

## **SELECTION OF FIRST GENERATION LINES WITHIN A TRADITIONAL RYE POPULATION UNDER ULTRA LOW DENSITY**

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### **ABSTRACT**

Over the last 30 years, the total amount of rye (*Secale cereale* L.) produced has been gradually decreasing not only in Greece and Europe but also in a global level. However, climate change, food crisis, as well as the differentiation in the eating habits of consumers force the reconsideration of the cultivation of rye which is considered a source of "environmentally resistant" genes and stands out for its nutritional value. For this reason, during the 2020-2021 growing season, an experimental field was established on the farm of the Department of Agriculture at the University of Western Macedonia, in order to evaluate a traditional population of rye grown in the area of Vevi, Northwest Greece. The experimental design used was the Non-Replicated Honeycomb (NRO) with a number of 1024 plants. During the growing season, at the individual plant level, various agronomic (March height, maturity height, days to ear emergence, number of tillers, length of spikes with and without awns, number of spikelets per spike, seed yield), qualitative (1000-grain weight, seed protein content) and physiological characteristics (leaf chlorophyll concentration, chlorophyll fluorescence and total photosynthetic capacity) were measured. Appreciable variability was found for most traits, while individual plants with high productivity and good values in the remaining agronomic, physiological and quality traits were identified and selected to be evaluated at low and normal seeding density in the 2021-22 cropping season, showing that there is a response to the selection.

**Keywords:** *Rye, traditional population, agronomic evaluation, honeycomb design.*

## INTRODUCTION

Common rye (*Secale cereale* L.) is an important cereal crops and its cultivated area could be important in Europe (Wrigley *et al.*, 2017). The main countries that produce rye are Russia, Poland, Germany, Belarus, Ukraine, China (mainland), Denmark, Canada, Turkiye and Spain (FAO, 2022). Over the past three decades, there has been a consistent decline in the worldwide production of rye, a phenomenon not limited to Greece and Europe but extending globally, as reported by FAO in 2022. Nevertheless, starting from 2011, fluctuations have become evident in global rye production, as documented by Schlegel in 2013. These fluctuations are attributed, on one hand, to the impact of climate change, which necessitates the exploration of resilient rye genotypes, and on the other hand, to a shift in consumer preferences towards healthier dietary choices. Rye unlike other cereals exhibits high winter hardiness, high tolerance to many abiotic and biotic stress factors and is suitable for nutrient-poor, sandy soils with low pH value (Al-Khayriet *et al.*, 2019). The main components of rye grain are starch (57.1%–65.6%), dietary fiber (14.7%–20.9%), protein (9.0%–15.4%), and ash (1.8%–2.2%) (Hansen *et al.*, 2004). Rye is rich in fibres (arabinoxylan, -glucan, cellulose, lignin, and fructan) the consumption of which reduces incidence of diabetes, cardiac problems, and cancer (El-Mahiset *et al.*, 2022).

Rye is a diploid ( $2n = 2 \times = 14$ ) species and the only cross-pollinating small-grain cereal and such outbreeding nature results in a high intraspecific diversity (Schlegel, 2014). As a result in the cultivation of rye, there are available populations and hybrid cultivars. The most commonly used are populations and some of them are cultivated and propagated in a traditional way. These populations are locally adapted, they have undergone little or no mass selection and they present higher levels of diversity than the officially registered modern varieties (Camacho Villa *et al.* 2005). From 1970, especially in Germany, hybrid breeding has gained much attention caused by higher grain yields (15-20% over populations) and a higher gain from selection compared to open-pollinated cultivars (Miedaner *et al.*, 2019). Furthermore hybrids have better uniformity as compared to population cultivars. However, hybrids do not always answer expectations under severe climatic conditions and are inferior to local varieties, especially in unfavorable years (Serenius *et al.*, 2005; Hakala *et al.*, 2003). Therefore, it is of major importance for each rural area involved in rye production to have local varieties that are adapted to the specific conditions. Breeding of modern varieties of rye is aiming to genotypes that combine a set of economically valuable characteristics (increased winter hardiness, reduced lodging through sortstemming, high and stable yield of the grain of good quality and resistance to adverse winter environmental factors) (Utkina *et al.*, 2018; Goncharenko *et al.*, 2019; Ponomarew *et al.*, 2017).

A different breeding approach is selection within landraces under ultra-low plant density, which seems to be a short-time effective procedure in cereals (Tokatlidis *et al.*, 2004; Koutsika *et al.*, 2010; Vlachostergios *et al.*, 2018; Ninou *et al.* 2021; 2022), since maximizes the phenotypic expression and differentiation of the

genotype. Also, this procedure applied in rye by Kyriakou and Fasoulas (1985) resulting in identifying high-yielding genotypes.

Considering the above, a new project started, aiming to the study of genetic variation of local rye population, with the ultimate goal of selecting superior genotypes for the improving of the initial population, also testing the effectiveness of breeding procedure of single plant selection under ultra-low plant density.

### MATERIALS AND METHODS

An experimental field was established on the farm of the Department of Agriculture at the University of Western Macedonia at the beginning of November of 2020. The plant material was a local unimproved rye population, called “Vevi”, adapted to the climate of the Florina area and specifically in low temperatures, poor soils and short biological cycle. The experimental design used was the Non-Replicated honeycomb design (NR0) with 32 rows of 32 plants (1024 plants in total), with interplant distance of 100 cm (Ultra-Low Density of 1.15 plants m<sup>-2</sup>). During the growing season were measured, at the individual plant level, various agronomic (March height, maturity height, days to ear emergence, number of tillers, length of spikes with and without awns, number of spikelets per spike, seed yield), qualitative (1000-grain weight, seed protein content) and physiological characteristics (leaf chlorophyll concentration, chlorophyll fluorescence and total photosynthetic capacity). In terms of yield and maturity height at the level of individual plants selection applied. Applying strict selection were isolated 7 high yielding and 2 low yielding individual plants. The selected genotypes were sown the next cultivated season 21/22, on Randomized Complete Block with three replications per entry. In the RCB design, except the 9 selected genotypes, was also sown the initial population “Vevi”. Because of the small amount of available seeds of selected individual plants, each plot consisted of three rows of 1m long and 25 cm inter row distance. The genotypes were evaluated in terms of seed yield protein content and height at maturity.

The protein content was measured by usage of the Infratec™ 1241 Grain Analyzer FOSS which use the Near-Infrared spectroscopy (NIR), a non-destructive technology (Font *et al.* 2006) which is widely used for routine analysis of nutrients in food products and crops, as it is an alternative to conventional methods of analysis and has the advantages of simplicity, accuracy and rapidity (Magwasa *et al.* 2016). Spad 502 Plus was used for measurement of the leaf chlorophyll concentration, OPTI SCIENCES OS5p was used for the measurement of the chlorophyll fluorescence and total photosynthetic capacity was measured with the LICOR 6400XT gas exchange system. The statistical analyses were conducted using SPSS (version 29.0; SPSS, Chicago) package.

### RESULTS AND DISCUSSION

From the table 1, observing the range of the values we conclude that the variation is quite large for almost all of the measured traits. The highest variability is for weight of spikes and stems and for seed yield also. On the contrary the lowest

variability is noted for days to ear emergence. So it is obvious that the local population of rye “Vevi”, has appreciable variability for most traits. According to Ninou *et al.*, (2014) the utilization of the genetic variability of traditionally cultivated and locally adapted farmer varieties, may lead to successful progress through selection.

Table 1. Maximum (max), minimum (min) and mean for all the measured traits of the first year of experiments

<b>Trait</b>	<b>max</b>	<b>min</b>	<b>mean</b>
Yield (gr)	101,8	0,09	30,86
Spikelets per spike (cm)	50	12	34,27
TKH (gr)	48,1	4,81	26,43
Number of tillers	63	2	24,26
Weight of spikes (gr)	425	7,5	61,34
Weight of stems (gr)	450	11	161,87
Length of spike with awns (cm)	21,5	6	15,48
Length of spike without awns (cm)	18,5	4,55	12,39
Days of ear emergence	200	179	191,46
Height of March (cm)	23	4	10,99
Total height (cm)	158	40	106,93
Protein content (%)	23,7	14,4	18,81
SPAD	64,4	25,1	48,14
Chlorophyll fluorescence	528	41	283,74
Photo out	17,4	0,65	9,59

Applying the honeycomb design were selected seven high yielding (101,8 gr to 53,4 gr) and two low yielding (13,4 gr to 11,1 gr) individual plants, some with high (142 cm) and some with low (80,8 cm) height (table 2). According to Kyriakou and Fasoulas (1985) selection efficiency depends on the magnitude of genetic variation and on the ability to identify phenotypically the few exceptional genotypes. Phenotypic identification of exceptional genotypes is possible by practicing selection in the absence of competition and by applying very high selection pressures. With the honeycomb design Kyriakou and Fasoulas (1985) manage to identify high-yielding genotypes in winter rye.

Table 2. Selected genotypes based on productivity and height at maturity

<b>Genotypes</b>	<b>Yield</b>	<b>Height</b>
1	53,4	118
2	59,3	110
3	66,5	142
4	68,5	130
5	84,0	120
6	77,6	126
7	101,8	112
8	11,1	124,5
9	13,4	80,8

From the ANOVA applied, it is concluded that high yielding genotypes were more productive than the low yielding ones. Also two of them (2 and 3) were significant more productive (with almost double yield) than the low yielding genotypes. Furthermore these two genotypes (2 and 3) outperformed the original population, without being significantly different. It must be noted that 2 and 3 genotypes did not differ significantly either from each other or from the original population in terms of final height.

Table 3. Post hoc tests for seed yield, protein content and plant height for the 10 genotypes of the RCB design of the second year of experimentation.

<b>Genotypes</b>	<b>Yield</b>		<b>Genotypes</b>	<b>Total height</b>	
9	168,62	a	4	151,00	a
8	176,96	ab	2	151,67	a
1	219,77	abc	1	156,33	ab
4	237,67	abcd	8	160,83	abc
6	267,49	abcd	Initial Population	162,50	abc
5	281,73	bcd	3	162,67	abc
7	284,58	bcd	7	164,00	abc
Initial Population	298,42	cd	6	164,17	abc
3	316,69	cd	9	168,17	bc
2	349,27	d	5	170,67	c

Regarding the results of the final height, although there were significant differences in height between genotypes, none were significantly different from the original population.

## CONCLUSIONS

In conclusion appreciable variability was found for most traits of the initial rye local population and especially for traits with high agronomic value like seed yield and plant height. In fact, were identified genotypes with high productivity and low total height, with the case of genotype 2 be one of the more characteristic. Furthermore response to selection was confirmed, as both low-yielding and high-yielding genotypes maintained their productivity the next generation. Finally, must be noted the adaptability of the initial population which performed very well at the special climatic conditions of the Florina area. Selection will continue at individual plant level for another year and with cross-site experiments progress by selection will be assessed.

## REFERENCES

- Al-Khayri M., Jain S. and Johnson D. (2019). Advances in plant breeding strategies: Cereals. Springer Nature Switzerland
- Camacho Villa ., Maxted ., Scholten . and Ford-Lloyd B. (2005). Defining and identifying crop landraces Plant Genetic Resources 3(3); 373–384.
- El-Mahis A., Baky M, and Farag M. (2022). How Does Rye Compare to other Cereals? A Comprehensive Review of its Potential Nutritional Value and Better Opportunities for its Processing as a Food-Based Cereal. Food Reviews International <https://doi.org/10.1080/87559129.2021.2023817>
- FAO. (2022) [org/faostat/en/#data/QCL/visualize](https://www.fao.org/faostat/en/#data/QCL/visualize). Accessed 20 July 2023
- Font R., Celestino M. and Bailon A. (2006). The use of near-infrared spectroscopy (NIRS) in the study of seed quality components in plant breeding programs. Industrial Crops and Products 24. 307–313. doi:10.1016/j.indcrop.2006.06.012
- Goncharenko A., Makarova A., Ermakova S., Semenovaa T., Tochilina V., Tsygankova N., Skatovaa S., Krakhmalevaa O. (2019). Ecological Stability of Short Stemmed Winter Rye Varieties. Russ. Agric. Sci., 45, 315–322.
- Hakala K., Pahkala K. (2003). Comparison of central and northern European winter rye cultivars grown at high latitudes. J. Agric. Sci., 141, 169–178.
- Hansen H., Møller B., Andersen S., Jørgensen J., Hansen Å. (2004) Grain characteristics, chemical composition, and functional properties, of rye (*Secale cereale* L.) as influenced by genotype and harvest Year. J. Agric. Food Chem. 52, 2282–2291. <https://doi.org/10.1021/jf0307191>.
- Koutsika-Sotiriou M., Mylonas I., Ninou E., Traka-Mavrona E., (2010). The cultivation revival of a landrace: pedigree and analytical breeding. Euphytica 176, 15–24. <https://doi.org/10.1007/s10681-010-0206-z>
- Kyriakou D. and Fasoulas A. (1985). “Effects of competition and selection pressure on yield response in winter rye (*Secale cereale* L.)” *Euphytica*, vol. 34, no. 3, pp. 883–895.
- Magwaza L., Messo Naidoo S., Laurie S., Laing M., Shimelis H. (2016). Development of NIRS models for rapid quantification of protein content in sweetpotato [*Ipomoea batatas* (L.) LAM.]. LWT - Food Sci. Technol. (Lebensmittel- Wissenschaft -Technol.) 72, 63–70

- Miedaner T. and Laidig F. (2019). Hybrid Breeding in Rye (*Secale cereale* L.). In Advances in Plant Breeding Strategies: Cereals; Al-Khayri, J.M., Jain, S.M., Johnson, D.V., Eds.; Springer: Cham, Switzerland,; Volume 5, pp. 343–372.
- Ninou E., Cook C., Papathanasiou F., Aschonitis V., Avdikos I., Tsivelikas A., Stefanou S., Ralli P., Mylonas I. (2021). Nitrogen Effects on the Essential Oil and Biomass Production of Field Grown Greek Oregano (*Origanum vulgare* subsp. *hirtum*) Populations. *Agronomy* 11, 1722. <https://doi.org/10.3390/agronomy11091722>
- Ninou E., Mylonas I., Karagianni I., Michailidou S., Tsivelikas A., Sistanis I., Avdikos I., Korpetis E., Papathanasiou F. (2022). Utilization of Intra-Cultivar Variation for Grain Yield and Protein Content within Durum Wheat Cultivars. *Agriculture* 12, 661. <https://doi.org/10.3390/agriculture12050661>
- Ninou E., Mylonas I., Tsivelikas A., Ralli P., Dordas C., Tokatlidis I. (2014). Wheat landraces are better qualified as potential gene pools at ultraspaced rather than densely grown conditions. *Scientific World Journal* 2014. <https://doi.org/10.1155/2014/957472>
- Ponomarew S., Ponomarewa M. (2017). Photosynthetic Peculiarities of Winter Rye Cultivars with Different Control of Dwarfness. *Zemledelie*. 7, 36–40
- Schlegel R. (2014). “Physiology” in Rye: Genetics, Breeding and Cultivation (Boca Raton, FL: CRC Press; Taylor & Francis Group) 51
- Schlegel, R. (2013) Rye: Genetics, Breeding and Cultivation; United States: Crc Press.
- Serenius M., Huusela-Veistola E., Avikainen H., Pahkala K. (2005). Effects of sowing time on pink snow mould, leaf rust and winter damage in winter rye varieties in Finland. *Agric. Food Sci.*, 14, 362–376.
- Tokatlidis I., Tsiatas J., Xynias I., Tamoutsidis E., Irakli M. (2004). Variation within a bread wheat cultivar for grain yield, protein content, carbon isotope discrimination and ash content. *Field Crops Research* 86, 33–42. [https://doi.org/10.1016/S0378-4290\(03\)00169-2](https://doi.org/10.1016/S0378-4290(03)00169-2)
- Utkina E., Kedrova L. (2018). Winter hardiness in winter rye: Problems and solutions. *Agric. Sci. Euro-North-East*, 62, 11–18.
- Vlachostergios D., Tzantarmas C., Kargiotidou A., Ninou E., Pankou C., Gaintatzi C., Mylonas I., Papadopoulos I., Foti C., Chatzivassiliou E., Sinapidou E., Lithourgidis A., Tokatlidis, I. (2018). Single-plant selection within lentil landraces at ultra-low density: a short-time tool to breed high yielding and stable varieties across divergent environments. *Euphytica* 214. <https://doi.org/10.1007/s10681-018-2139-x>
- Wrigley C., Bushuk W. (2017). Rye: Grain-Quality Characteristics and Management of Quality Requirements. In *Cereal Grains: Assessing and Managing Quality*, 2nd ed.; Wrigley, C., Batey, I., Miskelly, D., Eds.; Woodhead Publishing Ltd.: Cambridge, UK,; pp. 153–178.