Extraction, distribution and efficiency of nitrogen use in newly created genotypes of common winter wheat (*Triticum aestivum*)

Elisaveta Vasileva, Zlatina Uhr, Blagoy Andonov
Agricultural Academy, Institute of Plant Genetic Resources "Konstantin Malkov" - Sadovo 4122, Plovdiv, Bulgaria
E-mail*: elisaveta_vasileva@ipgr.org*

Citation
Vasileva, E., Uhr, Zl., & Andonov, Bl. (2022). Extraction, distribution and efficiency of nitrogen use in newly created genotypes of common winter wheat (*Triticum aestivum*). *Bulgarian Journal of Crop Science, 59*(5) 21-27.

Abstract
In a field experiment, sown according to the method of fractional plots after fallow, on a fertilizer background of 20kg/da P_2O_5, for three consecutive growing years (2018/2019 – 2020/2021), the impact of nitrogen fertilizer rates 0, 6, 12 and 18 kg/da at a seeding density of 500 and 550 g.s./m^2 on the genotypes Sadovo 1, Anapurna, Avenu, Iaizla, BA535, BA769, BA850, RU33/3244 and MH260/1175. The aim of the research is to characterize newly created genotypes of common winter wheat (*Triticum aestivum*) in terms of nitrogen extraction, distribution and utilization efficiency. The cumulative effect of the factors genotype, agrometeorological conditions and nitrogen fertilization is statistically significant with well above the typical effect size ($p \leq \alpha$; $\eta \geq 0.45$) on grain protein content, total nitrogen export with biomass, nitrogen consumption for formation of a unit of production, the nitrogen harvest index, the grain nitrogen supply index, the efficiency of nitrogen use from fertilizers and the return efficiency of nitrogen fertilization. Genotypes were differentiated by grain protein content, post-flowering accumulation, nitrogen export, nitrogen consumption per 100 kg of grain, grain collateral and nitrogen reutilization. Lines BA769, BA850 and RU33/3244 have the highest protein content in the grain, the most active post-flowering nitrogen accumulation, the largest nitrogen export with the biomass, the highest nitrogen consumption per unit of production and the highest efficiency of nitrogen recycling after flowering. At RU33/3244, the strongest post-flowering accumulation and the strongest reutilization of nitrogen were recorded simultaneously. These three lines have the greatest potential for use in view of the requirements of modern agriculture.

Key words: wheat (*Triticum aestivum*); breeding lines; nitrogen use efficiency

INTRODUCTION

The use in the production of varieties with more efficient assimilation of nitrogen is an important approach in the modern conditions of climate change and the global energy and raw material crisis. The efficient use of nitrogen plays an important role in the development of sustainable production systems, with reduced costs by farmers, without environmental pollution and increased greenhouse gas emissions (Atanasov et al., 2019; Mălinaş et al., 2022). In wheat, nitrogen uptake, assimilation, allocation and use efficiency are important, as are the interactions of genetics, environment and management. The efficiency of nitrogen uptake is a characteristic of the genotype and is due to the morphological, physiological and biochemical processes occurring in the plants. The full realization of the genetic potential of grain protein content varieties depends on nitrogen fertilization, the content of nutrients available to plants, the ratio and balance of nutrients in the soil and moisture in it (Ivanov, 2018). Bulgarian common wheat varieties grown in practice require intensive technology to obtain high yields and quality (Yordanova, 2020). Elite European winter wheat germplasm has demonstrated a trend toward improvement in NUE and NHI over the past 25 years (Lupini et al., 2021; Mălinaş et al., 2022).

Export and consumption of nitrogen to form a unit of main and additional production in wheat are basic agrochemical indicators (Ivanov, 2018). Nitrogen export is an indicator that integrates productivity and protein content, which are negatively correlated with each other (Sheheda et al., 2018; Kiriziy & Sheheda, 2019). The reduced consumption of nitrogen for the formation of 100 kg of grain is directly related to the efficiency of its use and increases the productivity coefficient. Effective from an agrochemical point of view are varieties with low nitrogen consumption per unit of production. The nitrogen harvest index is informative about the distribution of nitrogen by organs and is indicative of the economic use of plant-extracted nitrogen. Agrochemically valuable genotypes have up to 2 times higher fertilizer utilization rate and high nutrient remobilization efficiency (Ivanov, 2018; Atanasov et al., 2019). Protein content is a determining factor for bakery qualities (Hawkesford & Riche, 2020; Mălinaş et al., 2022) and is important for the concentration of other nutritional elements (Mn, Zn, Cu Fe), for functional and bioactive properties (antioxidant properties, flavonoid content, etc.), for thermotolerance and stress resistance (Punia et al., 2019; Kumar et al., 2020; Miroshnychenko et al., 2020).

Nitrogen (respectively protein) in grain is the result of two opposite processes – assimilation and reutilization (Zhang et al, 2020; Mălinaş et al., 2022). The efficiency of using the nitrogen-containing compounds deposited in the vegetative mass is an important physiological component of the wheat production process, as it significantly correlates with main economic-valuable signs such as yield and protein content in the grain (Shegeda et al., 2018; Morgun et al., 2021). Since the two traits are negatively correlated, an increase in the total amount of protein per unit of sown area is set as a selection objective. To increase this indicator, it is useful to optimize the donor-acceptor interactions between the wheat organs in the process of pouring the grain. Stronger expression of genes related to nitrogen transport, assimilation and remobilization are reflected in higher yields (Alpuerto et al., 2021). Nitrogen recycling efficiency is indicative of wheat resistance to interspecific competition (Asadi et al., 2020).

The aim of the research is to characterize newly created genotypes of common winter wheat (*Triticum aestivum*) in terms of nitrogen extraction, distribution and utilization efficiency.

MATERIALS AND METHODS

In a field experiment, sown according to the method of fractional plots after fallow, on a fertilizer background of 20kg/da P_2O_5, for three consecutive growing years (2018/2019 – 2020/2021), the influence of nitrogen fertilizer rates 0, 6, 12 and 18 kg/da at a seeding density of 500 and 550 g.s./m^2 on the genotypes Sadovo 1, Anapurna, Avenu, Iaizla, BA535, BA769, BA850, RU33/3244 and MX260/1175.

Average vegetation temperatures during the experimental period exceeded the norm for the region (multi-year data from the period before climate changes were taken into account - 1931 - 1985) by approximately 2°C. The average increase in temperatures is greatest in the 2019/2020 growing season (2,3°C). The largest increase during the spring vegetation period was recorded in the first year 2018/2019 (1,95°). The most uneven distribution of temperature deviations was observed in the third year 2020/2021, when at the beginning and end of the growing season the deviations had a negative sign, and the winter months were warmer by approximately 5°C.

The total amount of vegetation precipitation slightly exceeded the norm for the area in all three years of the experiment, and the distribution of precipitation was uneven. Relatively the most unfavorable is the first year, which is relatively dry throughout the growing season and in the month of June, during the ripening of the grain, there was natural precipitation in the amount of about 180 l/m^2. The second growing year is characterized by a relatively dry autumn-winter period, abundant spring rains and drought at the end of the growing season. The most favorable for the development of the plants and the formation of the composition of the grain is the third year, when abundant autumn-winter precipitation was recorded, leveling with the multi-year norm for the area in the spring and gradual drying towards the beginning of the summer (Figure 1).

The following indicators were calculated: Straw protein, kg/da; Grain protein, kg/da; PFAN, kg/da

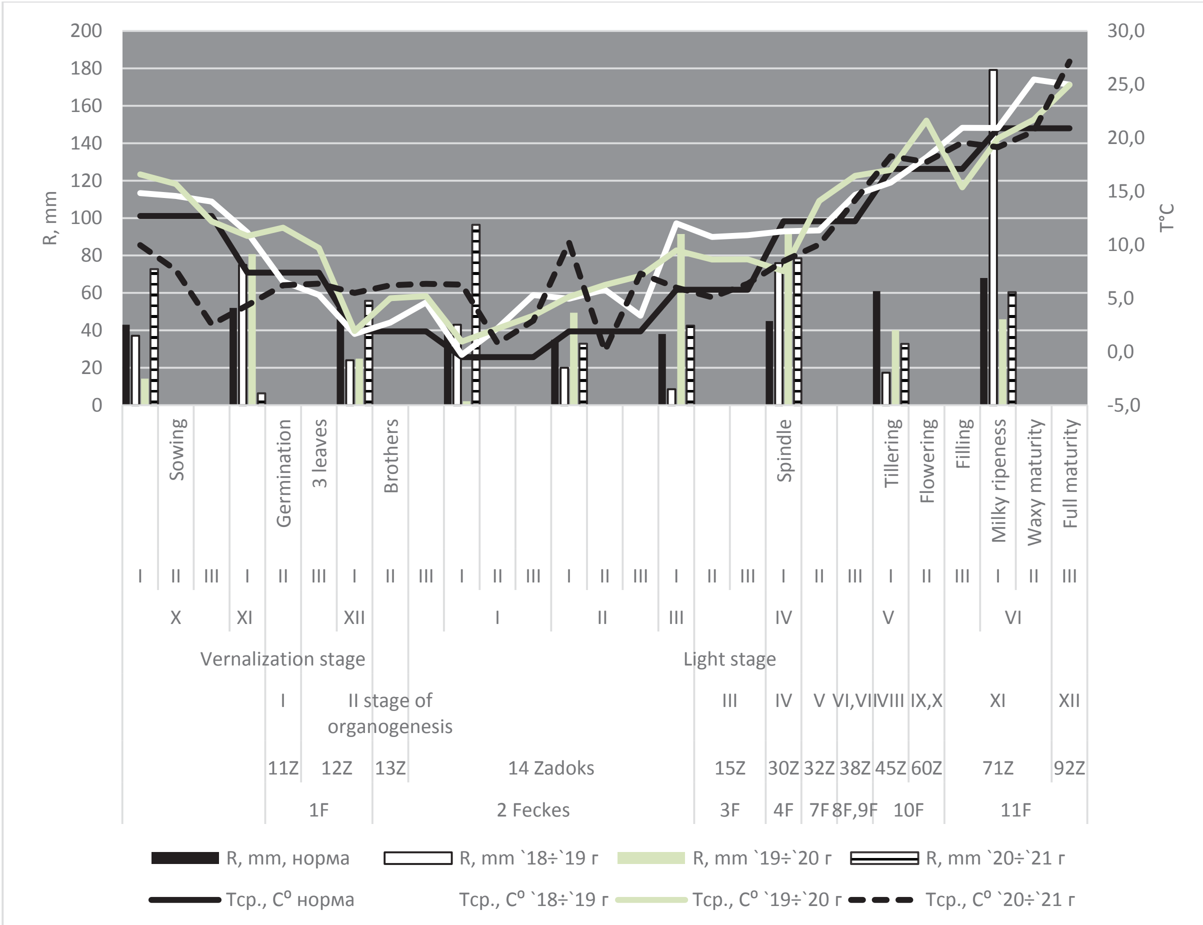

Figure 1. Agrometeorological conditions

– post-flowering accumulation of nitrogen (PFAN = EN - N kg/da flowering); EN, kg/da – nitrogen export (EN = N kg/da straw + N kg/da grain); CN/100 kg – nitrogen consumption per unit of production (CN = EN*100/yield kg/day of grain); NHI – nitrogen harvest index (NHI = N kg/da of grain / EN); GSIN – indicator of nitrogen supply of the grain (GSIN= EN/yield kg/da of grain); RN – reutilization of nitrogen, kg/da (RN= N kg/da flowering - N kg/da straw); ER – reutilization efficiency, % (ER= RN*100/ N kg/da flowering); NUE – nitrogen utilization efficiency from fertilizers (NUE= N kg/da fertilizer/ EN); REN – returnable efficiency of nitrogen fertilization (REN= (N kg/da grain with fertilization - N kg/da grain without fertilization) / N kg/da fertilizer). Univariate three-factor analysis of variance and regression analysis of the results were performed.

REZULTS AND DISCUSSION

Univariate three-way analysis of variance showed statistically significant results with well above the typical effect size ($p \leq \alpha$; $\eta \geq 0.45$) for the cumulative effect of the factors genotype, year (agrometeorological conditions) and nitrogen fertilization on grain protein content, total export of nitrogen with biomass, nitrogen expenditure for the formation of a unit of production, the nitrogen harvest index, the grain nitrogen supply indicator, the efficiency of nitrogen use from fertilizers and the return efficiency of nitrogen fertilization. Between 52% and 69% of the variance in the measures was predicted by the combined effect of the three experience factors (Table 1). For the post-flowering accumulation, the reutilization of nitrogen and its efficiency, the simultaneous effect of the factors genotype and level of

Table 1. Analysis of variance

Dependent variables	Factors	Genotype	Year	Fertilizer nitrogen, kg/da	Genotype * Year	Genotype * Fertilizer nitrogen	Year * Fertilizer nitrogen	Genotype * Year * Fertilizer nitrogen
Straw protein, kg/da	**p**	0,014	0,000	0,000	0,006	0,085	0,000	0,131
	η	0,183	0,817	0,863	0,251	0,282	0,343	0,345
Grain protein, kg/da	**p**	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	η	0,810	0,980	0,967	0,775	0,454	0,493	0,693
PFAN, kg/da	**p**	0,000	-	0,000	-	0,000	-	-
	η	0,649	-	0,976	-	0,743	-	-
EN, kg/da	**p**	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	η	0,780	0,979	0,973	0,709	0,424	0,561	0,654
CN/100 kg	**p**	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	η	0,513	0,826	0,948	0,554	0,514	0,650	0,591
NHI	**p**	0,000	0,000	0,365	0,000	0,000	0,000	0,000
	η	0,368	0,298	0,034	0,415	0,447	0,597	0,518
GSIN	**p**	0,000	0,000	0,000	0,000	0,000	0,000	0,000
	η	0,514	0,814	0,945	0,556	0,489	0,631	0,590
RN, kg/da	**p**	0,001	-	0,000	-	0,000	-	-
	η	0,496	-	0,767	-	0,707	-	-
ER, %	**p**	0,694	-	0,000	-	0,001	-	-
	η	0,134	-	0,701	-	0,671	-	-
NUE	**p**	0,000	0,000	0,000	0,000	0,000	0,011	0,000
	η	0,429	0,483	0,450	0,695	0,536	0,170	0,523
REN	**p**	0,000	0,000	0,000	0,000	0,000	0,011	0,000
	η	0,429	0,483	0,450	0,695	0,536	0,170	0,523

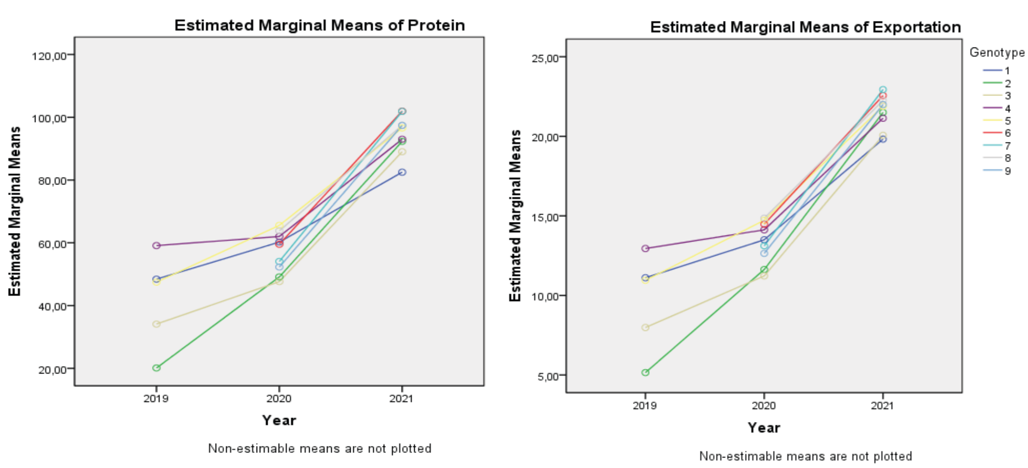

Figure 2. Illustration of the interaction effect between the independent variables on the indicators

nitrogen fertilization is statistically significant. The magnitude of the effect is well above typical and it predicts 67–74% of the variance of the indicators. Only for the indicator protein in straw no statistically significant main and combined effects of the independent variables were observed.

The main impact of the conditions of the year was statistically proven for all the studied indicators, with their values being the lowest in the first year. The reason for this is the high humidity and abundant rainfall during the second half of the growing season, during the spindle-heading period, when the largest amount of soil nitrogen is used and stem reserves are built up. The values of all indicators increased at all levels of fertilization in the second and were highest in the third year of the experiment, the most favorable from an agrometeorological point of view (Figure 2). This corroborates the observations of other authors (Zhaoyun et al., 2019) that rainfall is more decisive than the amount of fertilizer for the photosynthetic gas exchange characteristics of flag leaves at the grain pouring stage.

Genotypes were differentiated by grain protein content, post-flowering accumulation, nitrogen export, nitrogen consumption per 100 kg of grain, grain collateral and nitrogen reutilization. The main influence of the genotype on the indicated indicators is statistically significant with a much larger than typical effect size. The lines BA769, BA850 and RU33/3244 have the highest protein content in the grain, the most active post-flowering nitrogen accumulation, the largest nitrogen export with the biomass and, accordingly, the highest nitrogen consumption per unit of production, but also with the highest nitrogen recycling efficiency after flowering. As other studies have shown, high post-flowering nitrogen accumulation (Sheheda et al., 2018) and strong nitrogen reutilization (Zhang et al., 2021) are associated with high grain protein content.

In contrast to previous studies, in which the variety Avenu showed a large export of nitrogen (Yordanova, 2020), under the conditions of the present experiment, this variety had one of the lowest amounts of exported nitrogen with biomass. The most effective from an agrochemical point of view are the genotypes Avenu, BA535 and MH260/1175. They have the lowest nitrogen consumption per unit of production, and according to the research of other authors, this indicates that they are suitable for conditions of a biological system of cultivation (Ivanov, 2018). Interestingly, RU33/3244 had the highest post-flowering accumulation and the highest nitrogen reutilization, which resulted in the highest grain protein content. Another thing that is striking is that with the three genotypes BA769, BA850 and RU33/3244, the efficiency of using nitrogen from fertilizers is below the reference values for NUE 0.5 (Table 2).

The polynomial model of dependence shows that with an increase in fertilizer nitrogen by one kilogram, the regression step for the efficiency coefficient for the three genotypes established increases by 0.105-0.155, while for the rest of the genotypes, with the exception of MH260/1175, the increase is more than 0.208-0.434 (Figure 3). According to some studies, nitrogen extraction increases with increasing nitrogen fertilization (Dereje et al., 2019), and nitrogen use efficiency from fertilizers decreases with increasing fertilizer rate (Belete et al., 2018). Under the agrometeorological conditions of

Table 2. Derived indicators

Genotype	Grain protein, kg/da	PFAN, kg/da	EN, kg/da	CN/100 kg	NHI	GSIN	RN, kg/da	ER, %	NUE	REN
Sadovo 1	63,72	18,20	14,81	2,18	0,77	0,02	25,61	82,98	0,57	0,61
Anapurna	53,86	19,86	12,76	2,16	0,67	0,02	27,05	83,40	0,79	0,49
Avenu	56,92	18,49	13,09	2,01	0,76	0,02	22,51	84,07	0,66	0,46
Iaizla	71,38	19,64	16,07	2,13	0,79	0,02	22,82	82,71	0,50	0,47
BA535	69,87	20,32	15,86	2,11	0,78	0,02	24,72	83,66	0,52	0,43
BA769	80,75	21,01	18,52	2,21	0,76	0,02	24,66	84,24	0,44	0,57
BA850	77,97	21,32	18,03	2,33	0,76	0,02	25,98	83,80	0,45	0,57
RU33/3244	80,65	20,67	18,56	2,28	0,77	0,02	28,10	84,65	0,43	0,50
MH260/1175	74,85	20,52	17,32	2,12	0,76	0,02	23,20	82,74	0,48	0,58

the present study, this effect was not observed, due to the presence of sufficient water reserves in the soil, ensuring the accumulation of soil nitrogen. According to other authors, nitrogen use efficiency is greater in high-intensity cultivars (Kiriziy & Sheheda, 2019). With the modern trends for sparingly agriculture, the ability of the varieties for post-flowering accumulation and nitrogen reutilization are more important, which shows that the specified selection lines BA769, BA850 and RU33/3244 have a very high potential for use.

CONCLUSIONS

The cumulative effect of the factors genotype, agrometeorological conditions and nitrogen fertilization is statistically significant with a much higher than typical effect size ($p \leq \alpha$; $\eta \geq 0.45$) on the grain protein content, total nitrogen export with biomass, nitrogen expenditure for the formation of a unit of production, the nitrogen harvest index, the indicator for the provision of nitrogen to the grain, the efficiency of using nitrogen from fertilizers and the return efficiency of nitrogen fertilization.

Genotypes are differentiated by grain protein content, post-flowering accumulation, nitrogen export, nitrogen consumption per 100 kg of grain, grain collateral and nitrogen reutilization.

Lines BA769, BA850 and RU33/3244 have the highest protein content in the grain, the most active post-flowering nitrogen accumulation, the largest nitrogen export with the biomass, the highest nitrogen consumption per unit of production and the most high efficiency of nitrogen recycling after flowering. At RU33/3244, the strongest post-flowering accumulation and the strongest reutilization of nitrogen were recorded simultaneously. These three lines have the greatest potential for use in view of the requirements of modern agriculture.

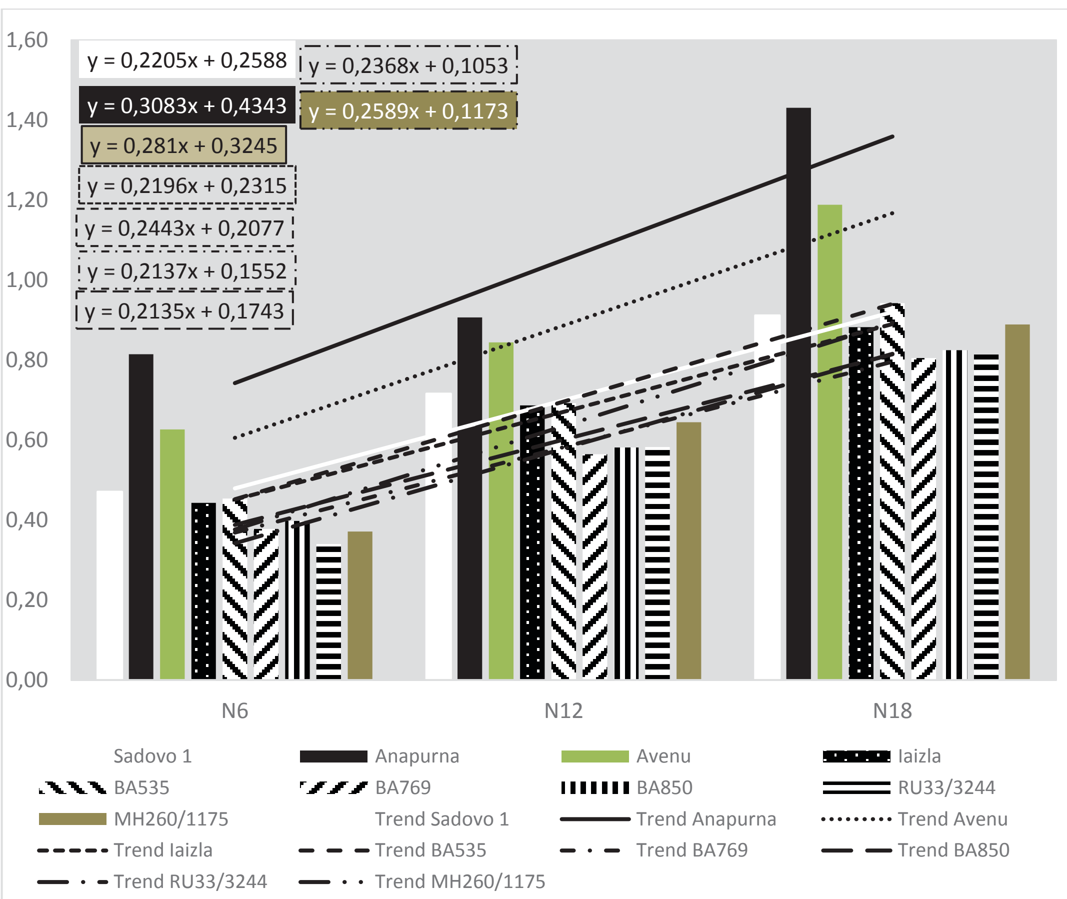

Figure 3. Regression analysis of the effect of nitrogen fertilization level on NUE

ACKNOWLEDGEMENT

The study was presented at the 2022 International Scientific Conference entitled "140 Years of Agricultural Science in Sadovo and 45 Years Institute of Plant Genetic Resources", which was held in Plovdiv from September 28 to September 29, 2022.

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