SD, min		DS, f.u.		NQCF		LVol	
	TS	CS	TS	CS	TS	CS	TS	CS	TS	CS	TS	CS	TS	CS
2017														
Minimum	34.00	29.00	16.30	13.10	2.00	1.90	2.00	1.80	33.00	33.00	74.00	0.00	555.00	550.00
Maximum	54.50	51.50	28.90	27.60	5.55	4.85	8.30	8.30	110.00	111.00	110.00	114.00	665.00	645.00
<b>Mean</b>	<b>44.04</b>	<b>40.22</b>	<b>23.28</b>	<b>20.42</b>	<b>3.37</b>	<b>3.08</b>	<b>5.50</b>	<b>4.83</b>	<b>80.47</b>	<b>71.39</b>	<b>89.72</b>	<b>72.22</b>	<b>615.00</b>	<b>604.17</b>
Std. Deviation	7.58	6.95	4.37	5.30	1.30	1.22	2.00	2.10	26.89	26.70	10.68	39.38	28.18	29.42
CV%	17.21	17.29	18.75	25.96	38.59	39.72	36.34	43.42	33.42	37.41	11.90	54.52	4.58	4.87
2018														
Minimum	8.00	6.00	0.00	2.10	2.95	3.00	2.10	2.20	41.00	46.00	203.00	192.00	500.00	500.00
Maximum	53.00	56.00	22.80	26.30	4.25	4.45	3.15	3.60	58.00	62.50	253.00	275.00	605.00	650.00
<b>Mean</b>	<b>32.89</b>	<b>36.17</b>	<b>12.29</b>	<b>14.82</b>	<b>3.40</b>	<b>3.79</b>	<b>2.62</b>	<b>2.80</b>	<b>49.65</b>	<b>51.51</b>	<b>229.11</b>	<b>225.72</b>	<b>557.22</b>	<b>564.72</b>
Std. Deviation	19.12	19.64	8.56	7.86	0.38	0.44	0.30	0.36	4.67	5.24	15.04	21.26	25.22	38.10
CV%	58.13	54.31	69.68	53.02	11.22	11.68	11.38	12.89	9.41	10.18	6.57	9.42	4.53	6.75
2019														
Minimum	32.00	32.00	4.30	4.10	1.70	1.70	1.80	2.00	29.00	28.00	59.00	55.00	575.00	535.00
Maximum	46.00	42.00	22.65	21.05	6.30	6.30	9.55	8.05	121.00	102.00	118.00	121.00	679.00	660.00
<b>Mean</b>	<b>39.28</b>	<b>36.94</b>	<b>14.77</b>	<b>12.00</b>	<b>2.48</b>	<b>2.41</b>	<b>5.94</b>	<b>5.10</b>	<b>75.22</b>	<b>67.17</b>	<b>86.56</b>	<b>88.50</b>	<b>628.00</b>	<b>601.94</b>
Std. Deviation	5.14	2.86	5.58	5.12	1.40	1.42	3.15	2.39	35.50	29.34	16.61	17.91	31.02	34.60
CV%	13.09	7.74	37.81	42.70	56.70	58.66	53.09	46.78	47.20	43.68	19.19	20.24	4.94	5.75

During the next two years of the study, we observed a highly changed reaction of the variety depending on the weather conditions. Its detailed characterization is presented in Nankova and Iliev (2023). The strong drought in critical phases of the spring vegetation in 2018 leads to the lower average values for SED, but with a much more pronounced variation between the maximum and minimum values of the indicator and in the 2 systems. Year conditions also contributed to lower absolute values of dough stability (SD) and degree of softening (DS) during the study. At the same time, the average values of the number for the flour quality factor (NQCF) marked its highest absolute values and lowest CV%.

Dough development time (DD) was characterized by relatively close values, regardless of the seeding machine systems used during the years of research. However, this fact is accompanied by a very large dynamic of variation, which is why the sample in 2019 is the most heterogeneous. Of all the indices studied over the years, the volume of the bread is the quality characteristic that is subject

to the lowest dynamics in this experimental setup. CV% values ranged from 4.58% to 6.75% during the study period.

During the years of research, the sedimentation of Enola variety flour is significantly influenced by the type of plant residues of the previous crop and the ways of their utilization (Figure 1). In the year with the most favorable combination of the main meteorological elements (2017), the bean predecessor increased the SED values and, regardless of the cropping systems, maize proved to be a better predecessor than sunflower. Definitely with higher SED<sup>s</sup> is the flour when using TS. The values for the rheological properties of the dough are subject to significant differentiation depending on the type of plant residues of the previous crop and the methods of their utilization in the agricultural technique used. In severe drought during the perennial vegetation (2018) it is distinguished by the highest fluctuation in the obtained SED results depending on the type of predecessor. It was found that regardless of the machinery system, after the maize predecessor, the flour had the highest SED and the bean the lowest. In 2019 (with rainfall below the climatic norm, but relatively evenly distributed), again the predecessors beans and corn are better compared to sunflower. The influence of the cropping systems used on the mean SED values was most pronounced after the predecessor bean and when TS was used. A stronger positive influence of CS was found after sunflower PHR.

The WGC index reached a maximum value (26.45%) for the entire study period in 2017, with the bean predecessor and TS leading to better results. Under conditions of severe moisture deficit (2018), the use of CS contributes to obtaining higher values (14.82%) compared to TS (12.99%). After the bean predecessor, the influence of the systems on the WGC values was practically equalized, while after maize and sunflower, the application of CS definitely gave better results compared to the traditional way of sowing. In 2019, better average scores were again obtained for the indicator after the implementation of TS (14.77%) compared to CS (12.00%).

During the first two years of the study, growing wheat under TS resulted in an increase in dough development time (DD) compared to CS. Under the conditions of a moderate moisture deficit, but relatively uniform distribution of precipitation, an approximately equal response was found in the values of this indicator for the tested sowing machine systems. Dough development (DD) took relatively longer after predecessor beans in 2017 and 2019. Conversely, severe drought conditions during grain formation lead to a reduction in DD when growing wheat after beans and an increase after sunflower and corn. In general, in 2019 the dough formation time was shorter compared to the other years of the study.

Dough stability (SD) values show that application of TS in 2017 and 2019 contributes to obtaining dough with higher stability (SD) compared to CS (5.50-4.83). In 2018, the contribution of CS to obtaining a more stable dough is greater compared to that of TS. Using the CS with all three predecessors consistently gives better results. Predecessor maize (2018) has the greatest contribution to obtain dough with high stability. 2019 is the year in which the dough has the highest stability. The greatest contribution to this is made by the plant residues of beans, followed by those of maize and sunflower, whose values are close. In the sunflower PHR, the difference in influence of the systems is unnoticeable, while in the maize PHR it is drastically in favor of TS.

The values for the degree of softening of the dough (DS f.u.) show a strong differentiation depending on the type of plant residues of the predecessor. Similar to dough stability (SD) in 2019, values obtained for the indicator were higher for TS compared to CS. For 2018, the trend for the strong influence of CS on the values of the indicator compared to the cultivation of wheat under TS has been preserved. Utilization of sunflower residues in 2017 contributed to increase the values for the degree of softening of the dough when applying CS compared to TS. In 2018 on its impact on DS f.u. predecessors are arranged in the following order: corn>sunflower>bean. Characteristically, in 2019, the application of CS after a sunflower PHR leads to obtaining higher values of the indicator compared to TS. In the case of predecessor maize, a sharp drop in the values of the indicator was found at CS (40.33) compared to TS (71.33).

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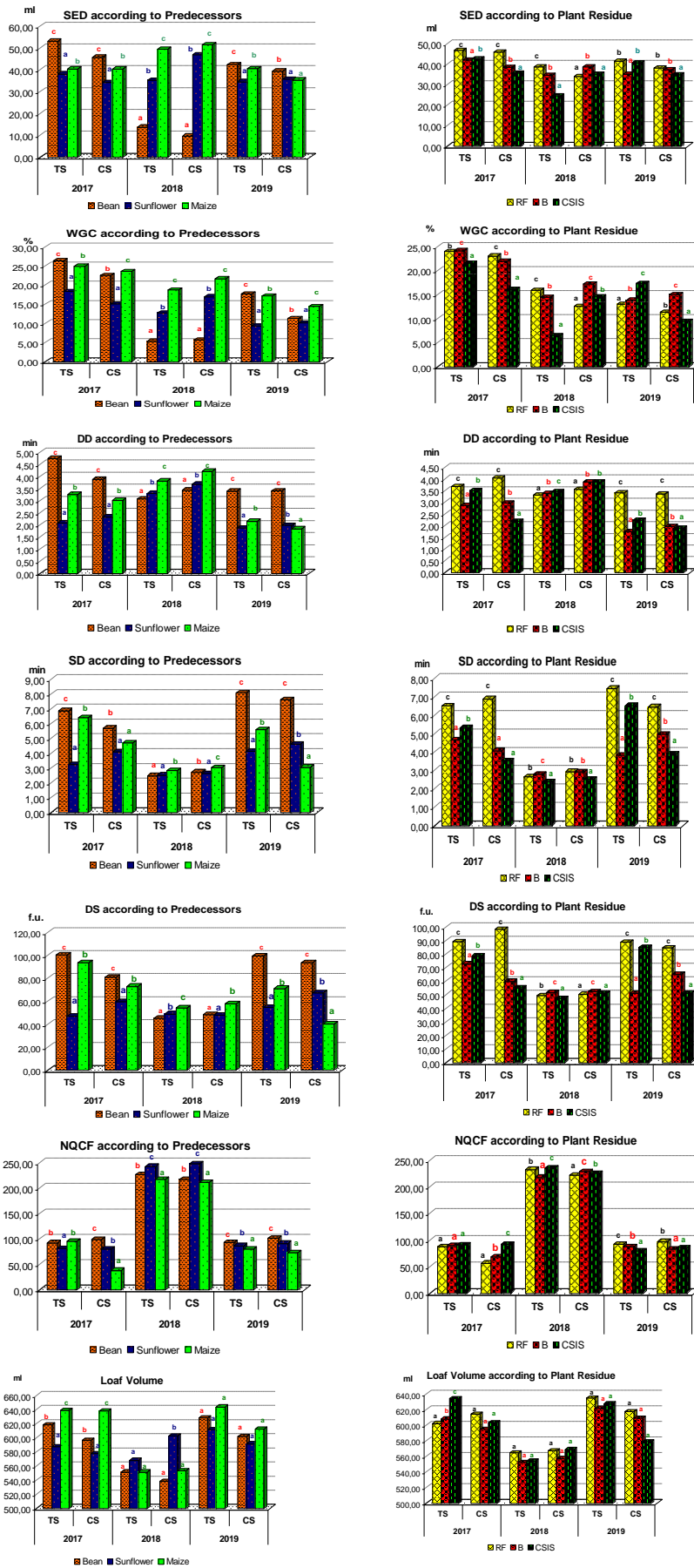


Figure 1. Dynamics in quality characteristics of flour and dough by years of research depending on the factors in the experiment



The differentiation in the values of the quality coefficient of the flour (NGCF) according to the type of plant residues of the predecessor varied according to the tested sowing machine systems and according to the weather conditions during the years of the study. In 2017, no strong differentiation was found depending on the type of plant remains of the predecessor in TS, while in CS the same was maximally manifested. In traditional sowing, bean and maize predecessors have approximately equal impact on NGCF, and after sunflower PHR the values of the indicator are the lowest. In the use of CS, the influence of predecessors was in the following order: bean>sunflower>maize. Regardless of this fact, the values of the indicator when using TS (89.72) are higher compared to those obtained with CS (72.22). This is the year with the lowest values of the index. The NGCF has the highest values for the experiment period in 2018. Differentiation between predecessors is reliable, with both systems having the highest values after sunflower, followed by beans and maize PHR. In 2019 NGCF has lower values compared to 2018. The differentiation between predecessors is also credible. In terms of influence, the contribution of predecessors PHR is in the following order: beans>sunflower>maize.

Statistically significant differentiation for the influence of plant residues from the predecessor on the volume of bread (LVol) was found only in 2017 when using TS. The impact of the tested PHR was practically equalized. In 2018 and 2019, the differences in LVol values were statistically implausible. The year with the lowest values for the volume of bread is 2018, and in 2019 - bread has the largest volume.

What will be our attitude to the plant residues of the predecessor and our agrotechnical solutions for their utilization over the years also have a significant impact on the quality characteristics of the grain, flour and the rheological properties of the dough.

The sedimentation value of flour (SED) is significantly influenced by weather conditions and methods of utilization of plant residues from the previous crop. In years with favorable conditions field RF and TS seeding are preferred. In the conditions of strong water deficit (2018), the burning of plant residues and the use of CS leads to the obtaining of higher SEDs compared to the other two ways of their utilization. Returning the entire amount of plant mass from the predecessor in suitable condition (CSIS) is only recommended when using TS for soil preparation and seeding. Under conditions of uniform water deficit during the growing season (2019), flour sedimentation has higher values at TS. In this sowing method, the best results were obtained in the following order RF>CSIS>B, while CS sowing method ranked the ways of using the post-harvest residues as follows: RF>B>CSIS.

Favorable weather conditions (2017) lead to obtaining more WGC for the study period and a significant contribution of the use of TS compared to CS. RF and B of PHR have relatively equal influence on gluten, while when they are plowed, especially CS there is a sharp drop in the values of the indicator - by more than 5 points compared to B and by more than 7 points - in RF. CS sowing has a significant advantage over TS in terms of WGC values under drought conditions. When applying TS, a sharp drop in the values of the obtained results was observed in the direction from RF to CSIS, while with CS, the highest values for gluten were obtained in the variants with B and the lowest - at RF. The positive influence of TS compared to CS on WGC values was again established in 2019, with both systems being the highest after B.

Dough formation time (DD) in RF and using CS exceeded the same in TS. In both sowing systems, B results in approximately the same values, while in CSIS the formation of the dough in TS is longer compared to CS. In the conditions of critical droughts (2018), regardless of the method of RO utilization, the use of CS leads to an extension of the dough formation time compared to TS. Within each of the systems, the differences between individual ways of treating PO are narrowed. RF in both systems speeds up dough development time. In 2019, the formation of the dough in both systems takes approximately the same time. The same is shortest in the PHR burn variant, followed by CSIS. The variant with RF is characterized by the longest time for forming the dough.

Higher dough resistance (SD) in 2017 was found in the RF variant, and for this fact the contribution of CS is significant. The stability of the dough is reduced when burning the PHR, and the difference between the two systems is too small. Severe water deficit (2018) lowers dough stability and reduces variation in PHR usage patterns. On the contrary, with a uniform water deficit during the growing season (2019), a strong differentiation is observed depending on the ways of using the predecessor PHR. In both systems, RF has the most significant contribution to increasing dough stability. For TS, CSIS also scores highly for this index, but CS is highly inadvisable for PHR incorporation.

For the degree of softening of the dough (DS f.u.) it is characteristic that under the conditions of moisture deficiency (2018 and 2019) the variants with B and use of CS obtained higher values for this indicator compared to TS. It was found that CSIS, especially those from maize, in 2019 resulted in a sharp decrease in the degree of softening of the dough. DS f.u. in 2017 it had the highest values at RF of PHR, and at CS of sowing the values were higher than those obtained at TS of sowing.

Higher flour quality coefficient (NGCF) was found in CSIS of PHR, followed by RF and lowest - in B. This trend was observed in 2018 in CS at sowing and in 2019 - TS. No differentiation was found depending on the method of utilization of plant residues in TS of sowing in 2017, while in CS the same was clearly manifested. The highest flour quality coefficient was found for CSIS and the lowest for the RF variant of PHR.

Within the experiment, there is a differentiation in the values for the volume of the bread only by years of study. On average, for this time period, neither the type of plant residues from the previous crop, nor the methods of their utilization reliably influence the volume of bread for the period 2017-2019.

Based on the statistical analysis, the reliability of the above-mentioned differences between the tested factors in the experiment and their influence on the quality characteristics of the grain, flour and the rheological properties of the dough has been established (Table 2). On average for the studied period, only LVol is not statistically reliably affected by the independent effect of the type of plant residues of the previous crop and the way of their use. Of all the types of interactions, the same indices is reliably affected only by the combined interaction *Year x Predecessor*.

The average values of the tested quality characteristics for the period 2017-2019 for the approach to each machine system show the strong influence of the conditions of the year (Table 3). For both tested seeding machine systems, the highest values for SED, ml and WGC,% indicators were obtained in 2017 when seeding was carried out in a traditional way. In terms of average data for dough development time, its stability and quality factor number, the values were the lowest in 2018. The same applies to the LVol, but the statistical analysis of the data shows that the differences found are unreliable. The year with extreme droughts in critical phases during the growing season and both systems are characterized by the highest values for the degree of softening of the dough.

The role of PHR of the predecessor is clearly expressed. With the traditional method of sowing, with the exception of the degree of softening of the dough (DS f.u.), all quality characteristics have the lowest values in the predecessor sunflower (Table 4). This trend is less pronounced for CS and does not affect indicators such as SED, WGC, DS f.u. and NGCF. The most significant difference in the influence of the two systems was found in the indices WGC and SED, where in the variants with the bean's PHR the superiority of TS compared to CS was more pronounced. For the specified quality characteristics, this increase is respectively 25.10% and 15.14%. Traditional sowing after PHR of corn greatly increased the values of the rheological properties SD, DS f.u. and NQCF. This increase compared to CS is respectively 37.10%, 27.98% and 21.76%.

Comparing the two systems of sowing after PHR of sunflower, the obtained results show that, despite the lower values after this predecessor, CS has a more pronounced positive influence on quality compared to TS. This fact is most pronounced in the indices SD (min) and DS f.u., whose values in TS are respectively 87.14% and 86.21% of those obtained in CS. It was also found that the quality results obtained after the sunflower predecessor were below the average of the three predecessors and this fact was most pronounced for the DS f.u. and WGC.

Table 2. Analysis of variances of bread making qualities according to the ways of PHR utilization over years and averaged for the period 2017 – 2019.

Source 2017-2019	Dependent Variable	df	TS			CS		
			Mean Square	F	Sig	Mean Square	F	Sig
Years (1)	SED	2	563.43	684.17	.000	83.39	82.41	.000
	WGC	2	598.34	249502.00	.000	330.73	86277.28	.000
	DD	2	4.96	4116.81	.000	8.51	7070.191	.000
	SD	2	58.51	30822.15	.000	28.56	181.09	.000
	DS f.u.	2	119284.13	21399.81	.000	127971.46	19744.17	.000
	NQCF	2	4893.59	5618.54	.000	1975.33	2343.84	.000
	LVol	2	25550.30	56.05	.000	8838.89	13.03	.000
Predecessor (2)	SED	2	318.25	386.45	.000	549.76	543.32	.000
	WGC	2	211.90	88361.33	.000	240.57	62756.40	.000
	DD	2	7.80	6481.39	.000	3.81	3168.12	.000
	SD	2	29.51	15546.15	.000	16.74	106.14	.000
	DS f.u.	2	245.13	43.98	.000	6134.46	946.46	.000
	NQCF	2	4769.52	5476.08	.000	1685.63	2000.09	.000
	LVol	2	2272.24	4.99	.014 <sup>NS</sup>	2279.17	3.36	.050 <sup>NS</sup>
PlantResidue (3)	SED	2	205.85	249.97	.000	82.34	81.37	.000
	WGC	2	35.70	14888.18	.000	99.33	25911.58	.000
	DD	2	2.94	2442.81	.000	4.85	4026.12	.000
	SD	2	14.27	7519.24	.000	21.17	134.22	.000
	DS f.u.	2	151.24	27.13	.000	428.13	66.05	.000
	NQCF	2	1403.68	1611.63	.000	3085.50	3661.10	.000
	LVol	2	562.24	1.23	.307 <sup>NS</sup>	1254.17	1.85	.177 <sup>NS</sup>
1 x 2	SED	4	1055.13	1281.25	.000	1417.93	1401.32	.000
	WGC	4	150.93	62937.28	.000	162.61	42419.39	.000
	DD	4	3.87	3214.39	.000	2.66	2205.46	.000
	SD	4	9.18	4833.73	.000	9.91	62.82	.000
	DS f.u.	4	650.35	116.67	.000	1610.82	248.53	.000
	NQCF	4	1755.97	2016.10	.000	1747.48	2073.47	.000
	LVol	4	1953.07	4.28	.008	5468.06	8.06	.000
1 x 3	SED	4	112.43	136.53	.000	73.06	72.20	.000
	WGC	4	82.21	34279.55	.000	32.47	8469.50	.000
	DD	4	1.32	1099.39	.000	2.35	1952.14	.000
	SD	4	6.24	3287.82	.000	4.36	27.66	.000
	DS f.u.	4	324.96	58.30	.000	993.16	153.23	.000
	NQCF	4	814.64	935.32	.000	979.63	1162.38	.000
	LVol	4	839.74	1.84	.150 <sup>NS</sup>	988.89	1.46	.242 <sup>NS</sup>
2 x 3	SED	4	264.18	320.80	.000	12.41	12.27	.000
	WGC	4	33.41	13929.49	.000	49.34	12871.65	.000
	DD	4	.70	579.64	.000	1.56	1298.54	.000
	SD	4	8.32	4382.93	.000	4.14	26.24	.000
	DS f.u.	4	503.13	90.26	.000	2210.82	341.10	.000
	NQCF	4	1163.14	1335.45	.000	906.04	1075.07	.000
	LVol	4	1687.52	3.70	.016 <sup>NS</sup>	1312.50	1.94	.133 <sup>NS</sup>
1 x 2 x 3	SED	8	105.28	127.84	.000	26.76	26.45	.000
	WGC	8	67.42	28112.95	.000	39.49	10302.48	.000
	DD	8	2.46	2044.90	.000	2.41	1999.07	.000
	SD	8	6.97	3671,10	.000	2.52	16.00	.000
	DS f.u.	8	452.35	81,15	.000	866.98	133.76	.000
	NQCF	8	848.97	974,74	.000	390.78	463.69	.000
	LVol	8	595.85	1,31	.282 <sup>NS</sup>	410.76	.61	.765 <sup>NS</sup>

The ways of using the plant residues also have a significant dynamic in the values of the investigated quality characteristics (Table 5). The sedimentation value of flour averaged over the period has the highest values in RF and TS sowing. The excess compared to CS is 7.58%. The burning and incorporation of plant mass leads to a gradual decrease in the values of this index. The average excess when using TS compared to CS is 2.53%.

Table 3. Mean data of the values of the end use quality depending on the years of investigation (2017-2019)

Years	SED, ml	WGC, %	DD, min	SD, min	DS, f.u.	NGCF	LVol, ml
TS							
2017	44.04 c	23.28 c	3.37 b	5.50 b	89.72 b	80.47 c	615.00 b
2018	32.89 a	12.29 a	3.40 c	2.62 a	229.11 c	49.65 a	557.22 a
2019	39.28 b	14.77 b	2.48 a	5.94 c	86.56 a	75.22 b	628.00 b
CS							
2017	40.22 c	20.42 c	3.08 b	4.83 b	72.22 a	71.39 c	604.17 b
2018	36.17 a	14.82 b	3.79 c	2.80 a	225.72 c	51.51 a	564.72 a
2019	36.94 b	12.00 a	2.41 a	5.10 c	88.50 b	67.17 b	601.94 b

Table 4. Mean data of the values of the bread-making quality depending on the kind of the previous crop PHR (2017-2019)

PHR Predecessors	SED, ml	WGC,%	DD, min	SD, min	DS, f.u.	NGCF	LVol, ml
TS							
Bean	36.54 a	16.53 b	3.74 c	5.82 c	137.61 b	81.85 c	599.44 ab
Sunflower	36.08 a	13.48 a	2.43 a	3.30 a	136.89 b	50.33 a	589.17 a
Maize	43.58 b	20.33 c	3.08 b	4.94 b	130.89 a	73.17 b	611.61 b
CS							
Bean	31.73 a	13.21 a	3.58 c	5.35 b	139.22 b	74.51 c	578.89 a
Sunflower	39.03 b	14.09 b	2.67 a	3.78 a	139.72 b	58.39 b	590.56 ab
Maize	42.57 c	19.94 c	3.03 b	3.60 a	107.50 a	57.17 a	601.39 b

In both systems, the CSIS had the most unfavorable effect on the WGC values. In TS, there is practically no difference between the RF and B variants, but in CS, residue burning results in the highest average values in the experiment (18.11%). Regarding the values of this index, TS leads to an average excess compared to CS by 6.57%

The rheological properties of the dough are also, although to a different extent, influenced by the use methods of the plant residues of the predecessor. Regardless of this fact, no significant differences were found in DD on average for the 2 sowing systems. In the variants with RF and B, the extensibility of the dough has higher values in CS compared to TS, while in CSIS a significant excess of TS compared to SC was found - on average by 16.04%. It was also found that the inclusion of the entire amount of plant residues leads to an increase in the stability of the dough (SD) in the TS by 43.36% compared to the values obtained in the combined one. The average excess of TS compared to CS by 10.42%. A similar trend was also found for the indicator DS f.u. in the variant with incorporation (CSIS) and use of TS, where the excess compared to CS is 33.83%. With this index, the average increase in values is respectively 8.04% compared to CS.

Table 5. Mean data of the values of the bread-making quality depending on the way of utilization of the previous crop PHR (2017-2019)

Plant Residue	SED, ml	WGC, %	DD, min	SD, min	DS, f.u.	NGCF	LVol, ml
TS							
RF	42.57 c	17.66 c	3.49 c	5.54 c	137.72 c	76.04 c	600.78 a
B	37.44 b	17.53 b	2.68 a	3.77 a	132.00 a	58.76 a	594.17 a
CSIS	36.19 a	15.16 a	3.09 b	4.75 b	135.67 b	70.56 b	605.28 a
CS							
RF	39.57 c	15.71 b	3.67 a	5.43 c	125.33 a	77.98 c	599.72 a
B	38.35 b	18.11 c	2.95 b	3.99 b	126.72 a	59.36 b	587.22 a
CSIS	35.41 a	13.41 a	2.66 a	3.31 a	134.39 b	52.72 a	583.89 a

In terms of NQCF and incorporation of plant residues, the difference between the 2 sowing systems is insignificant. The values of this indices are highest in the variants with RF and B when using CS. In these variants, however, TS leads to their increase and the excess compared to CS is respectively 9.88% and 4.16%. Thus, on average, for the tested ways of using plant residues, the

traditional method of sowing exceeds the sowing with a combined machine system by an average of 4.90%. As already mentioned, the volume of bread, regardless of the established differentiation in values, is statistically unreliable.

What makes an impression is that in different systems the meteorological factor affects the values of the tested quality characteristics with different strength (Figure 2). Its independent influence on TS has a determining power of impact on the indicators DS f.u., LVol, WGC, SD and SED, which varies from 95.85% to 12.84%, respectively. The type of plant residue of the predecessor has a determining force on the values of DD at TS. The power of the ways of using plant residues is well expressed on the values of SD, DD and NQCF, but still this is the factor whose power of influence on the quality characteristics of the variety is less pronounced compared to the independent action of the other two (*Year and Predecessor*). A similar tendency, for the leadership influence of the meteorological factor, was also found for CS, where the variation in the impact strength was 86.68% DS f.u. to 2.18% (SED). The strength of the independent influence of the type of plant residue of the predecessor is greatest in SED (14.36%), and in the way of their use - NQCF (19.81%).

Of all the types of combined interactions, the strongest in its impact is the *Year x Predecessor* combination. The strength of its impact varies from 74.06% SED to 2.18% DS f.u. This tendency is also preserved for TS, but the strength of the indicated combined interaction is weaker - from 48.27% SED to 1.05% NQCF.

On average for the research period, regardless of the seeding machine systems used, the type of plant residues of the predecessor has a significant influence on the tested parameters characterizing the quality of flour, dough and bread (Figure 3).

Sedimentation and wet gluten content had the highest values after the maize PHR.

Although with an unreliable difference between the predecessors, this trend is also observed for the volume of bread. For the rheological properties of the flour, plant residues from the precursor bean had a stronger positive influence compared to those from sunflower and maize.

The use of the plant residues of the predecessor in the three ways indicated affects the wide range of quality indicators of the produced production. Cleaning the field of residues has a positive effect on sedimentation (SED), development (DD-min) and dough stability (SD-min) as well as on the degree of its softening (DS f.u.). For the study period, these indices have higher average values compared to the other two ways of using plant residues.

The annual burning of plant residues is an extremely inadvisable agrotechnical practice, because affects the environment as a whole. Here, our attention is focused on some technological and baking qualities of wheat. Its influence on some rheological properties of the dough - stability and degree of softening of the dough (SD and DS f.u.), as well as on the values of the number of the quality factor (NQCF) is extremely unfavorable. A similar tendency is observed in other indicators, where it occupies an intermediate position. Only in this variant were the highest average values of wet gluten yield (WGC) reported.

The number of the quality coefficient (NQCF) as one of the most important quality characteristics shows that, on average for the period, the Enola variety achieves the highest values precisely when the plant residues are returned to the soil. The influence of this way of using the plant residues was definitely negative for the values of the indices of sedimentation (SED) and yield of wet gluten (WGC).

In each of the research years, significant dynamics were found in the values of the correlation dependences between the tested quality indicators and the level of their statistical reliability (Table 6). In the year with the most favorable conditions for the development of wheat during the studied period (2017), the SED is in a clearly expressed positive relationship with the DD and with its stability (SD) and degree of softening DS f.u. in both tested systems seeding machines. This index is also highly correlated with the WGC in CS. The yield of wet gluten in the grain (WGC) shows a well-expressed differentiation in its correlative dependences with the other parameters in the tested systems of sowing machines. The use of CS was distinguished by higher values of correlation coefficients in the relationship between SED with DD and LVol compared to traditional sowing (TS). When using the latter, however, the relationship between WGC with dough stability (SD) and degree of softening

DS f.u. is stronger compared to that found with CS. It can be seen that the relationship between DD time and its stability and degree of softening is pronounced in both seeding systems, but the correlation values are higher when using TS compared to CS.

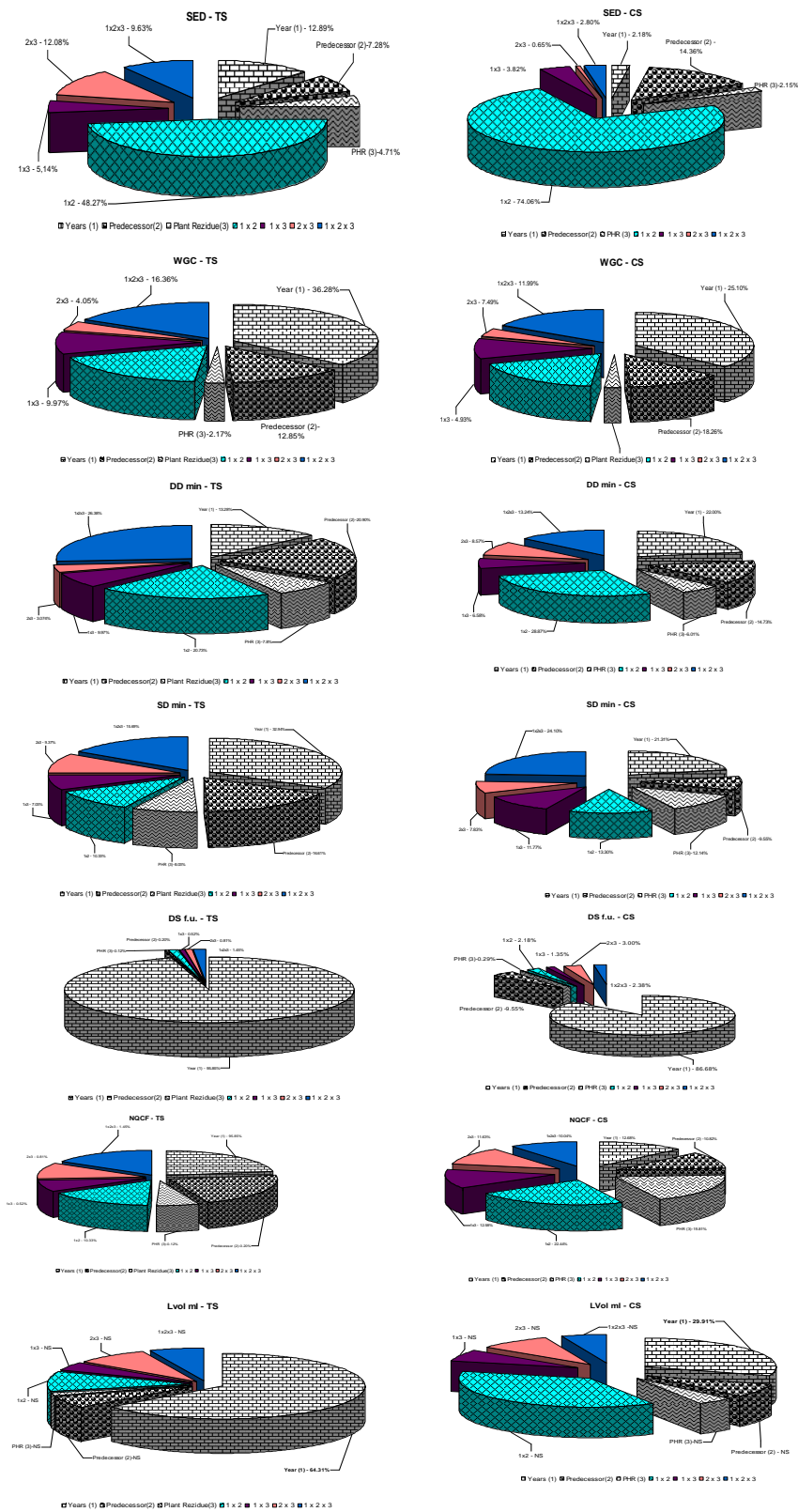
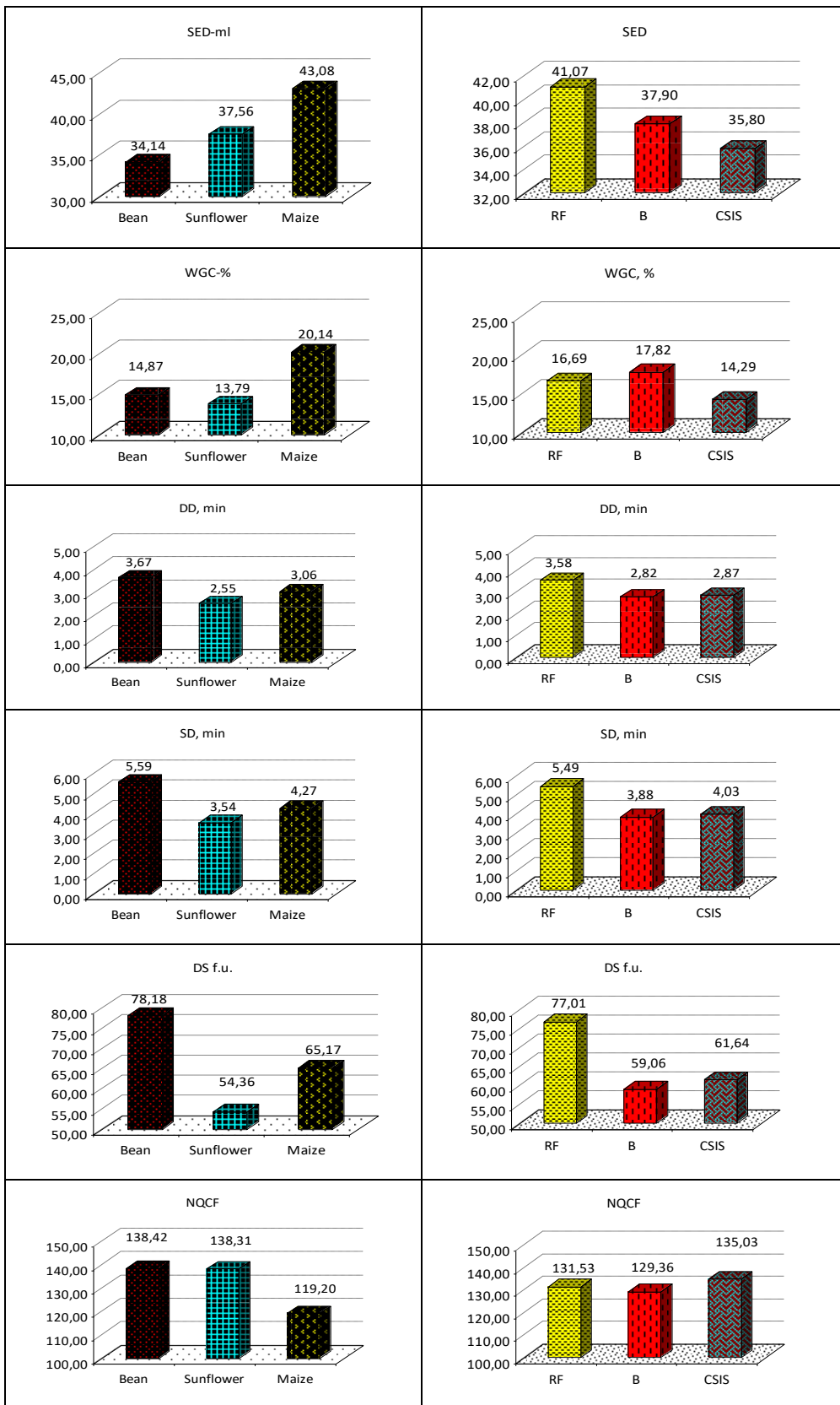


Figure 2. Strength of effect of the factors and their combinations average for the period 2017-2019 on the bread making qualities of wheat according to sowing systems



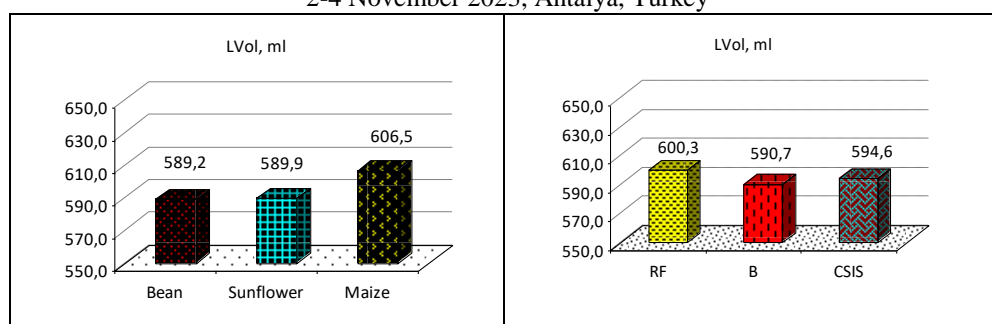


Figure 3. Average values for the quality characteristics of flour, dough and bread of the Enola variety depending on the type of plant residue and ways of their use, 2017-2019

The highest level of positive correlation in 2017 was found between dough stability (SD) and degree of softening (DS f.u.) - 0.983\*\* (TS) and 0.953\*\* (CS).

The number of quality coefficient (NQCF) is in a very well expressed positive correlation with the volume of bread (LVol) when using TS (0.719\*\*), and when using CS the relationship is negative (-0.528\*). For this index, no statistically reliable correlations with the other investigated quality indicators have been established. Under the conditions of water deficit for the growing season, accompanied by extreme droughts in critical phases of development (2018), the positive correlation between SED and WGC is most pronounced in both systems, respectively 0.910\*\* (TS) and 0.922\*\* (CS), followed by that of DD and DS f.u. This tendency holds true for both seeding systems tested. When applying traditional seeding, high levels of positive correlation between the WGC and the degree of softening of the dough (DS f.u.) - 0.872\*\*, as well as with its stability - 0.854\*\* and its development time - 0.597 \*\*. The correlation between SD and NGCF was strongly negative for both seeding systems. A similar negative correlation distinguishes the relationship between the NGCF and the degree of softening of the dough (DS f.u.).

The results show that the volume of bread (LVol) in traditional sowing is in most cases negatively correlated with most of the quality indicators, and statistically unreliable. Only in the case of sowing with a combined machine system, statistical reliability was established with the flour sedimentation (SED - 0.472\*) and the number for the quality factor (NGCF-0.511\*).

In 2019, statistically significant correlation dependencies are much less compared to the other two years and at a relatively lower level of correlation coefficient values. The most significant is that between SD) and DS f.u. - respectively for TS seeding 0.994\*\* and CS seeding 0.968\*\*. In the next position are the correlations between SED and dough stability (TS - 0.836\*\* and CS-0.846\*\*), as well as between SED and the degree of softening of dough (TS - 0.828\*\* and CS -0.748\*\*). A positive correlation with the bread volume indicator (LVol) was found only with the yield of wet gluten in the flour under a traditional sowing system (0.657\*\*).

The correlation dependences averaged over the study period for each of the systems separately show a very strong correlation between the stability of the dough and the number for its quality, regardless of the system of machines for soil preparation and sowing (Figure 4). A similar trend is observed in the correlation dependences between the SED and the WGC. The correlation coefficients between the degree of softening of the dough (DS f.u.) with the DD with all the other quality indicators in both machine systems, the correlations were negative. Loaf volume (LVol) correlated strongly negatively with DS f.u. and positively with dough stability (SD), especially in the TS.



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Table 6. Pearson Correlations between quality indices according to systems of sowing by years of investigation (n=18)

Indices	SED		WGC		DD		SD		NGCF		DS f.u.		LVol	
	TS	CS	TS	CS	TS	CS	TS	CS	TS	CS	TS	CS	TS	CS
2017														
SED	1	1	0.485*	0.789**	0.837**	0.876**	0.726**	0.783**	0.056	-0.038	0.732**	0.822**	0.001	0.424
WGC	0.485*	0.789**	1	1	0.582*	0.701**	0.633**	0.447	0.465	-0.331	0.683**	0.485*	0.504*	0.659**
DD	0.837**	0.876**	0.582*	0.701**	1	1	0.858**	0.611**	0.107	0.040	0.841**	0.695**	0.344	0.201
SD	0.726**	0.783**	0.633**	0.447	0.858**	0.611**	1	1	0.111	-0.079	0.983**	0.953**	0.373	0.266
NGCF	0.056	-0.038	0.465	-0.331	0.107	0.040	0.111	-0.079	1	1	0.226	-0.065	0.719**	-0.528*
DS f.u.	0.732**	0.822**	0.683**	0.485*	0.841**	0.695**	0.983**	0.953**	0.226	-0.065	1	1	0.452	0.336
LVol	0.001	0.424	0.504*	0.659**	0.344	0.201	0.373	0.266	0.719**	-0.528*	0.452	0.336	1	1
2018														
SED	1	1	0.910**	0.922**	0.722**	0.565*	0.616**	0.172	-0.261	0.243	0.767**	.486(*)	-0.045	0.472*
WGC	0.910**	0.922**	1	1	0.681**	0.676**	0.854**	0.250	-0.469*	0.044	0.872**	.637(**)	-0.109	0.360
DD	0.722**	0.565*	0.681**	0.676**	1	1	0.597**	0.337	-0.464	-0.386	0.818**	.846(**)	-0.161	-0.073
SD	0.616**	0.172	0.854**	0.250	0.597**	0.337	1	1	-0.790**	-0.630**	0.895**	.665(**)	-0.127	-0.171
NGCF	-0.261	0.243	-0.469*	0.044	-0.464	-0.386	-0.790**	-0.630**	1	1	-0.648**	-.668(**)	0.367	0.511*
DS f.u.	0.767**	0.486*	0.872**	0.637**	0.818**	0.846**	0.895**	0.665**	-0.648**	-0.668**	1	1	-0.095	-0.210
LVol	-0.045	0.472*	-0.109	0.360	-0.161	-0.073	-0.127	-0.171	0.367	0.511*	-0.095	-.210	1	1
2019														
SED	1	1	0.636**	0.416	0.375	0.581*	0.836**	0.846**	-0.265	0.599**	0.828**	0.748**	0.430	0.165
WGC	0.636**	0.416	1	1	0.310	0.217	0.286	0.013	0.020	0.015	0.321	-0.080	0.657**	0.462
DD	0.375	0.581*	0.310	0.217	1	1	0.409	0.500*	0.535*	0.674**	0.416	0.431	0.378	0.378
SD	0.836**	0.846**	0.286	0.013	0.409	0.500*	1	1	-0.406	0.597**	0.994**	0.968**	0.162	0.107
NGCF	-0.265	0.599**	0.020	0.020	0.535*	0.674**	-0.406	0.597**	1	1	-0.424	0.482*	0.137	0.121
DS f.u.	0.828**	0.748**	0.321	-0.080	0.416	0.431	0.994**	0.968**	-0.424	0.482*	1	1	0.179	0.076
LVol	0.430	0.165	0.657**	0.462	0.378	0.378	0.162	0.107	0.137	0.121	0.179	0.076	1	1

\*\* Correlation is significant at the 0.01 level (2-tailed).

\* Correlation is significant at the 0.05 level (2-tailed).

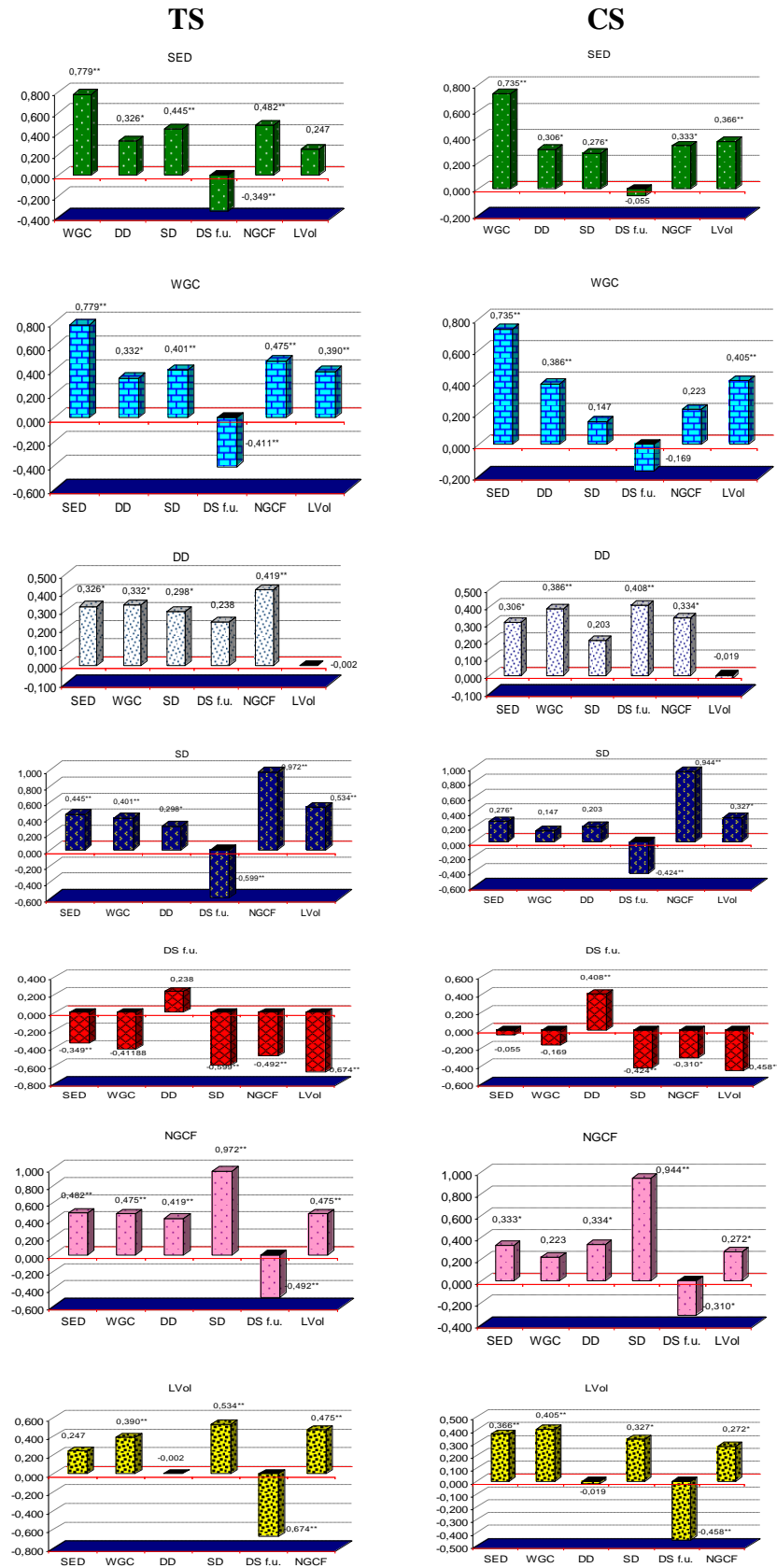


Figure 4. Pearson Correlations between quality indices average for 2017-2019 (n=54)

## CONCLUSIONS

The present study gives us the opportunity to obtain information about the dynamics in the values of a number of quality characteristics of flour and dough as a result of the influence of current agrotechnical practices with a main emphasis on the ways of using different plant residues of wheat predecessors.

The beans PHR under both soil preparation and sowing systems was found to increase farinographic stability (SD) and dough development time (DD), degree of softening (DS f.u.) and flour quality coefficient number (NQCF). Except for the softening degree data, the traditional system contributes higher data values for the listed indicators than the combined system. Sedimentation and wet gluten content were highest after maize precursor and again in traditional system (TS).

The removal of plant residues from the field in both sowing systems leads to an increase in the values of flour sedimentation, dough stability, especially rheological properties, as well as the yield of wet gluten in the grain in the traditional sowing system. Conversely, incorporation the PHR into the soil is the reason for the noticeable decrease in the values of these indices. In a combined sowing system, however, the inclusion of the entire amount of the non-economic part of previous production leads to an increase in the values of the degree of softening. It was also found that the burning of the post-harvest residues in the combined system provoked the obtaining of higher wet gluten values in the grain and increased the dough development time.

Irrespective of weather conditions, the correlative dependences between flour sedimentation value (SED) and wet gluten yield (WGC), its development time (DD), its stability (SD) and degree of softening (DS f.u.) are most stably expressed. This tendency is more pronounced with traditional seeding for the area compared to the use of the combined system of seeding machines.

Bread volume is the quality characteristic that is practically not significantly affected by the way of using the post-harvest residues and by its interactions with other factors in both tested systems. Its values in both systems are mainly influenced by the meteorological factor, and this fact applies to a much greater extent to traditional sowing compared to that with a combined system of machines.

The years with an optimal combination of the main meteorological elements (2017) have a significant contribution to obtain higher values for sedimentation, wet gluten and the number for the quality coefficient. In years with insufficient rainfall combined with higher temperatures in critical phases of the perennial wheat vegetation (2018), the dough development time and degrees of softening were higher compared to the others.

Multiple correlations were established between the tested quality characteristics by years of research and average over the period. They differ both in the direction of interaction and in the strength of the correlation dependences by sowing systems.

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## IMPROVEMENT OF BEAN PLANT TRAITS BY INDUCED MUTAGENESIS

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### ABSTRACT

*Phaseolus vulgaris* is a leguminous plant with very high nutritional values and widely spread in Albania. Climatic changes have affected this plant to reduce its production and this experiment was designed to shorten the flowering time of Shijak variety bean plants, collected in Central Albania. We used as physical mutagens Cs-137 gamma radiation and we irradiated with 50 Gy, 100 Gy and 150 Gy. Also, we choose as chemical mutagens dES (Diethyl Sulphate), and we treated seeds with three concentrations: 0.0025M, 0.005M and 0.010M. The irradiated seeds were planted in the experimental field and the greenhouse during two consecutive years 2021 and 2022. The effect of physical and chemical mutagens on bean plants and pods was compared with untreated parental materials. Diverse traits were analyzed as germination ability, pigment change, chlorophyll content, pod characteristics, root system development etc. The best performance for pod length and width, seeds number and weight were taken for the 50 Gy gamma radiation dose. This dose has increased production and the weight per unit. The chlorophyll content in M1 and M2 generation had evident differences according to irradiation doses. Photosynthetic pigments differ from one generation to another due to the action of physical mutagen. Since the chlorophyll content is an indicator of plant health, we observed that 100 Gy irradiation dose improves the condition of plants in the experimental field. For the first two dES concentrations the plants had a better development of the root system than the control.

**Keywords:** Induced mutagenesis, gamma radiation, dES, white bean

### INTRODUCTION

Beans have exceptional energy values and contain almost all essential amino acids (Velu et al., 2012). Beans contain calcium, iron, phosphorus, magnesium, zinc and sodium, and it is an excellent source of protein, but beans are low in fat and contain no cholesterol (Coyne et al., 2003). Induced mutagenesis for a long time is considerate as a precious direction to create high quality and sustainable crop plants to environmental conditions (Sofkova et al., 2021). The experience of different research groups has shown that mutagenesis is an important for the creation of new plant variety especially at cereal, leguminous and many other crops. Also, the physical and economic possibilities of food are the important criteria of food security.

Induced mutations have played a major role in increasing food security, but for some bean varieties, radiation with doses from 300 Gy to 500 Gy are lethal (Singh et.al., 2005). In Albania the bean is important in the leguminous group. Climatic changes, especially in the recent years, have had a major impact on the production of this leguminous plant, affecting negatively it's production. For several years, the application of induced mutations has turned into the main way to create new cultivars with improved features by comparing them with the parents. Induced mutagenesis technology has been recently recognized as a valuable additional tool to

create improved cultivars in agriculture (Horn et al., 2017). Mutant crop varieties are more adaptable to the environment, require less agricultural contributions, and are therefore more economical to grow and contribute to more environmentally friendly agriculture (Mba, 2013).

## MATERIAL AND METHOD

The selected plant material was Shijak variety bean (*Phaseolus vulgaris*) seeds obtained from the National Seed and Seedling Entity which is the Genetic Bank of Albania. The experimental work was based to special protocols of the IAEA. The experimental work is organizing respectively in the Mutagenesis Laboratory, in an experimental field in the Fieri District (Albania) and in the greenhouse, part of the Department of Biotechnology, Faculty of Natural Sciences, University of Tirana. In the greenhouse experiment the seeds were planted in pots with soil with control of temperature and humidity. (Ylli et al., 2018; FAO / IAEA, 2018). For the treatment of the bean seeds was used the gamma radiation with Cs-137, which were applied in three different doses, 50 Gy, 100 Gy and 150 Gy (FAO / IAEA, 2018). The irradiated seeds and the control material were planted in the experimental field in randomized complete block (3m x 0,7m) and four replications, while in the greenhouse the seeds were planted in two replications.

We choose as chemical mutagens dES (Diethyl Sulphate), and we treated seeds with three concentrations: 0.0025M, 0.005M and 0.010M. The seeds were treated with this mutagenic solution for one hour, based in FAO / IAEA protocols (FAO / IAEA, 2018; Ylli, et al., 2013). CCM-200 type chlorophyll meter was used for the measurement and analysis of the chlorophyll pigment of the third level leaves of bean plants. Measurements of photosynthetic pigments were made in 25 plants for each dose.

## RESULTS AND DISCUSSIONS

Measurements were done according to the above described protocol for all material taken from the experimental field in Fieri District and the greenhouse in Tirana, during two consecutive years 2021 and 2022. The analyzes of the bean plants materials *Phaseolus vulgaris* irradiated with three doses of gamma radiation 50 Gy, 100 Gy, 150 Gy as well as the control were done in the Induced Mutations Laboratory of the Biotechnology Department. The treated seeds were planted in the greenhouse and in experimental field (Figure 1) in the M1 generation and then in the M2 generation to see the changes in their development stages.



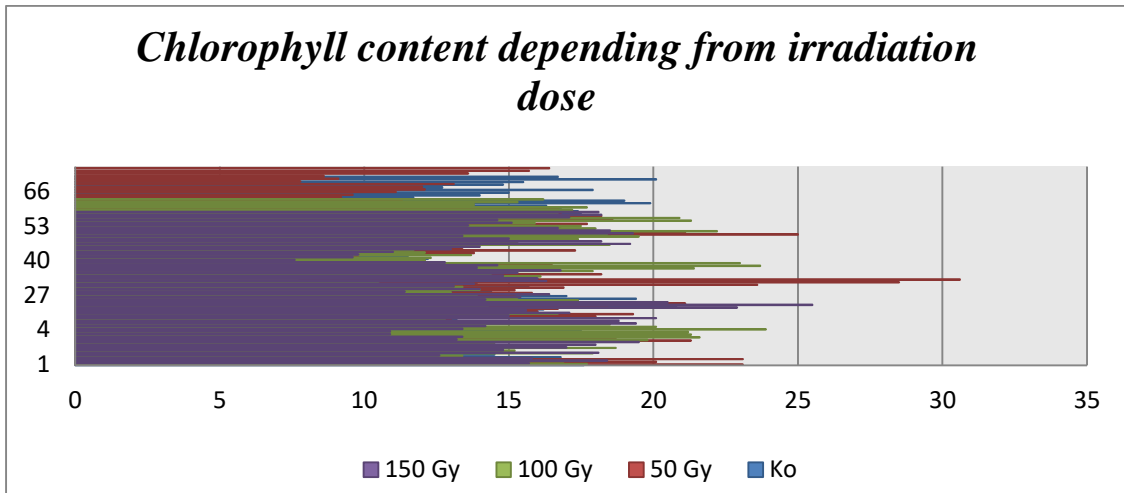
**Figure 1.** Bean seeds treated with gamma radiation planted in the greenhouse in two replications and in the experimental plot in four rows for each treatment dose

Different stages of plant development can be seen in Figure 2, which in doses of 50 Gy had set flowers 4 days earlier than the control, while in the dose of 150 Gy, flowers with a different color appeared compared to the control that has white flowers (Borkar et.al., 2010). During the vegetation period, have been recorded the phenological phase (germination, branching, flowering, beans, etc.).



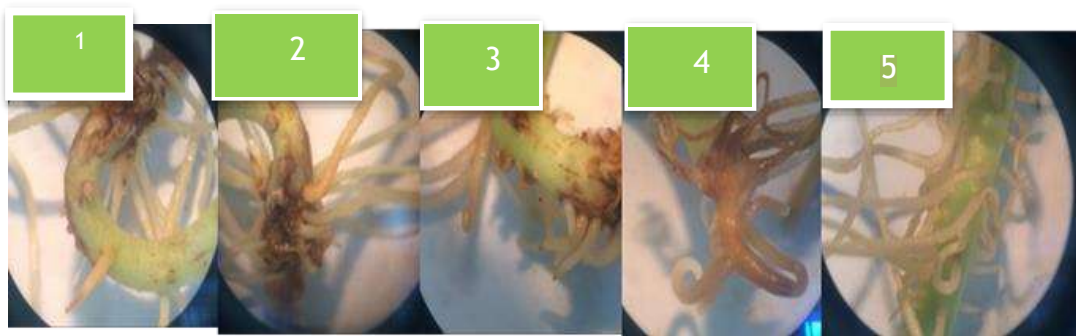
**Figure 2.** Phases of phenological development of bean plants with different doses of radiation

Germination capacity was determined by observing the consecutive germination of each plants each week and by calculating the percentage of germination. By determining germination capacity, it's possible to determine the optimal dose that causes lower plant mortality, but at the same time causes highest desired percentage of mutagens. From the greenhouse material turns out that 100 Gy dose (83,3%) has the highest germination capacity in M1 generation, while 150 Gy dose (87,5%) has the highest germination capacity in M2 generation. Meanwhile in the experimental field 100 Gy dose (92,1%) has the highest germination capacity. These results show that the germination was affected by the plant material, genetic potential, seeds size, seeds germination ability etc. During this experimental work we have evaluated and measured chlorophyll pigment, and we observed different mutations under the influence of mutagens. We used CCM-200 chlorophyllmeter for measurements and analysis of photosynthetic pigments of bean leaves. Changes in the photosynthesis pigments content allow to evaluate their modification / reduction. Changes in the photosynthetic units respond to mutagenic as a protective mechanism in the most tolerant and resistant species. Since the chlorophyll amount was an indicator of plant health, we evaluated that 100 Gy irradiation dose improves the condition of plants in the greenhouse or in the experimental field. Figure 3 shows the large differences in the photosynthetic pigments measured in plants irradiated with the three doses used.



**Figure 3.** Chlorophyll content depending from irradiation dose

We also analyzed the root system of bean plants with different doses and from the data obtained, stress caused by high temperatures develops a deeper root system that absorbs more water, but the growth of the root system decreased the productivity.



**Figure 4.** Analysis of the root system in plants treated with a dose of 50 Gy 15 days after planting

It's necessary to multiply roots hair to be able to use the same biomass more effectively or to stimulate a higher acid production in roots, to be able to maintain the same production or increasing it. Low phosphorus (P) availability and drought are the main constraints to common bean production.

The root system is an important factor for plant productivity in leguminous plants. During the experiment we followed the development of the roots after 12 days and after 15 days from germination measuring the length of the root. Stereomicroscopy with cameras was used to analyze the root system of plants and their development. As can be seen in the figure 4, 5 and 6, there are differences in their development depending on the doses of gamma radiation used.

From the measurements carried out in the laboratory concerning the plant germination capacity, we concluded that compared to the control, the seeds treated with the mutagen had longer roots. The seeds treated with the dose of 50 Gy were more developed than the control plants and had a greater length, so the treatment with the dose of 50 Gy affects the development of the plants. The 150 Gy dose reduces plant survival and is not effective in growing plants in Petri dishes.





**Figure 5.** Analysis of the root system in plants treated with a dose of 100 Gy 15 days after planting



**Figure 6.** Analysis of the root system in plants treated with a dose of 150 Gy 15 days after planting

From the measurements of leaf's, it was found that dES chemical mutagen with three different doses (0.0025M, 0.005M and 0.010M), affected the length of the leaves of the bean plants, but did not affect their width. The first dose of diethylsulfate (0.0025M), brought a higher increase compared to the control, where we get that with the increase of this dose, it brings a decrease from this average value (Kodhelaj *et al.*, 2021; FAO / IAEA, 2018). By processing the measurements performed on the leaves with the Anova program, we have analyzed the influence of the dES mutagen on the dimensions of the leaves of bean plants.

**Table 1.** Linear regression for leaf length after treatment with dES in M1 generation

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.206 <sup>a</sup>	.043	.034	1.62251

a. Predictors: (Constant), dose of mutagen dES

In the linear regression analysis, it is determined that there is a stable relationship between the doses of the chemical mutagen and the length of the leaves because according to the Anova analysis, the significant value is less than 0.05 (Table 2). This relationship is determined by the correlation coefficient where only 4.3% of the length is determined by the dose of the chemical mutagen (Table 1). We can also determine this with the equation of a linear line where we have  $y = 6.777 + 0.303x$ . Thus, with the increase of one unit of the dose of the mutagen, the length of the leaf increases by 30.3% (Table 3).

**Table 2.** Analysis of variance for leaf length after dES treatment in M1 generation

Model	Sum of Squares	Df	Mean Square	F	Sig.
1 Regression	13.802	1	13.802	5.243	.024 <sup>b</sup>
Residual	310.638	118	2.633		
Total	324.440	119			

a. Dependent Variable: the length of the leaf M1 with dES,  
b. Predictors: (Constant), dose of mutagen dES

**Table 3.** Correlation coefficients for the association between dES and leaf length in M1

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
1 (Constant)	6.777	.248		27.343	.000
dose of mutagen dES	.303	.132	.206	2.290	.024

a. Dependent Variable: leaf length M1/dES

Even in the linear regression analysis, it is determined that there is a stable relationship between the doses of the chemical mutagen and the length of the leaves, because according to the Anova analysis, the significant value is less than 0.05. This relationship is determined by the correlation coefficient where only 4.3% of the length is determined by the dose of the chemical mutagen. We can also determine this with the equation of a linear line where we have  $y=6.777+0.303x$ . We conclude that with the increase of one unit of the dose of the mutagen, the length of the leaf increases by 30.3%.

**Table 4.** Linear regression for leaf width after treatment with dES in M1 generation

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.082 <sup>a</sup>	.007	-.002	.82072

a. Predictors: (Constant), dose of mutagen dES

**Table 5.** Analysis of variance for leaf width after dES treatment in M1

Model	Sum of Squares	Df	Mean Square	F	Sig.
1 Regression	.534	1	.534	.793	.375 <sup>b</sup>
Residual	79.482	118	.674		
Total	80.016	119			

a. Dependent Variable: leaf length M1/dES  
b. Predictors: (Constant), dose of mutagen dES

**Table 6.** Correlation coefficients for the association between dES and leaf width in M1

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
1 (Constant)	4.245	.125		33.863	.000
Dose of mutagen dES	-.060	.067	-.082	-.890	.375

a. Dependent Variable: the width of the leaf M1/dES

As for the width, the correlation percentage is very low, where 0.7% of the width is determined by the dES dose (Table 4). This very weak relationship is also determined by the Anova analysis where the significant value is greater than 0.05 (Table 5).

If a linear line is constructed, it can be determined with the equation  $y = 4.245 - 0.060x$ , where with the increase of one unit of the dose, the values of the width of the leaves decrease by 6%. So in the four cases of materials obtained from treatment with chemical mutagens, the leaves had a difference in their length and in width they did not have any difference from the control, the leaves appear long compared to the leaves of plants pretreated with physical mutagens with the three doses (Table 6).

While for the width, the correlation percentage is very low where 0.7% of the width is determined by the dose of dES. This very weak relationship is also determined by the Anova analysis where the significant value is greater than 0.05. If we were to construct a linear line, we could define it with the equation  $y = 4.245 - 0.060x$ , where with an increase of one unit of the dose, the values of the width of the leaves decrease by 6%.

## CONCLUSIONS

Induced mutagenesis on seeds of *Phaseolus Vulgaris* Shijak variety, treated with chemical mutagen dES with three concentrations as well as irradiated with gamma radiation of Cs-137 source with three doses has given positive influence on the change of traits and quality of the bean plant. The treatment with physical mutagens in the three doses of gamma radiation presents a higher average value for the body height and the development of the root system in the bean plants compared to the control, and this is also proven by the statistical analysis performed.

The use of induced mutagenesis for plant materials of physical mutagen treatments affected both values as leaf length and width, leading them to a circular shape. This was observed for the three doses used with the Cs-137 gamma radiation source.

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## POST-HARVEST RESIDUE TREATMENT EFFECT ON THE WINTER WHEAT PRODUCTIVITY

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### ABSTRACT

The investigation was carried out at Dobrudzha Agricultural Institute during 2017-2019. The effects of the traditional (TS) and combining (CS) soil tillage systems and sowing machines, the type of the previous crop post-harvest residue (common bean, maize, sunflower) and their treatment on the yield from cv. Enola (*Triticum aestivum* L.) was investigated in six-field crop rotation. The spring crop post-harvest residues (PHR) were utilized in three different ways - removed from the field (RF); burned (B) and chopped and subsequently incorporated into the soil (CSIS). During the studied period, a wide dynamic was established in the productivity of the wheat, depending on the type of the predecessor and the method of utilization of its plant residues. The traditional system of soil preparation and wheat sowing for the Dobrudzha region provides higher yields compared to the combined system in all years of the study. The average increase is 352.17 kg/ha (6.61%). The role of the predecessor also has a stronger impact on productivity in the TS. The cv. Enola was expected to have the highest yields in both systems after the predecessor bean. The lowest productivity in the experiment was found after the predecessor sunflower, where the biggest difference between the systems was also found - 552.30 kg/ha in favor of the TS. To obtain the maximum expression of the productive possibilities of the wheat, a differentiated approach to the ways in which we will use the post-harvest residues (PHR) of the predecessor and the technical means for this is required. In areas with minimal presence of PHR, the CS of soil preparation and sowing contributes to obtaining 403.3 kg/ha more compared to TS one. However, it is extremely unsuitable in cases of burning the residues or their complete plowing. The use of the TS in such situations provides higher yields compared to the CS with 314.1 kg/ha (B) and 1145.8 kg/ha (CSIS), respectively. The TS definitely contributes to obtaining a larger grain compared to the CS one of the tested predecessors and ways of utilizing their plant residues. The grain hectoliter is mainly influenced by the weather conditions of the years, but not by the sowing systems. Its values do not always follow the established trends for the 1000 kernel weight and are characterized by a weaker, although reliable, dynamics. Strength of effect of each of the tested factors on the values of the studied indicators was determined. The correlative dependence between the productivity of the variety and the test weight of the grain is reliable. The correlation with TS has higher coefficient values (0.615\*\*) compared to that with CS (0.486\*\*). The relationship between the yield and the and the 1000 kernel weight is negative and unreliable. There is a positive and reliable correlative relationship between the two indicators characterizing grain physics.

**KEY WORDS:** utilization of post harvest residue, wheat, yields, grain physical properties

### INTRODUCTION

Crop residues worldwide are estimated in billions of tons. A significant part of them are post-harvest residues after harvesting. They are the carriers of various chemical compounds

extracted from the soil and converted into organic matter, as well as a large amount of energy as a result of photosynthesis. In one of his studies, [Scheffer \(2004\)](#) points out that the formed plant mass on the planet concentrates 10 times more solar energy than the needs of people. The return of plant residues contributes to the control of erosion processes, recycling of nutrients and their availability to plants, phytosanitary status, soil fertility and others ([Yadvinder-Singh, 2005](#)). Scientific research at home and abroad has long proven that post-harvest residues are a natural resource of extreme importance, contributing to soil structure and fertility, as well as crop productivity ([Nankova & Yankov, 1997](#); [Filcheva, 2004](#); [Nankova M., 2011](#); [Iliev et al., 2018](#); [Nankova & Filcheva, 2020](#)). In the detailed study of [Filcheva et al. \(2005\)](#), the benefits of the intelligent approach to the use of plant residues through the prism of knowledge - Waste or Wealth are clearly indicated.

However, crop residue decomposition has both positive and negative impacts on crop production. Scientific research is the basis of highlighting the effects of different crop residue management practices and improving the positive impact on the environment ([Lu, 2020](#), [Gupta et al., 2022](#)).

One of the ways to free the field from "unnecessary" plant residues is their burning. On a global scale, farmers use fire precisely as a tool for their removal ([Zhang et al., 1996](#); [Yevich and Logan, 2003](#); [Chen et al., 2005](#); [Korontzi et al., 2006](#); [Yan et al., 2006](#); [Sahai et al., 2007](#); [Yang et al., 2008](#)). In the USA, burning crop residues is a common practice for pest and weed control in preparing fields for seeding. Almost universally, burning is considered a quick and cheap tool to remove plant material after harvest ([Canode and Law, 1979](#); [Smiley et al., 1996](#); [Ball et al., 1998](#); [Eiland, 1998](#)).

According to [Stoynev \(2004\)](#), the "temporary benefit" of burning plant residues leads to a permanent deterioration of soil fertility. Crop residue burning has also been identified as a significant hazard to human health, causing significant air pollution problems and soil health degradation ([Manu et al., 2020](#)). Numerous studies prove that crop residues have the potential to improve soil fertility.

This depends not only on their biochemical properties, but also on the preparation for their return to the soil, as well as the use of the most appropriate technique for this ([Viator et al., 2009](#); [Coulibaly 2020](#)). The application of unreasonably intensive agricultural practices in recent decades often cause serious disturbances not only in soil fertility but also in the uneven depletion of various natural resources, which intensifies the process of desertification in many areas of the world. The management of crop residues, i.e. their return to the soil is a key step in modern agricultural production aimed at protecting the environment and obtaining satisfactory production in terms of size and quality.

The article discusses the role and ways of using crop residues from spring field crops on the productivity and physical characteristics of wheat grain. The aim of the study is also the used seeding machine systems.

## **MATERIALS AND METHODS**

The investigation was carried out at Dobrudzha Agricultural Institute during 2017-2019 on Haplic Chernozems. The trial was performed on 5 ha area in 4 replicates. The effect of the traditional systems of soil tillage and sowing machines and the type of the previous crop post-harvest residue treatment on the yields and grain physical properties were investigated in six-field crop rotation. The crops were arranged in crop rotation as follows: grain maize – wheat – sunflower – wheat – common bean – wheat.

Soil preparation and sowing of the wheat (cv. Enola) after sunflower, maize and common bean was done according to the traditional system (TS). According to this system, the sowing of wheat is carried out with a seed drill SZU - 3.6 after double disking at a depth of 10-

12 cm. In the case of the combined system (CS), a single disking of the area is performed in advance. It includes the aggregation of a Fendt 820 Vario tractor and a Horsch 6.0 sowing machine. The second disking in this system is carried out together and immediately with the sowing itself. In both systems, sowing was carried out at a rate of 500 germinating seeds/m<sup>2</sup>. The mineral fertilization of wheat is consistent with the predecessor. After beans, the same is N<sub>90</sub>P<sub>100</sub>K<sub>0</sub>, and after the other predecessors - N<sub>120</sub>P<sub>100</sub>K<sub>0</sub>.

The post-harvest residues (PHR) from the previous spring crops were utilized in three different ways: they were removed from the field (RF); they were burned (B) and they were chopped and subsequently incorporated into soil (CSIS).

The statistical analysis of the data was carried out according to the type of the design (ANOVA), running the analysis of variance, and comparison of means using LSD 0.05. Combined analysis was carried out for the three seasons using SAS 2000.

## RESULTS AND DISCUSSION

On the basis of the statistical analyzes made by years of research, the influence of the methods of PHR in TS is statistically unreliable for each of the years (Table 1). In this system, however, the role of the predecessor on the productivity of the Enola variety is decisive.

Table 1. Analysis of variances of wheat productivity according to the ways of predecessors PHR utilization over years

Source	df	2017		2018		2019	
		F	Sig.	F	Sig.	F	Sig.
TS							
Predecessors (1)	2	29.13	0.000	34.20	0.000	29.02	0.000
Utilization of PHR (2)	2	1.13	0.360 <sup>N</sup> <sub>s</sub>	0.90	0.442 <sup>N</sup> <sub>s</sub>	1.14	0.362 <sup>N</sup> <sub>s</sub>
1 x 2	4	0.65	0.640 <sup>N</sup> <sub>s</sub>	3.37	0.060 <sup>N</sup> <sub>s</sub>	0.65	0.643 <sup>N</sup> <sub>s</sub>
CS							
Predecessors (1)	2	50.55	0.000	118.01	0.000	51.15	0.000
Utilization of PHR (2)	2	32.42	0.000	49.94	0.000	32.76	0.000
1 x 2	4	1.30	0.340 <sup>N</sup> <sub>s</sub>	3.87	0.043 <sup>N</sup> <sub>s</sub>	1.32	0.332 <sup>N</sup> <sub>s</sub>

In CS, the independent influence of both factors is statistically significant at the maximum level. The results show that the combined influence between the two factors is statistically unreliable. On average for the research period, the two systems of soil preparation and crop sowing significantly differed in their impact on productivity both in the individual and in the combined interaction between the factors (Table 2). In both tillage and sowing systems, the influence of the *Year* and *Predecessor* factors has been conclusively statistically proven.

In TS, the way PHR was used and the types of interaction between the factors were statistically unreliable. However, the use of CS shows that single and double combinations of the tested factors strongly influence the productivity of the crop.

Over the years, the dynamics of productivity values depending on the type of predecessor and the way of using PHR varies widely (Table 3). With the traditional method of

tillage and sowing machines, the yields, depending on the type of predecessor, range from 4115.6 kg/ha (2018) to 6801.7 kg/ha (2017) and 6800.0 kg/ha (2019). In all three years of the study, the lowest yields were obtained after the predecessor sunflower.

Table 2. Analysis of variances of wheat productivity according to the ways of predecessors PHR utilization averaged for the period 2017 – 2019.

Source	df	TS'2017-2019		CS'2017-2019	
		F	Sig.	F	Sig.
Years (1)	2	88.970	0.000	173.259	0.000
Predecessors (2)	2	91.121	0.000	194.171	0.000
Utilization of PHR (3)	2	3.077	0.063 <sup>NS</sup>	103.651	0.000
1 x 2	4	1.538	0.219 <sup>NS</sup>	9.946	0.000
1 x 3	4	0.007	1.000 <sup>NS</sup>	5.001	0.004
2 x 3	4	0.733	0.577 <sup>NS</sup>	4.335	0.008
1 x 2 x 3	8	2.459	0.038 <sup>NS</sup>	0.973	0.477 <sup>NS</sup>

With the combined system, the yield variation is from 3546.8 kg/ha (2018) to 6589.7 kg/ha (2017) and 6586.7 kg/ha (2019). Again, sunflower is the predecessor whose residues have a more pronounced negative impact on the yields obtained.

Table 3. Mean productivity of wheat over years of investigation, according to trail indices

Indices	2017		2018		2019	
	TS	CS	TS	CS	TS	CS
By Predecessors						
Common Bean	6801.7 c	6589.7 c	5866.6 b	5685.7 b	6800.0 c	6586.7 c
Sunflower	5505.0 a	4961.4 a	4115.1 a	3546.8 a	5503.3 a	4958.3 a
Maize	6006.7 b	5923.0 b	4538.8 a	3797.6 a	6005.0 b	5923.3 b
By Treatments of Plant Residue						
RF	5954.8 a	6506.9 c	4669.9 a	4776.0 b	5953.3 a	6505.0 c
B	6175.8 a	5767.6 b	4914.7 a	4788.9 b	6173.3 a	5765.0 b
CSIS	6182.8 a	5199.6 a	4936.0 a	3465.1 a	6181.7 a	5198.3 a

The established differences in productivity depending on the ways of using PHR in TS are unreliable. However, with CS, the differentiation in the values of the yields obtained is very well expressed.

The highest average yields, regardless of weather conditions during the years of study, were obtained when part of the PHR (at the harvest height of the predecessor) was removed from the field (RF). It has been proven that the obtained yields are the lowest in the variant with their plowing (CSIS).

The obtained results for the wide dynamics in the productivity of the variety require a characterization of the meteorological conditions of the years of study. During the period 01.10.2016 - 31.03.2017 in the area of the Dobrudhza Agricultural Institute - the town of Gen.Toshevo the precipitation is 252.7 mm/m<sup>2</sup>, an amount that is above the average annual norm (Fig.1).



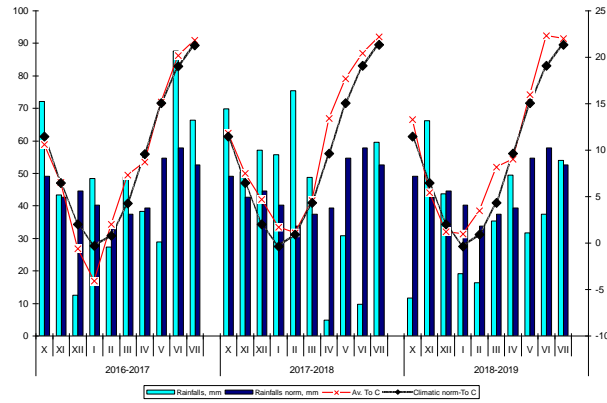


Figure 1. Dynamics of precipitation and temperature values over months during the vegetative growth, and mean long-term norm for 1953-2015

Weather conditions during the winter months are favorable for the normal entry of the culture into the initial phases of its development. Vegetation precipitation is  $16.9 \text{ mm/m}^2$  above the climatic norm. The year 2017 is distinguished by intense rainfall in the month of June. The total amount of precipitation is  $20 \text{ mm/m}^2$  above the climatic norm (1953-2015) and contributes to obtaining a normal wheat yield.

During the autumn-winter vegetation of the 2018 harvest, the precipitation was  $357.6 \text{ mm/m}^2$ , which is above the average the climatic norm of  $110.0 \text{ mm/m}^2$ . The 2018 perennial spring growing season was characterized by drought at critical stages of development (spinning and heading) and heavy rainfall at harvest.

The 2019 harvest is characterized by a precipitation deficit of  $55.1 \text{ mm/m}^2$  during the autumn-winter vegetation and  $33.4 \text{ mm/m}^2$  less precipitation during the period of permanent spring vegetation until harvest. The total precipitation deficit in the 2019 harvest is below the climatic norm with  $88.5 \text{ mm/m}^2$ . Regardless of this fact, the precipitation that fell is relatively evenly distributed.

In terms of temperature, the conditions during the growing season of crops are distinguished by higher temperatures on average for the growing season of wheat in 2018 and 2019 by  $1.5^\circ\text{C}$ - $1.2^\circ\text{C}$ . The indicated years are also characterized by significantly higher average monthly temperatures during the permanent spring vegetation. The average temperature for the growing season of wheat during the 2017 harvest period is as close as possible to that of the climatic norm. It is characterized by negative average monthly temperatures in December and January, which distinguishes it from the other years included in the study.

Of the three research years, 2018 stands out with the lowest yields, regardless of the fact that the amount of precipitation for the wheat vegetation period slightly exceeds the same for the period 1953-2015 (Fig. 2). The main reason for this fact is the drastic drought since the beginning of the permanent spring vegetation in combination with significantly higher temperatures compared to the perennial average.

The productivity of the Enola variety under both tillage and sowing systems was approximately the same in 2017 and 2019. The entire study period was characterized by higher yields using TS compared to CS. The excess by year is respectively: 2017 - 4.80%; 2018 - 11.40% and 2019 - 4.81%. For the studied period, the average yield in TS was  $5682.47 \text{ kg/ha}$ , and in CS -  $5330.30 \text{ kg/ha}$ , which equals an increase of  $352.17 \text{ kg/ha}$  (6.61%). The superiority of TS over CS is also preserved across progenitor species. Expected highest yields in both systems were obtained after predecessor bean, the difference between the systems being  $202.10 \text{ kg/ha}$  in favor of TS. Under the conditions of the experiment, sunflower is the most unfavorable predecessor for the amount of yield, and the difference between the systems is the largest -  $552.30 \text{ kg/ha}$ . In the case of predecessor corn, it is  $302.10 \text{ kg/ha}$  in favor of TS.

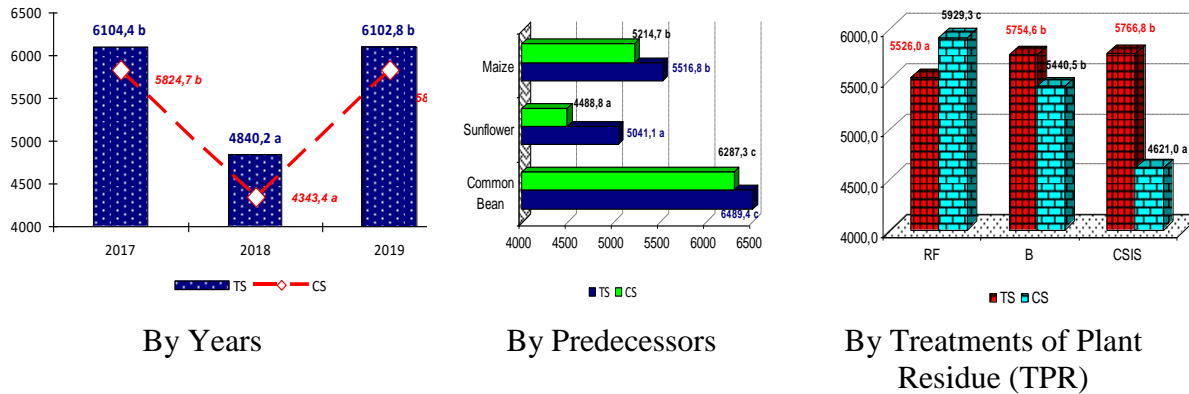


Figure 2. Yields dynamics according to factors of the trial, average for the 2017-2019, kg/ha

Of particular interest are the average results over the period depending on our decision on what to do with the post-harvest residues of the predecessor and how to approach sowing. The most widespread practice based on the removal of crop residues resulted in lower yields when using TS compared to CS by 403.3 kg/ha, or the superiority of CS by 7.30%. In the variants with burning (B) and plowing of plant residues (CSIS), average yields were obtained practically equal in value. In these cases, there is a significant superiority of TS over CS. The same is expressed in obtaining 314.1 kg/ha (5.77%) when burning (B) the residues and 1145.8 kg/ha (24.80%) when completely plowing (CSIS) the non-economic part of the previous crop.

The effect on the yield of the tested ways of using plant residues is strongly influenced by the agricultural technique used when sowing the crop. When using the traditional method of sowing for the region, the average increase in yield compared to the option with removing the residues is +228.6 kg/ha in the option with their burning and +240.8 kg/ha in the option with their complete return to the soil. Qi et al (2019) also reported a positive effect amounting to an average of 8.29% higher yield when crop residues were returned to the soil compared to removal. For the entire study period, the use of a combined machine system is recommended only when removing plant residues from the field. With this system, lower yields were obtained when burning (B) plant residues (- 488.8 kg/ha) and when they were completely plowed (- 1308.3 kg/ha).

The strength of influence of the factors by year, as well as their statistical significance, varies depending on the type of preparation and sowing system used (Fig. 3). The yields in the traditional way of carrying out these activities in the favorable conditions of 2017 and 2019 are more than 90% influenced by the type of the predecessor. In the year with less favorable conditions, the strength of this influence drops to 81.74% of that found in the CS. The strength of post-harvest residue treatment methods and their interaction with the predecessor is subject to statistically unreliable dynamics.

In the combined system (CP), the influence of both factors is essential for yield. In years with a favorable combination of the main meteorological components, about 60% of the yield is determined by the predecessor and over 35% - by the ways of utilization of the post-harvest residues. Under unfavorable conditions for the development of culture, the credibility of the power of influence of the two factors is preserved, but that of the predecessor increases to 67.10%.

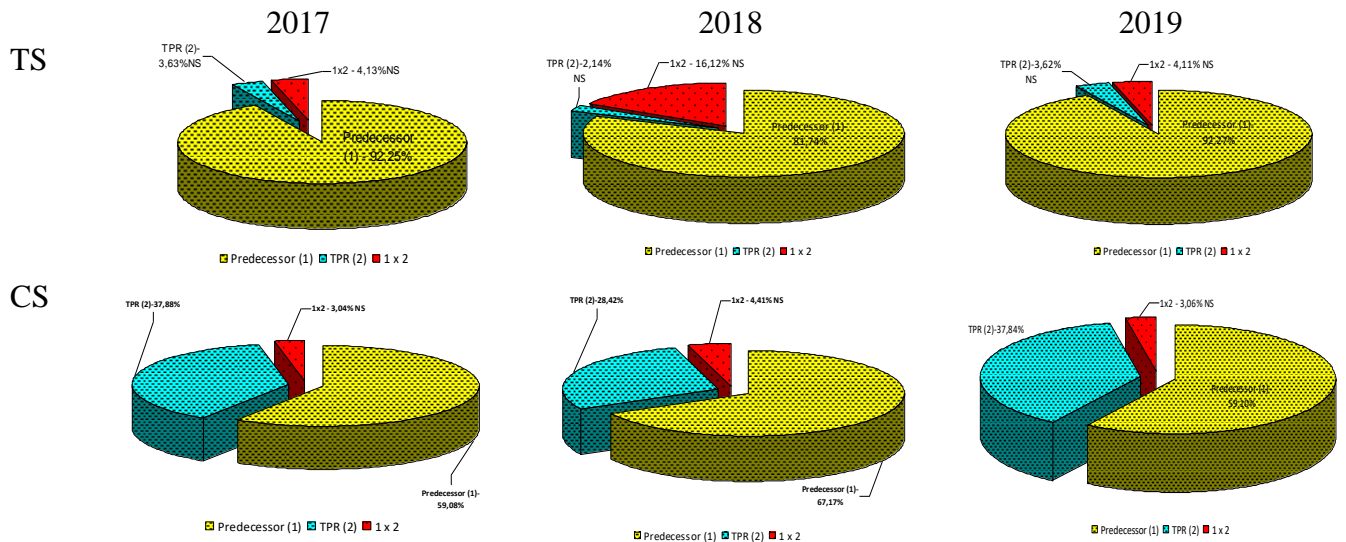


Figure 3. Strength of effect of the factors *Predecessor* and *TPR* on the wheat productivity by years, %

Averaged over the study period, for both tillage and sowing systems tested, the strength of the influence of the predecessor on the yield size was the greatest, followed by that of the weather conditions of the years studied (Fig. 4). In CS, there is a serious redistribution of the power of influence of the three factors, due to the fact that in this system the methods of treatment of plant residues (TPR) have a reliable power of influence both independently and in interaction with the other factors.

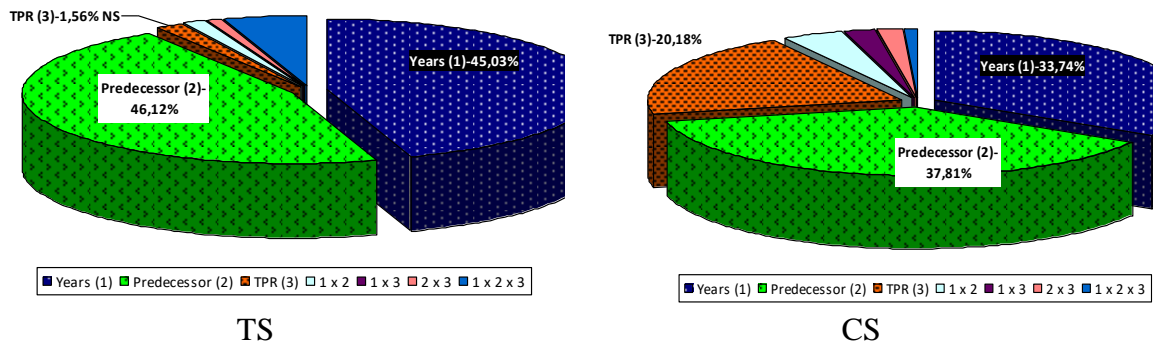


Figure 4. Strength of effect of the trail factors on the wheat productivity for the 2017-2019, %

The values of the physical properties of the grain of the Enola variety are also subject to significant dynamics by year, depending on the type of predecessor and the way of treatment of its post-harvest residues (Table 4). In the tested systems, the reliability of this dynamic varies significantly from year to year depending on the predecessor and the ways of using its plant residues.

In 2017, CS did not lead to significant changes in the 1000 kernel weight depending on the type of predecessor, while in TS the differentiation was very clearly expressed in favor of the bean (Table 5). In this system, the differences between the ways of utilization of plant residues are unproven, and in CS a slight increase in grain size was found in the option with burning (B) of the residues. In 2018, the type of plant residues and the way they are used have little influence on the values of this index when using TS. The application of CS has an unreliable influence on the values of the indicator, but the way they are used leads to a complete

differentiation in the values again in favor of burning (B). The data shows that in 2019 the systems show a clear differentiation in the values depending on the tested factors in the study.

In general, the dynamics of changes in the values of the test weight are less pronounced compared to those of the 1000 kernel weight (Table 6). The highest average values of this index were obtained in 2017 for the variant with plowing of the residues (CSIS) - 81.22 kg (TS) and 81.30 kg (CS).

Table 4. Analyses of variances of physical wheat grain properties according to the ways of PHR utilization over years

Source	Dependent Variable	df	2017		2018		2019	
			F	Sig.	F	Sig.	F	Sig.
<b>TS</b>								
Predecessors (1)	1000 KW	2	19.643	0.001	4.993	0.035 <sup>NS</sup>	70.711	0.000
	TW	2	3.946	0.059 <sup>NS</sup>	170.708	0.000	28.545	0.000
Utilization of PHR (2)	1000 KW	2	0.849	0.459 <sup>NS</sup>	10.239	0.005	69.324	0.000
	TW	2	29.327	0.000	21.064	0.000	7.331	0.013
1 x 2	1000 KW	4	2.638	0.104 <sup>NS</sup>	0.751	0.581 <sup>NS</sup>	21.233	0.000
	TW	4	6.043	0.012	19.055	0.000	2.383	0.128 <sup>NS</sup>
<b>CS</b>								
Predecessors (1)	1000 KW	2	1.338	0.310 <sup>NS</sup>	0.665	0.538 <sup>NS</sup>	6.044	0.022 <sup>NS</sup>
	TW	2	0.941	0.426 <sup>NS</sup>	825.113	0.000	14.968	0.001
Utilization of PHR (2)	1000 KW	2	7.754	0.011	20.557	0.000	25.035	0.000
	TW	2	36.618	0.000	39.500	0.000	146.585	0.000
1 x 2	1000 KW	4	10.589	0.002	5.751	0.014	0.347	0.839 <sup>NS</sup>
	TW	4	4.691	0.025 <sup>NS</sup>	35.750	0.000	1.803	0.212 <sup>NS</sup>

Table 5. Values of 1000 kernel weight by years of study depending on the predecessor and the way of treatment of the residue in the tested systems, g

Factors	2017		2018		2019	
	TS	CS	TS	CS	TS	CS
<b>By Predecessors residue</b>						
Bean	46.64 c	44.71 a	43.17 a	43.57 a	40.90 a	42.05 a
Sunflower	45.32 a	45.05 a	44.84 b	43.32 a	42.62 c	41.84 a
Maize	46.10 b	45.36 a	44.52 b	43.67 a	42.21 b	42.87 b
<b>By Utilization of Plant Residue</b>						
RF	45.90 a	44.40 a	42.84 a	42.54 a	41.08 a	41.20 a
B	45.98 a	45.90 b	45.37 b	44.55 c	42.85 c	42.14 b
CSIS	46.17 a	44.82 a	44.31 b	43.47 b	41.79 b	43.41 c

In addition to the significant differences in the values of the test weight during the individual years of the study, the plant residues of the sunflower have a pronounced negative effect on the values of the index.

Table 6. Values of the test weight by years of study depending on the predecessor and the way of treatment of the residue in the tested systems, kg

Factors	2017		2018		2019	
	TS	CS	TS	CS	TS	CS
By Predecessors						
Bean	80.58 a	80.98 a	75.08 c	75.27 c	77.62 b	77.31 a
Sunflower	80.74 ab	80.93 a	73.27 a	73.18 a	77.17 a	77.53 b
Maize	80.93 b	81.06 a	74.76 b	74.74 b	77.78 b	77.67 b
By Utilization of Plant Residue						
RF	80.24 a	80.54 a	74.17 a	74.17 a	77.42 a	76.91 a
B	80.79 b	81.13 b	74.76 b	74.38 b	77.71 b	77.56 b
CSIS	81.22 c	81.30 b	74.18 a	74.64 c	77.44 a	78.03 c

During the research, the trends of the tested factors in the two used tillage and sowing systems on the values of the physical characteristics of the grain are clearly highlighted (Table 7). The 1000 kernel weight at TS is reliably affected by the independent action of the factors and some of the interactions between them. Using CS excluding the *Year x Predecessor* interaction results in statistically significant effects of the factors and their interactions. The tested factors in both systems have a proven influence on changes in test weight values.

Table 7. Analyses of variances of physical wheat grain properties according to the ways of PHR utilization average for period 2017-2019

Source	df	1000 Kernel Weight				Test weight			
		TS'2017-2019		CS'2017-2019		TS'2017-2019		CS'2017-2019	
		F	Sig.	F	Sig.	F	Sig.	F	Sig.
Years (1)	2	199.15	0.000	99.88	0.000	5337.96	0.000	12373.29	0.000
Predecessors (2)	2	7.60	0.002	5.058	0.014	94.20	0.000	148.24	0.000
Utilization of PHR (3)	2	25.10	0.000	31.53	0.000	31.55	0.000	178.53	0.000
1 x 2	4	12.15	0.000	1.162	0.349 <sup>NS</sup>	46.03	0.000	157.18	0.000
1 x 3	4	6.41	0.001	8.484	0.000	17.42	0.000	11.78	0.000
2 x 3	4	1.63	0.195 <sup>NS</sup>	5.573	0.002	5.93	0.001	6.70	0.001
1 x 2 x 3	8	2.47	0.037 <sup>NS</sup>	6.775	0.000	11.16	0.000	10.94	0.000

Average values of 1000 kernel weight were strongly influenced by weather conditions during the years of study (Figure 5). For both tillage and sowing systems tested, the same were highest in 2017. In general, TS contributes to obtaining a larger grain - on average by 0.43 g compared to CS. The conditions of 2019 are the most unfavorable for the formation of a large grain.

The test weight of the grain is also the highest in 2017, and the conditions of 2018 are extremely unfavorable for the formation of heavy grain. Another characteristic feature is that the difference in average test weight values between the two systems is insignificant in each of the years of the study.

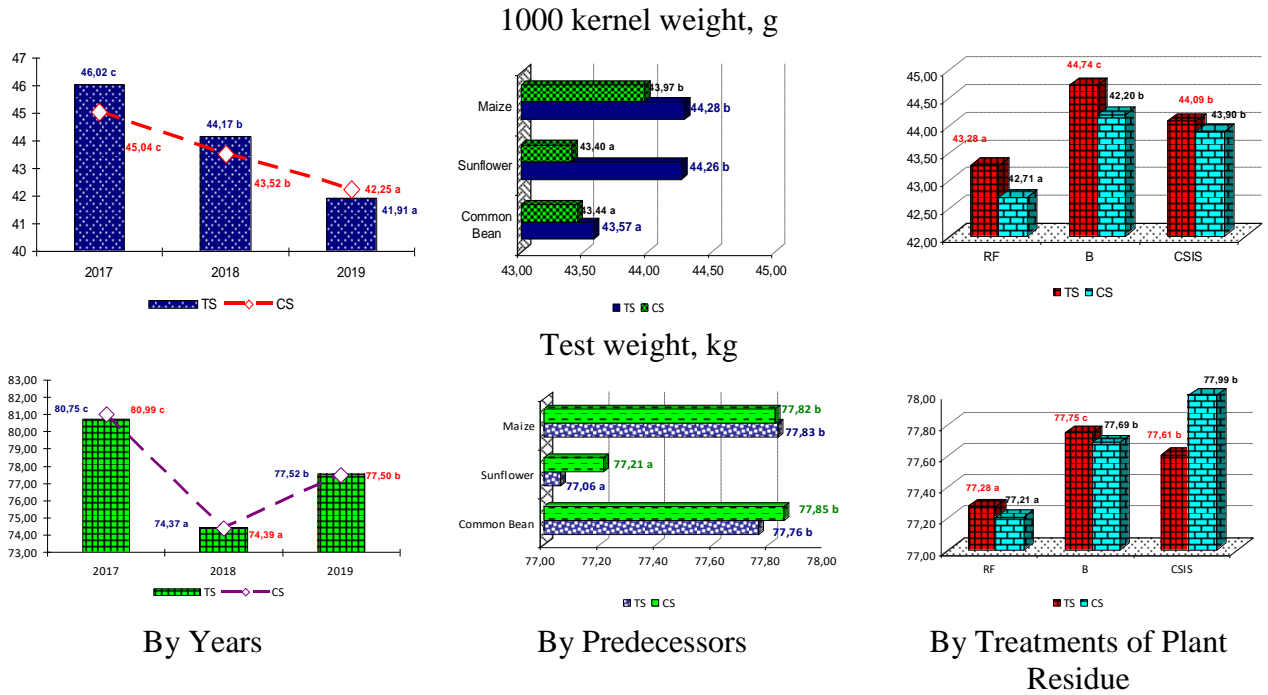


Figure 5. Average values of grain physical characteristics depending on the tested factors in the experiment

The role of the preceding culture on grain physics is very pronounced. Definitely, for both indices, sunflower residues have an extremely negative effect, which is particularly strongly manifested in the values of the test weight at TS. For 1000 kernel weight, except after sunflower predecessor, in both systems, the values of the index are low and after beans. Practically regardless of the technique used for soil preparation and sowing, the largest grain was obtained after a maize predecessor. Using TS after all three predecessors resulted in a grain with a higher 1000 grain weight compared to CS. However, the latter has a more pronounced contribution to obtaining a heavier grain compared to TS. In addition, the positive influence of the predecessors corn for grain and beans on the test weight values is practically equalized.

Depending on what destiny we choose for the post-harvest residues of the predecessor, we also find some changes in the physical characteristics of the grain. They are definitely the lowest in the variant with their removal from the field (RF). This is true for both systems, but it is more noticeable for the 1000 kernel weight, whose average values are higher in TS compared to CS ones. The tendency for better results for grain size in TS was preserved in both the burning of the predecessor crop residue and the chipping and plowing (CSIS) variant. Overall, the largest grain average for the period regardless of tillage and sowing system was obtained by burning (B) the residues (44.47 g), followed by plowing them (44.00 g).

The most unfavorable effect on grain size is their removal from the field (RF). The overall average variation in the values of the test weight depending on the ways in which we use the post-harvest residues is insignificant - from 77.25 kg to 77.80 kg. However, looking at each of the options separately, we find that when removing (RF) or burning (B) the residues, it is preferable to use TS. Averaged over the whole experiment, the heaviest grain was obtained with full tillage of the residues (CSIS) combined with the use of CS.

The physical characteristics of the grain, although relatively conservative, due to its genetic determination for each variety, can be significantly changed depending on the agricultural techniques we apply. The strength of their influence by years of study is clearly manifested and valued as a result of the statistical processing of the results (Figure 6). The

power of influence of each of the factors by years of research and its statistical significance in each of the systems giving an initial start to the plants is graphically expressed.

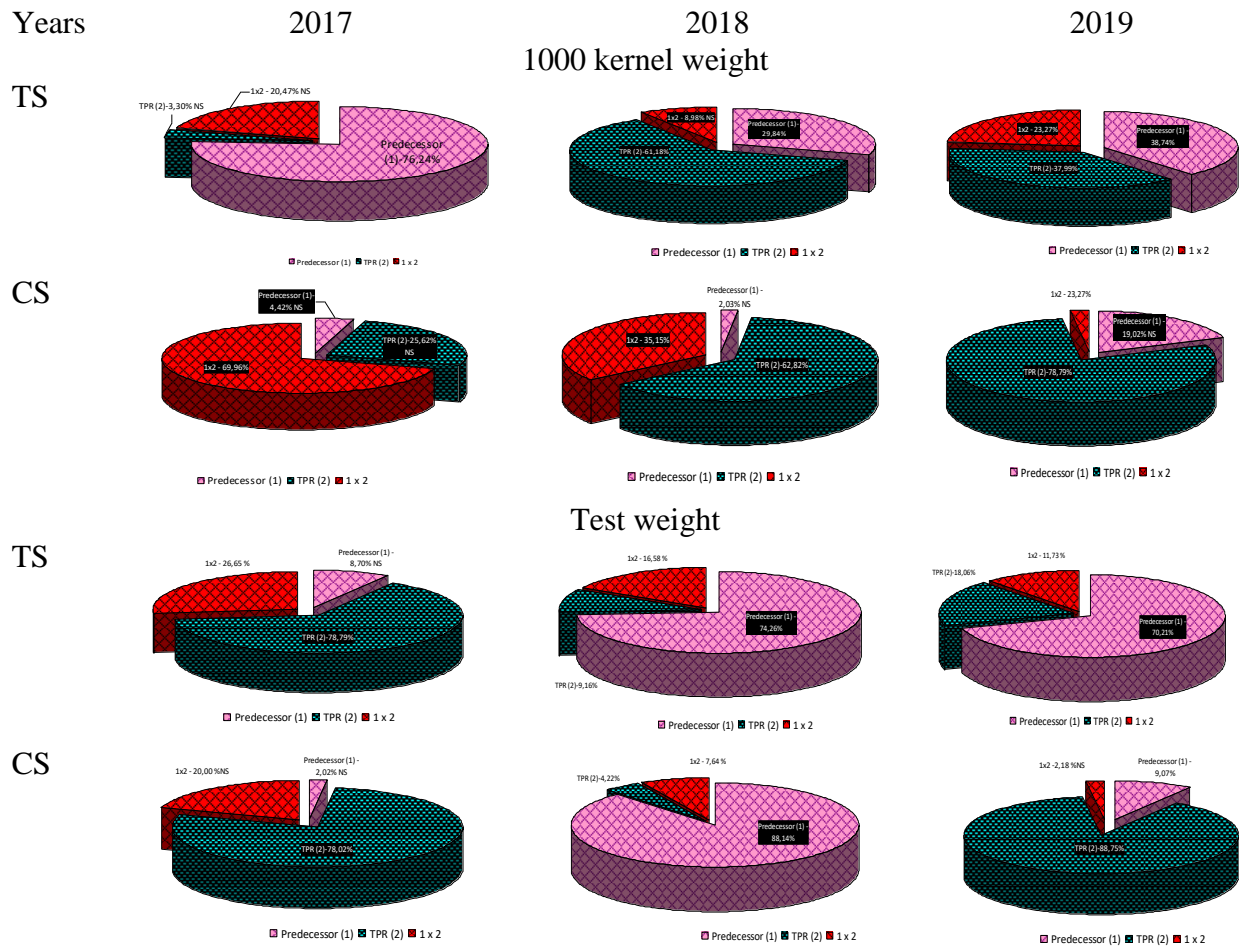


Figure 6. Strength of effect of the factors by years on the grain physical properties of wheat, %

In years with favorable conditions for the development of the crop, the determining force of influence on the 1000 kernel weight in TS is the predecessor, and in CS - the interaction between the predecessor and the way in which plant residues are used. In the favorable conditions of 2017, the test weight values were mainly formed under the strong influence of CSIS in both systems. For the conditions of 2018, a complete synchronicity in the influence of the systems is again established, but this time in favor of the predecessor. For 2019, TS most strongly affects the predecessor, while CS this applies to CSIS.

The established correlation dependences between the studied indices show that their values are subject to significant dynamics in the years of study (Table 8).

Table 8. Pearson correlations between grain yields and grain physical properties by years (n=18)

Indices	2017			2018			2019		
	Yields	1000 KW	TW	Yields	1000 KW	TW	Yields	1000 KW	TW
TS									
Yields	1	,700(**)	-,099	1	-,462	,578(*)	1	-,488(*)	,452
1000 KW	,700(**)	1	,136	-,462	1	-,052	-,488(*)	1	,087
HW	-,099	,136	1	,578(*)	-,052	1	,452	,087	1
CS									
Yields	1	-,138	-,447	1	,154	,524(*)	1	-,364	-,692(**)
1000 KW	-,138	1	,398	,154	1	,301	-,364	1	,831(**)
HW	-,447	,398	1	,524(*)	,301	1	-,692(**)	,831(**)	1

\* Correlation is significant at the 0.05 level (2-tailed); \*\* Correlation is significant at the 0.01 level (2-tailed)

The most pronounced correlation was found in 2017 between yield and mass of 1000 grains ( $r=0.700^{**}$ ) at TS. All other correlations in this year are statistically unreliable. When using CS, a significant correlative dependence was observed between yield and hectoliter mass in 2018 and 2019.

This means that under the conditions of the experiment in years with unfavorable conditions during the period of permanent spring vegetation, there is a proven relationship between productivity and test weight, while the one with 1000 kernel weight is unreliable.

On average for the research period, this fact has been confirmed for both systems. The correlation with TS has higher coefficient values ( $0.615^{**}$ ) compared to that with CS ( $0.486^{**}$ ).

## CONCLUSIONS

The result of the present study found wide dynamics in the productivity of the Enola variety depending on the type of the predecessor and the method of utilization of its plant residues. The traditional system of soil preparation and wheat sowing for the Dobrudja region provides higher yields compared to the combined system in all years of the study. The average increase was 352.17 kg/ha (6.61%). The role of the predecessor has a more pronounced positive influence on the productivity of wheat in the traditional sowing system compared to the combined one. The Enola variety is expected to have the highest yields after its predecessor beans, regardless of the applied agricultural technique during sowing.

Sunflower plant residues have a clear negative impact on wheat productivity. With this predecessor, the biggest difference between the systems was found - 552.30 kg/ha in favor of the traditional system. To obtain the maximum expression of the productive possibilities of the Enola variety, a differentiated approach to the ways in which we will use the plant residues of the predecessor and the technical means for this is required. In areas with minimal presence of plant residues, the combined system of soil preparation and sowing contributes to obtaining 403.3 kg/ha more compared to the traditional one. However, it is extremely unsuitable in cases of burning the residues or their complete plowing. The use of the traditional system in such situations provides higher yields compared to the combined system with 314.1 kg/ha (burning) and 1145.8 kg/ha (plowing), respectively.

In the tested predecessors and methods of utilization of their plant residues, the traditional system definitely contributes to obtaining a larger grain compared to the combined one. The test weight values is mainly influenced by the weather conditions of the years, but not



by the sowing systems. The values of the test weight do not always follow the established trends for the 1000 kernel weight and are characterized by a weaker, although reliable, dynamics.

The power of influence of each of the tested factors on the values of the investigated indices was established. The correlative dependence between the productivity of the variety and the hectoliter weight of the grain is reliable. The correlation with traditional sowing has higher coefficient values (0.615\*\*) compared to that with combined sowing (0.486\*\*). The relationship between the yield and the 1000 kernel weight is negative and unreliable. There is a positive and reliable correlation between the two indices characterizing the physics of the grain.

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## NEGLECTED AND UNDERUTILIZED A CROP IN TURKEY: LINSEED (*LINUM USITATISSIMUM* L.)

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### ABSTRACT

Linseed (*Linum usitatissimum* L.) is the only economically important crops species of the Linaceae family, which includes 13 genus and 300 species. It has two different types, is an annual industrial crop used in fiber and oil production. The tall, high branching types with strong fibers are grown for fiber production and the short, partially low branching types are grown for oil production. Linseed contain 30-45% oil and are an important raw material for the dye and varnish industry as the oil has a natural drying characteristic. Linolenic fatty acid (omega-3) content of linseed oil is high and in recent years, its varieties with high quality oil for edible use have been developed using some breeding methods. Despite the many uses of linseed, it has remained a minor or alternative oilseed crop in Turkey. Therefore, scientific research on this crop is necessary and popularize it as a commercial crop for edible oil, dye and varnishes industry, source of  $\alpha$ -linolenic acid, quality and cool keeping fabric. In addition, if the cultivation of oilseed flax can be expanded in our country and cultivated primarily in fallow areas, it can be one of the alternative oilseed crops that have the potential to close the vegetable oil deficit of Turkey.

**Keywords:** Oilseed crops, Linseed, Flax, Neglected crops

### INTRODUCTION

The neglected and underutilized crops are a rich source of secondary metabolites, vitamins, and micronutrients and thus have the potential to bring dietary diversity with high nutrient value and production for low-income consumers (Baldermann et al., 2016; Hunter et al., 2019). However, most of neglected or underutilized crops are due to their highly low sowing area, production, relatively low yield, consumption, and demand.

Linseed (*Linum usitatissimum* L.) is the only economically important crops species of the Linaceae family, which includes 13 genus and 300 species. The word *usitatissimum*, which means most useful, is appended because of its traditional usefulness as an agricultural crop. (Chand and Fahim, 2008; Koçak and Bayraktar, 2011). It is grown either for its fiber (fiber flax) or for its oil (oilseed flax) (Hall et al., 2016). The tall, high branching types with strong fibers are grown for fiber production and the short, partially low branching types are grown for oil production (Yıldırım and Arslan, 2013). In past, linseed was main source to get industrial oil like dye, linoleum, polish, inks and cosmetic (Green and Marshall, 1984). Linseed contain 30-45% oil and are an important raw material for the dye and varnish industry as the oil has a natural drying characteristic. Linolenic fatty acid (omega-3) content of linseed oil is high and in recent years, its varieties with high quality oil for edible use have been developed using some breeding methods (Green, 1986). Since the Republican Period, our country has experienced an unstable development process in linseed and in recent years it has almost completely disappeared in agricultural terms. On the other hand, since 2018, remarkable attempts have been

made regarding linseed. Locally scaled developments have taken place in this area, such as the promotion of planting and the reproduction of traditional fabrics (Şahin and Yıldız, 2022). This review focuses on the uses of linseed, the characteristics of its oil, the importance of its fiber, its cultivation and production in Turkey and its future.

## **LINSEED USES**

### **Oil**

Linseed oil, extracted from seeds of flaxseed (*Linum usitatissimum* L.), a crop widely cultivated in Europe for fiber or oil for industrial use (Prasad, 1997). Its seed consist of about 25% indigestible fibre, 25% protein and 30-45% oil, which contains mostly unsaturated omega-3 fatty acids (Siva Kumar et al., 2017). Linseed oil contains approximately 9-11% saturated (5-6% palmitic acid and 4-5% stearic acid) and 75-90% unsaturated fatty acids (50-55% linolenic acid, 15-20% oleic acid). The fatty acid composition of linseed oil is dominated by linolenic acid (Bayrak et al., 2020) and it is the best source of the n-3 fatty acid,  $\alpha$ -linolenic acid, which constitutes nearly 55 % of its total fatty acids (Bloedon and Szapary, 2004).

*Alpha*-Linolenic acid (ALA) is an omega-3 ( $\omega$ -3), essential fatty acid. ALA is found in many seeds and oils, including linseed, walnuts, chia, hemp, and many common vegetable oils (Chen et al., 2002). Omega-3 fatty acids have many beneficial effects. It is thought to decrease the risk of heart disease by helping to maintain normal heart rhythm and pumping (Blondeau et al., 2015). Linseed cultivation has resumed in Turkey in the last few years. Especially in the last two years (2021 and 2022), the cultivation area has increased from 10 da to 95 da (TSI, 2022). Therefore, with the expansion of the cultivation area in the future, the potential to provide raw materials to the oil industry can be reached.

### **Fiber**

Flax fiber is obtained from the bast or skin of the stem of flax plant. It is consisting of 70-80% cellulose and 20-30% non-cellulose compounds such as hemicellulose, lignin, pectin, waxes and fats, mineral salts, natural coloring matter and water-soluble compounds (Chand and Fahim, 2008). The fiber is used in textiles, weaving, automotive industry, dye, paper and dietary products (Karimah et al., 2021). Flax fiber is preferred for weaving summer dress fabrics because of its ability to keep cool (Lisson, 2003). Despite all these superior properties of the fiber, developments in the artificial fiber industry have recently shifted linseed production more towards seed production. But the precious and special fiber of flax should not be ignored. Because Turkey has many years of experience in flax cultivation, flax processing and flax fiber industry.

High labor force, lack of mechanization, cheaper synthetic and staple fibers and the inability to compete with cotton plants have led to the extinction of flax in Turkey. Linseed is a potential alternative oilseed and fiber crop for the future as it is cold and drought resistant. It can reach the potential to provide raw materials to the oil and fabric industry by expanding its cultivation area in the future in Turkey.

### **Global Distribution**

Although the origin of linseed is not known exactly, according to the researches, it is stated that it was cultivated in Mesopotamia in 3500-4000 BC and the first cultivated linseed was found near Switzerland (Lay and Dybing, 1989; Özüstün, 2001). Flax fabrics were worn in ancient Egypt and it has even been found in mummy tombs (Bakır, 2005). There is also evidence that linseed oil was used in embalming (James, 2005).

Currently, linseed is grown in about 50 countries, occupying over 4 million hectares of agricultural land and producing over 3.3 million tons of seed. The top 10 leading countries for

linseed production are Russia (1.300.173 t), Kazakhstan (775.568 t), Canada (345.708 t), China (340.000 t), India (111.000 t), Ethiopia (82.000 t), United Kingdom (71.000 t), France (72.940 t), USA (68.790 t) and Ukraine (42.230 t) (FAOSTAT, 2021). On the other hand, only 8 tons of linseed was produced in Turkey in 2022 (TSI, 2022). Linseed has unique drought tolerance; in extreme conditions, it can complete its life cycle in climates in which annual rainfall is only 200 mm (Li and Wang, 2016). Currently, linseed cultivation is increasing in arid and semi-arid regions due to the crop's drought, heat and cold tolerance properties (Yadav et al., 2022; Arslan and Culpan, 2023).

## CONCLUSIONS

Compared to other oilseed crops, linseed is minor and underutilized in Turkey, despite its growing importance in the world. The importance of this crop must be emphasized and highlighted in Turkey as in the world. There are four locally registered linseed varieties (Karakız, Beyaz Gelin, Sarı Dane and Yılmaz) in our country. There is a need to increase the number of varieties due to the different climate and soil characteristics in our country and the increasing vegetable oil deficit every year. In addition, if the cultivation of oilseed flax can be expanded in our country and cultivated primarily in fallow areas, it can be one of the alternative oilseed crops that have the potential to close the vegetable oil deficit of Turkey. Although flax fiber has come to the forefront with its superior qualities (especially fiber fineness and strength) compared to other vegetable fibers, it has continuously declined and its share in natural fibers has continuously shrunk due to its inability to compete economically with other fibers, especially cotton. The economic value of flax is increasing year by year with the diversity of its uses, and its importance increases even more when the production of various industrial products, especially food supplements, cosmetics and paper industry, is added to its oil and fiber production. Therefore, scientific research on this crop is necessary and popularize it as a commercial crop for edible oil, dye and varnishes industry, source of  $\alpha$ -linolenic acid, quality and cool keeping fabric.

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## FATTY ACID COMPOSITIONS AND YIELD COMPONENTS OF HEMP (*CANNABIS SATIVA* L.) GENOTYPES OF DIFFERENT ORIGINS CULTIVATED IN LATVIA

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### ABSTRACT

Hemp is an important eco-friendly and multipurpose crop. The present study aim is focused on the variability in seed fatty acid profile and yield components of two types and eight varieties/lines of *Cannabis sativa* for increasing further the diversity of genetic resources of hemp in order to promote the use of this crop in the food chain for human health and animal welfare in Latvia. Genotypes from diverse European and Latvian origins of oil hemp varieties/lines 'Adzelvieši', 'Pūriņi', KA-2-2011, KA-3-2020, 'Finola', 'Henola' and fiber hemp varieties 'USO 31', 'Austa' were observed. Data for hemp genotypes were collected from the field trials conducted over three years (2020–2022) in the Institute of Agricultural Resources and Economics Department of Plant Breeding and Agroecology at Vilani located in the East part of Latvia in the mild continental humid climate. Results indicated that linoleic acid (C18:2 n-6) was the predominant fatty acid in all the analyzed oils which covers up 65% of the total fatty acids.  $\alpha$ -Linolenic acid (C18:3 n-3) was the second dominant fatty acid followed by oleic acid (C18:1 n-9) and palmitic acid (C16:0). A well-balanced  $\omega$ -6 to  $\omega$ -3 fatty acid ratio (2.3 – 2.7:1) was determined in all genotypes. All the measured parameters strongly varied under the influence of growing years and genotypes. The most promising hemp genotypes exhibited 'Henola' significantly ( $p \leq 0.05$ ) highest seed yield (3.2 t ha<sup>-1</sup>), highest  $\alpha$ -linolenic (24%) and oleic acid (16%), 'Adzelvieši' linoleic (65%),  $\alpha$ -linolenic (28%) and arachidic acid (1.1%), 'Pūriņi' 1000 seed weight (13.48 g),  $\gamma$ -linolenic (5%), palmitic (6.3%) and cis-vaccenic (0.9%) acid as well KA-3-2020 have highest stearidonic (1.8%) acid compared to all genotypes. Hemp oil contents range from 35.8 ('Austa') to 39.25% (KA-3-2020) identified.

**Keywords:** hemp seeds, oil contents, fatty acid profile, 1000 seed yield, genotypes

### INTRODUCTION

Industrial hemp (*Cannabis sativa*) is well adapted for growing in different European climates (Tang et al., 2017, Musio et al., 2018). In Europe, France is the largest agricultural area (with almost 18,000 ha), followed by Lithuania (over 9,000 ha), Estonia (4555 ha), and 10th place Latvia (875 ha) in 2019 (Statista, 2023). According to Latvia's data in 2022, the hemp area (1263 ha) is already higher (RSS, 2023). In Latvia, its production is expected to increase because EU policy focuses more on the 'European Green Deal' objectives.

Hemp is an eco-friendly and multipurpose crop that provides raw materials for a large number of traditional and innovative industrial applications (Ahmed et al., 2022, Zimniewska, 2022). Moreover, tasks set maximizing the use of Latvia's renewable natural raw materials in the production of various industrial, food and feed products in the Latvian Bioeconomy Strategy for 2030 require (BSL, 2023).

Hempseed has commonly been claimed as one of the most nutritionally complete food sources due to its high nutritive traits. It can be consumed as such (whole, hulled seed) or dehulled (hempseed kernel), as well as its processing products, including oil, flour, and protein powder. Despite the fact that some studies highlighted high variability in the hempseed composition according to the genotypes and environmental factors (Galasso et al., 2016, Irakli et al., 2019, Vonapartis et al., 2019, Lan et al., 2019). Hemp seeds contain 25–35% oil, 25–35% lipids with a unique and perfectly balanced fatty acids composition; 20–25% proteins easy to digest and rich in essential amino acids; 20–30% carbohydrates, a great part of which are constituted in dietary fiber, mainly insoluble as well as vitamins, and minerals (Farinon et al., 2020).

The hemp seed oil is valuable to human and animal diets (Cottrell et al., 2020). Additionally, the oil can be used for cooking or processed into cosmetics and fuels (Hua et al., 2019, Sarker et al., 2013). The residual seed cake (“flat cakes”) can be used for protein-rich animal feed (30% proteins, 10% fats). These are used in singles or in concentrated fodders to feed those animals subjected to fattening (600 g “flat cakes” are equivalent, as nutritional value, to 1000 g cereals grains). Hemp seeds have new sources of feed ingredient inputs, especially lipids and protein. For example, fishmeal is primarily made from forage fish and by-products of the commercial fishery industry. With the global supply of forage fish at a plateau, the aquaculture industry has heavily shifted to the use of plant-based proteins and oil to reduce dependency on conventional fishmeal (Cottrell et al., 2020, Hua et al., 2019). The inclusion rate of dietary fishmeal and fish oil used within salmon feeds has significantly reduced to 15–18% and 12–13%, respectively, of the total diet (Hua et al., 2019). In the case of pregnant cows, “flat cakes” must be restrainedly utilized because they can produce abortions. After refination, the oils can be used in the preserves industry.

The valuable and functional food or feed with hemp seeds is increasing. However, studies on seed yield components, oil content, and fatty acid profile of hempseed in Latvia are still unclear. As a semi-domesticated crop, many traits of hemp seed and oil yield require improvement. Selection for genetically stable cultivars with larger seeds will be important for increasing hemp grain yields. The aim of this study is to focus on the seed fatty acid profile and yield components potential of hemp varieties/lines in Latvian climatic conditions. Results are valuable for increasing further the diversity of hemp genetic resources in plant breeding for hemp seed quality characteristics.

## **MATERIAL AND METHOD**

The field trial was carried out at the Institute of Agricultural Resources and Economics, Department of Plant Breeding and Agroecology at Vilani in the middle of Latgale (56°34'10"N, 26°58'01"E) Latvia from 2020 to 2022.

Experimental material for the study consisted of 8 hemp genotypes: seed hemp varieties/line 'Adzelvieši', 'Pūriņi', KA-2-2011, KA-3-2020, 'Finola', 'Henola' and fibre hemp varieties 'USO 31', 'Futura 75', 'Austa' (Table 1.).

The field trials were laid out in a randomized block design of four replicates, the one plot size was 25 m<sup>2</sup>. Seeds were sown during the first to the second decade of May using an experimental sowing machine (SN-16) with an inter-row distance of 12.5 cm and a seeding rate of 60 kg ha<sup>-1</sup>. Complex fertilizer Yara Mila NPK(S) 18-11-13(7) 300 kg ha<sup>-1</sup> was applied after the first cultivation of the soil, 35 days after sowing, and was fertilized with 60 kg ha<sup>-1</sup> of nitrogen, as recommended in a previous study (Tang et al., 2017). Hemp was grown in a Humic Gleyic Podzol (Table 2).

Harvesting was carried out using a grain harvester Sampo SR 2035 and reaper KD-210 (duplex type). Seeds were cleaned with “MLN” sample cleaner. The yield of seeds was weighed



and then re-calculated to weight by 100% purity and 8% humidity. Seed oil content was determined on the grain quality analyzer “Infratec 1241” (FOSS, Denmark). The seeds were counted with the CONTADOR seed counter, weighed with electronic scales, and determined the 1000 seed weight.

Table 1. Industrial hemp varieties/ lines

Variety/line	Type	UPOV species registration country*	Maturity group	Crop type	Reported THC
Adzelvieši	Monoecious	Latvia	Early<120 days	Oil	<0.02%
Pūriņi	Monoecious	Latvia	Early<120 days	Oil	<0.02%
KA-2-2011	Monoecious	Latvia	Early<120 days	Oil	<0.02%
KA-3-2020	Monoecious	Latvia	Early<120 days	Oil	<0.02%
Finola	Monoecious	Finland	Early<115 days	Oil	<0.02%
Henola	Monoecious	Poland	Early<120 days	Oil	<0.02%
USO-31	Dioecious	Netherlands	Early<130 days	Fiber	<0.02%
Austa	Dioecious	Lithuania	Early<130 days	Fiber	<0.02%

\*<https://ec.europa.eu/food/plant-variety-portal/index.xhtml>

Table 2. The main agrochemical parameters of the arable soil layer

Year	Organic matter contents, %	Soil acidity (pH <sub>KCl</sub> )	Available P <sub>2</sub> O <sub>5</sub> , mg kg <sup>-1</sup>	Available K <sub>2</sub> O, mg kg <sup>-1</sup>
2020	7.41	6.61	151	112
2021	6.60	7.30	199	183
2022	7.0	6.85	169.5	143.5

Fatty acid analysis was performed according to the chromatographic analysis and its identity was verified by mass spectrometry in 2020 and 1H NMR spectroscopy data in 2021. The seed oil was obtained by stirring the crushed seeds in hexane at room temperature (4 h), then solidified particles only filtered off and the extract evaporated to a dry residue. The obtained oil is dissolved in CDCl<sub>3</sub>, 1H is taken up the NMR spectrum, and calculated fatty acid composition. The calculation was made according to the Carneiro et al., (2005) methodology.

Meteorological conditions. Agro-meteorological conditions characteristics were used by the Rezekne hydrometeorological station. The calculations were performed by applying the formula (Selyaninov, 1928):

$$HTC = \Sigma x / \Sigma t \times 10, \quad (1)$$

where  $\Sigma x$  and  $\Sigma t$  – sum of precipitations and temperatures in the period, when the temperature has not been lower than 10°C (1).

Ranges of values (Skowera et al., 2014):  $HTC \leq 0.4$  extremely dry;  $0.4 < HTC \leq 0.7$  very dry;  $0.7 < HTC \leq 1.0$  dry;  $1.0 < HTC \leq 1.3$  relatively dry;  $1.3 < HTC \leq 1.6$  optimal;  $1.6 < HTC \leq 2.0$  relatively humid;  $2.0 < HTC \leq 2.5$  humid;  $2.5 < HTC \leq 3.0$  very humid;  $HTC > 3.0$  extremely humid.

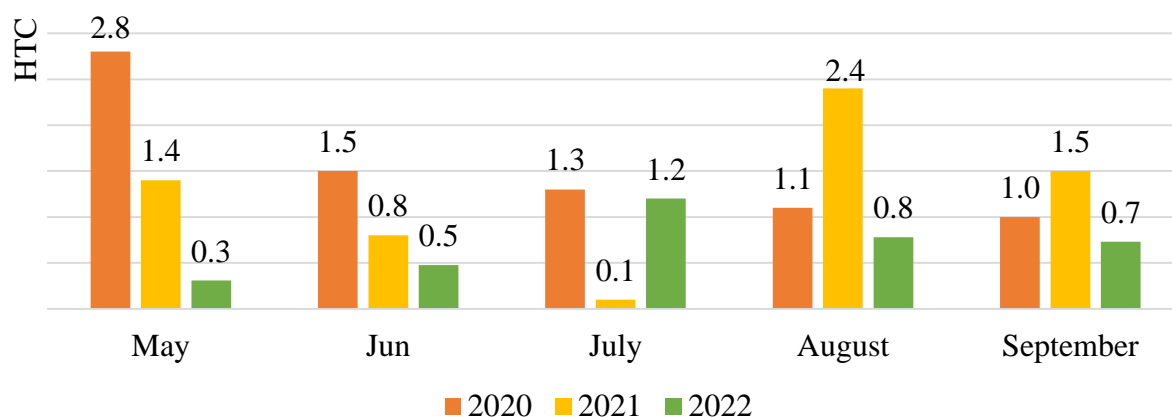


Figure 1. Hydrothermal coefficients (HTC) during the hemp vegetation period from 2020 to 2022

Statistical analysis. Software Excel (Microsoft, USA) was used for data statistical analysis. The difference between the yield properties was determined using analysis of variance (ANOVA). Significant differences among the measured characteristics of hemp were compared by Fisher's protected least significant difference (LSD) tests ( $p \leq 0.05$ ). The coefficient of variability (CV) was used (Döring and Reckling, 2018) to describe stability for each genotype as a stability parameter for seed yield, oil contents, and 1000 seed weight.

## RESULTS AND DISCUSSION

The seed yield of the tested hemp varieties exhibited significant differences between varieties/lines. Hemp genotypes of the seed yield showed higher in 2022 what is means that was a good condition for plant development. In 2021 HTC (Table 2.) was 0.8 as dry conditions in June and 0.1 as extremely dry conditions in July were observed, which affected early flower blooming and lower seed yield. Analyzing coefficient of variation (CV) ranged from 1.67 ('Pūriņi') to 58.85% 'USO-31') between years. The genotypes were harvested significantly ( $p \leq 0.05$ ) with higher seed yield of the variety 'Henola' and more plastic, stabile yield by CV 7.28%.

Table 3. Hemp varieties/line of the seed yield from 2020 to 2022

Variety/line	Seed yield, t ha <sup>-1</sup>			Average	CV
	2020	2021	2022		
Adzelvieši	2.17	1.78	2.12	2.02b	8.56
Pūriņi	2.25	2.16	2.20	2.20b	1.67
KA-2-2011	2.12	1.84	2.61	2.19b	14.53
KA-3-2020	2.46	1.14	2.82	2.14b	33.75
Finola	2.68	1.80	2.75	2.41b	17.94
Henola	2.96	3.26	3.54	3.25a	7.28
USO-31	0.36	0.78	1.69	0.94c	58.85
Austa	0.61	0.81	1.15	0.86c	26.02
			<i>LSD<sub>0.05</sub></i>	0.84	

The research included the seed hemp genotypes that have dioecious and fiber hemp varieties monoecious plants. Research proved that the significantly highest yield performed, and depended on the hemp variety type of used (Table 1., 3.). The seed yield of genotypes amount ranged from 0.86 to 3.25 t ha<sup>-1</sup>. According to Stramkale et al., (2023) previously results,

the hemp varieties with shorter plant heights have the highest seed yield, and fiber varieties with higher heights have lower seed yield in the mild continental humid climate. However, the most perspective genotype with the highest plant height and seed yield is the variety ‘Henola’. Overall compared to another study, Tang et al., (2016) concluded that there was a wide range of hemp seed productivity (0.3 to 2.4 t ha<sup>-1</sup>) for 14 commercial hemp cultivars when they were compared in four contrasting environmental conditions in Europe (Italy, Latvia, Poland) and the highest seed yield (2.4 t ha<sup>-1</sup>) and seed harvest index were found for the earliest maturing cultivar.

In this study, a significant highest of 1000 seed weights of genotypes ‘Pūriņi’ and ‘Austa’ was observed (Table 4.). Compared results were obtained that for oil hemp varieties or fiber, it is not higher and it is dependent more form variety genetic heritability. 1000 seed weight ranges from 10.98 to 13.48 g. Compared between years was showed a lower coefficient of variability of seed weight for both two new potential lines and the highest variety ‘Henola’.

Table 4. Hemp varieties/line of the 1000 seed weight from 2020 to 2022

Variety/line	1000 seed weight, g			Average	CV
	2020	2021	2022		
Adzelvieši	11.83	13.09	14.27	13.06ab	7.63
Pūriņi	14.35	12.14	13.97	13.48a	7.14
KA-2-2011	11.17	10.81	10.95	10.98b	1.35
KA-3-2020	12.12	10.11	11.31	11.18ab	2.85
Finola	12.56	11.82	12.57	12.32ab	9.01
Henola	11.90	14.25	11.77	12.64ab	19.75
USO-31	8.60	10.69	13.92	11.07b	7.39
Austa	13.60	14.25	11.66	13.17a	8.37
<i>LSD<sub>0.05</sub></i>				2.37	

The oil content was influenced by variety and a significant ( $p \leq 0.05$ ) highest amount was recorded of KA-3-2020 at 39.3%. However, the analyzed oil content of CV variations could be variable between genotypes, and it ranges to 0.73% (USO-31) and 10.19% (‘Henola’) over the years (Table 5.). In literature, for example, Farinon et al., (2020) hemp seed oil contents range mostly from 25 to 35% but in this study consist of genotypes with most highest 35% oil contents compared between years and average value.

Table 5. Hemp varieties/line of oil contents from 2020 to 2022

Variety/line	Oil contents, %			Average	CV
	2020	2021	2022		
Adzelvieši	36.1	39.1	37.6	37.6ab	3.23
Pūriņi	37.0	39.2	38.1	38.1ab	2.33
KA-2-2011	36.8	39.1	37.9	37.9ab	2.42
KA-3-2020	38.9	39.6	39.3	39.3a	1.07
Finola	37.5	38.5	38.0	38.0ab	0.80
Henola	38.1	38.9	38.5	38.5ab	10.19
USO-31	28.3	36.4	32.3	32.3c	0.73
Austa	34.2	37.3	35.8	35.8b	3.54
<i>LSD<sub>0.05</sub></i>				2.99	

Fatty acid profile and amount in seed oils vary depending on genotypes and years (Figure 2.). According to Peiretti, (2009) fatty acid profiles can vary in addition developmental stage of hemp plants, age, and environmental conditions. In hemp oil, the first highest from 47 to 65% in 2020 and 53 to 53.9% in 2021 linoleic acid from unsaturated fatty acids was represented. The second highest is  $\alpha$ -linolenic acid amount ranged from 22 to 28% in 2020 and from 20.6 to 22.4% in 2021. In studied Truřa et al., (2009), the  $\alpha$ -linolenic acid was detected only in seeds, this compound lacking in the other phenophases.

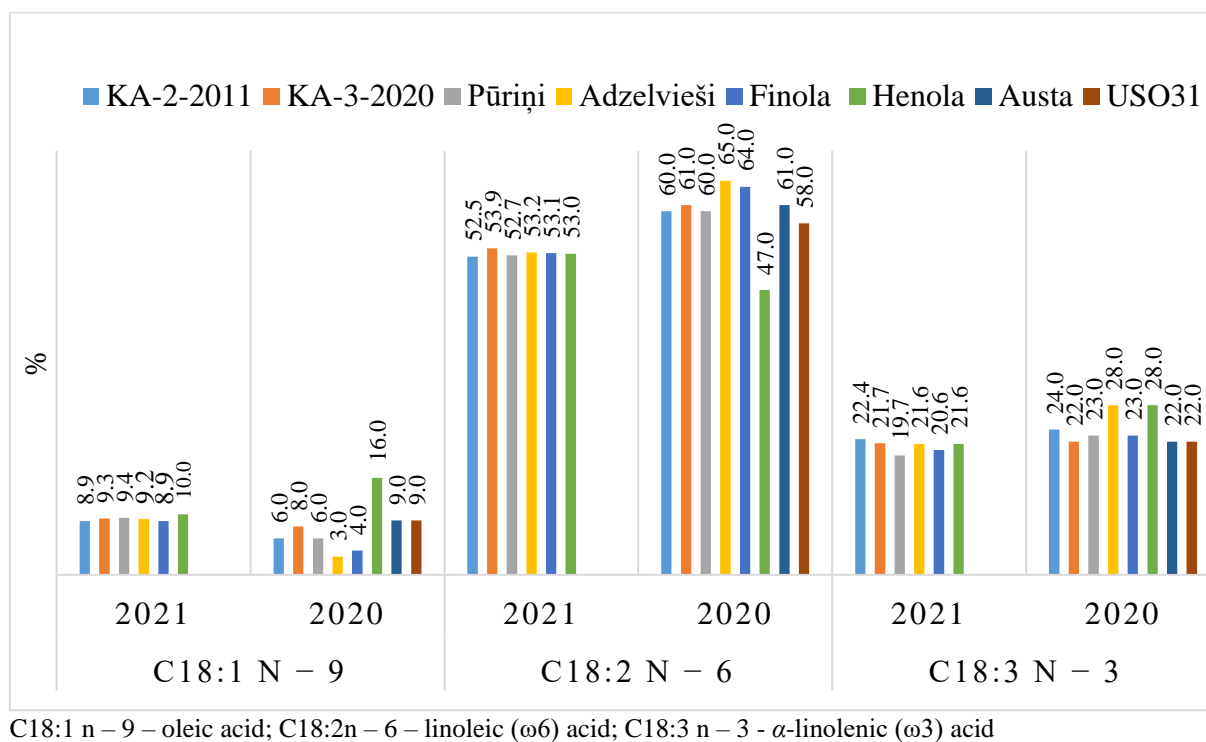


Figure 2. Oil fatty acid profile/content in hemp seeds 2020 and 2021

The major monounsaturated fatty acid was oleic acid (18:1, n-9) which has been shown to reach the range from 3 to 16% in 2020 and from 8.9 to 10% in 2021 between genotypes. The highest oleic acid of variety ‘Henola’ in both years was shown. Generally, the amount of oleic acid in hempseed oil is shown to be higher than that found in chia seed (7%) by Marineli et al., (2014) and comparable to those present in linseed (15%) by Teh and Birch, (2013).

Hemp seeds are the unique source of  $\gamma$ -linolenic acid, and it ranges from 2.5 to 5% between genotypes (Table 6.). The highest  $\gamma$ -linolenic acid of the variety ‘Pūriņi’. In addition, Kiralan et al., (2010) reveal that hempseeds from regions with a mild or warm climate contain small amounts of  $\gamma$ -linolenic acid, whereas hempseeds from temperate or even cold regions have a large amount. The palmitic acid amount ranges from 5.1 to 6.3%, stearic from 2.1 to 2.8%, cis-vaccenic from 0.7 to 1.0%, stearidonic from 1.2 to 1.8%, arachidic from 0.8 to 1.1%, and lowest cis-11-eicosenoic with amount 0.5%. Depending on genotype highest amount has ‘Pūriņi’, ‘Finola’ of palmitic acid, ‘Henola’ of stearic acid, ‘Finola’ of cis-vaccenic, KA-2-2011 of stearidonic and ‘Adzelvieši’ of arachidic acid. According to Leizer et al., (2000) hempseed oil is also one of the few botanical sources of  $\gamma$ -linolenic acid and stearidonic acid, which are of increasing pharmaceutical interest, making the nutritional value of hempseed superior to other seed oils. Furthermore, Callaway et al., (1996), Guil-Guerrero, (2007) reveal that both  $\gamma$ -linolenic acid and stearidonic acid act as precursors for the rapid synthesis of longer chain fatty acids, such as eicosanoids, in the human body.

Porto et al., (2015) investigated oil production in the north-east of Italy and noted that seed oils from different hemp varieties ('Felina 32', 'Chamaeleon', 'Uso31', and 'Finola') contained 2.89–5.88% palmitic, 1.76–2.37% stearic, 9.25–12.31% oleic, 55.98–59.37% linoleic, 3.19–6.42%  $\gamma$ -linoleic, 17.34–19.70%  $\alpha$ -linoleic and 0.74–1.58% cis-11-eicosenoic acids. Compared results of oil acid profile and amount in Italy (Porto et al., 2015), Turkey (Özdemir et al., 2021), with Latvia are similar only  $\alpha$ -linoleic acid is higher, and cis-11-eicosenoic acid is lower in Latvian conditions. The Golimowski et al., (2022) results in Poland compared range  $\alpha$ -linoleic acid of 'Finola' (16%) also obtained lower contents as in Latvia the same variety. Concluded that the oil acidity profile could be different depending on climatic conditions. Another study proved that the climate and geographical area also influence the fatty acid composition. Taaifi et al. (2021) evaluated the composition of hemp seed oil of two varieties of hemp grown in four different regions from Morocco and they determined that the observed variability in the composition was not only related to the cultivar variety but also to the growing area and the interaction of the climatic conditions. Furthermore, Irakli et al. (2019) reported that the most affected fatty acids by the genotype are  $\alpha$ -linoleic acid and oleic acid, while linoleic acid is less affected by the genotype variation. However, Galasso et al. (2016) besides the variability of  $\alpha$ -linoleic acid, observed a genotype variability in linoleic acid as well.

Table 6. Fatty acid (FA) profile/content in hemp seeds in 2020

Composition of fatty acid, %	Chemical Structure	KA-2-2011	KA-3-2020	Pūriņi	Adzelvieši	Finola	Henola
$\gamma$ -Linolenic ( $\omega 6$ )	C18:3 n – 6	4.2	3.6	5.0	4.4	4.4	2.5
Palmitic	C16:0	6.0	5.1	6.3	6.2	6.3	6.1
Stearic	C18:0	2.1	2.4	2.6	2.6	2.5	2.8
<i>cis</i> -Vaccenic	C18:1 n – 7	0.9	0.7	0.9	0.8	1.0	0.9
Stearidonic	C18:4 n – 3	1.8	1.7	1.7	1.7	1.7	1.2
Arachidic	C20:0	0.8	0.8	1.0	1.1	0.9	0.9
<i>cis</i> -11-Eicosenoic	C20:1	0.5	0.5	0.5	0.5	0.5	0.5
Total Unsaturated Fatty Acid	-	91.2	91.4	90.0	90.2	90.2	90.2
$\omega 6:\omega 3$ ratio	-	2.3	2.5	2.7	2.6	2.6	2.4

Overall, the literature data showed that hempseed oil is characterized by high polyunsaturated fatty acids content and low saturated fatty acids amounts. The genotypes of hempseed oil contained up to 90% unsaturated fatty acids (Table 6.). Compared to different literature results Vonapartis et al., (2015), Crescente et al., (2018) Leonard et al., (2020) are similar.

Results showed that the hemp genotypes in oil  $\omega 6:\omega 3$  ratio value ranged from 2.3 to 2.7. The highest amount between the variety of 'Pūriņi' (2.7). Simopoulos, (2008) reveals that the n-6/n-3 ratio represents an important index to ensure the maintenance of an optimal state of health, and to prevent the onset of chronic-degenerative diseases characterized by a chronic inflammation status, like cardiovascular and neurodegenerative diseases, as well as cancer. According to Callaway, (2004) the ideal n-6/n-3 ratio has been established as 3:1 to 5:1, that is also the ratio found in the traditional Japanese and Mediterranean diets, where the incidence of coronary disease has been historically low. In the study by Siano et al., (2018) the fat component of hempseeds makes them a food matrix with the ideal, low n-6/n-3 ratio ranging precisely from 3:1 to 5:1, which is useful for reducing the n-6/n-3 ratio in diets, especially of industrialized countries, where there is an unhealthy average n-6/n-3 ratio of about 10:1, due to the too high n-6 and too low n-3 amount in the people's diets.

## CONCLUSIONS

Climatic conditions of the experimental years had the strongest effect on hemp agronomically important seed yield traits.

Linoleic acid (C18:2 n-6) was the predominant fatty acid in all the analyzed oils which covers up 65% of the total fatty acids.  $\alpha$ -Linolenic acid (C18:3 n-3) was the second dominant fatty acid followed by oleic acid (C18:1 n-9) and palmitic acid (C16:0). A well-balanced  $\omega$ -6 to  $\omega$ -3 fatty acid ratio (2.3 – 2.7:1) was determined in all genotypes.

In Latvian local climatic conditions identified most perspective the hemp genotype ‘Henola’ significantly ( $p \leq 0.05$ ) highest and more plastic, stabile seed yield (3.2 t ha<sup>-1</sup>), highest  $\alpha$ -linolenic (24%) and oleic acid (16%), ‘Adzelvieši’ linoleic (65%),  $\alpha$ -linolenic (28%) and arachidic acid (1.1%), ‘Pūriņi’ 1000 seed weight (13.48 g),  $\gamma$ -linolenic (5%), palmitic (6.3%) and cis-vaccenic (0.9%) acid as well KA-3-2020 have highest stearidonic (1.8%) acid compared to all genotypes. Hemp oil contents were identified as valuable range from 35.8 (‘Austa’) to 39.25% (KA-3-2020).

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