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Analysis of some morphological, productive and qualitative characteristics variability in DH wheat

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Abstract This experiment was conducted to evaluate some morphological, productive and qualitative characteristics variability of 18 mutant/recombinant lines and their parental forms in order to identify new and valuable material for grains yield and its components or of quality.

Field experience was conducted during two vegetation period in South part of Romania, a region where climatic conditions are often very restrictive.

Analysis of variance showed highly significant differences among the parental wheat genotypes and doubled haploid lines for all the agronomic traits. The results indicated that the line Ai-II 152 (6757.25 Kg/ha) outrun both parental forms for grain yield (5955 Kg/ha Izvor variety and 6687.5 Kg/ha F00628-34 line). The Ai-II 55 and Ai-I 77 lines were also significantly different from the two parents as concern TKW, while Ai-I 75 and Ai-Ii 126 lines for protein content and Ai-I 69 and Ai-I 27 lines for starch content.

These results show that doubled haploid wheat production via mutagenesis can generate lines that have the potential for commercial production as well as lines that can be incorporated into a breeding program.

Key words

mutant/recombinant lines, characteristics variability, wheat

Wheat (*Triticum aestivum L.*) is one of the most important cereal and the plant which occupy the largest surfaces on the world. Romania occupies place five in EU at surface cultivate with wheat while the area cultivated with cereals decreased with 1.5% and yield increase with 8.3% [18].

Continuously wheat breeding programs have shown an excellent number of releasing improved varieties that have not only helped stabilize production, but also increase it to a moderate extent. The advances in the development and use of newer techniques to assist wheat breeding programs have been rapidly developed. While most the wheat breeders are aware of these developments and agree that their programs need to be adapted to incorporate these technologies, their priorities regarding importance of different techniques vary significantly [10]. Several programs use the development of doubled haploids as complementary to their conventional breeding efforts. The use of anther culture, wheat x maize hybridization, induced mutagenesis and DH technology by NARDI Fundulea conducted to the generation of a large spectrum of variability and a genetic stock of mutated/recombinant lines [5]. In Romania, out of 8 cultivars released of wheat breeding program of NARDI Fundulea in the last years, four were of doubled haploids, obtained using *Zea* method [14].

The experiences realized until now showed that through biotechnological and classical methods of breeding it can be created varieties of different species with superior issues.

Doubled haploid plants (DH) production is valuable in plant biotechnology because it enables breeders to obtain homozygous lines or hybrid individuals [1]. Also, the potential of induced mutagenesis to create genetic variability which can be used in crop improvement is now better exploited. Lately these methodologies have become very successful in numerous developing countries because it simplify and shorts breeding programs, its only limitation being the selection of the desired phenotypes from segregating generations.

Mutation breeding techniques for crop improvement are mainly used by advanced laboratories to obtain new genetic sources for biotic and abiotic stress or herbicide resistance and for improvement of specific quality. In the recent years, mutation induction became also a powerful tool for the investigation of gene function and expression.

Mutagenesis is described as the exposure or treatment of biological material to a mutagen physical or chemical agent that rains the frequency of mutations above the spontaneous rate. In a research paper who aims the investigation of the mutagenic efficiency of X-rays upon groundnut, [8] had shown that mutagenic efficiency is inversely proportional to the size of seeds of the tested varieties.

This paper reports some results obtained by few lines realized through a specific mutagenic protocol which included two modern wheat genotypes, two irradiation cycles application, hybridization and DH technology using the *Zea* system.

Material and Method

18 mutant/recombinant lines and their parental forms were tested in field conditions from ARDS Caracal which is placed in South Romanian Plain and has as geographical coordinate - 44°06' north latitude, 24°21' east longitude and 98 m altitude. Here, as concern the quantity of precipitation, it can be noted uneven distribution in the vegetation period and a reduced number of days with precipitation, particularly in summer season, which means unsatisfactory values to ensure requirements of plants, especially during critical periods. The amount of average annual rainfall means a moderate to drought, annual fluctuations of precipitation causing significant variations in yield of agricultural crops. The quantity of precipitation in the cold season, respectively November-March, corresponds to the period of accumulation of water in the soil, which is favorable to the starting in the vegetation active in the spring of the winter cereal crops. For the warm season of the year (April-October) which corresponds to the active period of vegetation, it is specific to moderate character (353.6 l/m^2) with the lowest values in July and August (106.9 l/m^2) and September-October (64.5 l/m^2) with negative effects on growth and development of the plants, especially in the extreme years.

The experiment was set up in a complete randomized block design with three replications during 2015-2017. The seeds were sown in plots of 9 m long and 2 m wide. Morphological characteristics variability was evaluated at harvest, for each character analyzing 10 plants; Stem length of the main tiller of each plant was measured in centimeters from ground level to the tip of the spike. Number of fertile tillers/plant was counted from each of the tagged plants. Spike length of main tiller of selected plants was measured in centimeters from base of the awn to its tip. From the main spike of guarded plants the numbers of spikelets per spike of were counted excluding the basal sterile spikelets. Three samples of 500 grains were taken from randomly selected plants and weight was determined in grams by weighing with the help of electric balance. Also, yield was established by weighing the grains resulted from 7 m and quality was determined using a Perten Quality Analyzer. Determinations values were calculated using analysis of variance method and least significant difference (LSD) test was used for the mean comparisons. Correlation coefficient between the experimented characteristics was also calculated.

Results and Discussions

Analysis of variance reveled highly significant differences between the 18 wheat doubled haploid lines and their parental forms for the studied characteristics (plant height, spike length, no. of spikelets, no. of grains/spike, grains weight/spike, TKW, grain yield, HW, protein and starch).

Stem lenght ranged from 74.70 to 108.00 cm with an average of 86.35 cm. The tallest line was Ai-I 77 (100.40 cm), while the shortest line was Ai-I 27 (81.70 cm) Stem lenght for parents Izvor and F00628-34 were 87.90 and 88.50 cm, respectively (table 1).

Number of fertile tillers varied from 8.00 to 13.80 with an average of 11.00. Biggest value of fertile tillers presented Ai-II 55 line (13.00) and the smallest Ai-I 18 line (8.50). Parental forms registered values of 10.70 (Izvor) and 9.90 (F00628-34). [7] reported similar values both for a series of mutagenic lines of wheat and for the Izvor variety and F00628-34 improved line as concern number of fertile tillers.

Spike length was significantly different among the wheat lines. Line Ai-I 131registered the shortest spike length (8.25 cm), while Ai-I 77 the longest (11.40 cm). Parental forms presented values of 10.10 cm (Izvor) and 9.75 cm (F00628-34). Variation amplitude presented a value of 6.40. Similar values of spike length reported [4] in an experiment of two years with 23 double haploid lines of winter barley.

Number of spikelets ranged from 17.60 to 22.60 with an average of 19.96. The highest value was registered in Ai- II 55 and Ai- I 77 lines (21.95 and 21.60, respectively), while the smallest was presented by Ai- I 69 (17.90). Parental forms presented values of 20.00 (Izvor) and 17.85 (F00628-34). Number of grains/spike ranged from 44.60 to 69.60 with an average of 58.02. The parent Izvor had a value of 53.15, while parent F00628-34 had 55.90.

Grains weight/spike varied from 2.21 g to 3.52 g with an average of 2.83 g. Parental forms presented 2.42 g (Izvor) and 3.015 g (F00628-34). There were few lines which presented values under those registered by parental forms (Ai- II 152 - 2.30 g, Ai- II 152 - 2.37 g) and there were also lines with higher values such as: Ai- II 123 (3.465 g), Ai- II 27 A (3.315 g), Ai- II 47 (3.065 g).

Some authors sustains that TKW is considered to be less influenced by environment than the other yield components, such as number of spikes per unit area or grain number per spike [13] while others says that very much dependent on temperatures during grain filling [17]. TKW results indicate that mutant/recombinant lines do not differ much from parental forms also there were few lines which registered higher values. Among there are Ai- II 55 - 45.38 g, Ai- II 223 - 44.925 g, Ai- I 77 – 44.475 g and Ai- I 75 – 44.13 g. Variation amplitude registered a value of 18.89 g. The results are in accordance with [3] who reported similar results for a number of 85 mutant/recombinant DH lines of winter wheat. Variability found by the mentioned researcher was of 21.46g – 41.80g in 2014 and 42.29g – 58.94g in 2015 as concern TKW. In the context future climate changes, the reduction of grain dimensions compensated by the improvement of grain density and/or form suggest possibilities of breeding to reduce the effect of higher temperatures during grain filling and increase the stability of grain weight [11].

Table 1

Variation of the main analyzed indexes of the experimented lines (average 2015-2017)

Character / Genotype	**Stem length (cm)**	**No. of fertile tillers**	**Spike length (cm)**	**No. of spikelets**	**No. of grains/spike**	**Grains weight/spike (g)**	**TKW (g)**	**Yield (Kg/ha)**	**HW (Kg)**	**U (%)**	**Protein (%)**	**Starch (%)**
Ai-I 18	85.40	8.50	9.70	19.40	60.10	2.70	40.41	6410	77.50	11.65	12.90	72.90
Ai-I 27	81.70	10.10	11.35	21.50	60.45	2.98	40.335	6335	76.05	12.15	11.75	74.35
Ai- I 75	83.10	11.30	10.45	20.00	61.90	3.31	44.13	5822.5	79.80	12.40	13.55	72.10
Ai- I 69	87.00	10.00	10.83	17.90	44.85	2.34	39.415	6410	76.35	13.10	11.25	74.50
Ai- I 77	100.40	11.80	11.40	21.60	63.50	2.77	44.475	6452.5	77.85	13.40	12.30	73.00
Ai- II 27 A	97.40	11.00	11.20	20.65	62.20	3.315	38.84	5970.5	78.60	12.95	12.55	73.25
Ai- II 47	87.20	11.90	8.40	21.40	66.60	3.065	40.265	5566.25	73.05	12.60	13.05	71.65
Ai- II 55	85.40	13.00	9.55	21.95	51.00	2.305	45.38	6343.75	76.80	12.95	12.70	72.15
Ai- II 107	84.90	12.00	9.45	19.00	53.60	2.685	43.99	6330	77.40	13.45	12.70	73.05
Ai- II 123	89.70	12.70	10.80	20.05	67.85	3.465	43.195	6500.5	78.10	13.05	12.35	73.15
Ai- II 126	87.60	11.35	9.55	19.95	56.65	2.60	42.905	5932.25	79.20	13.15	13.35	72.70
Ai- II 131	78.45	9.90	8.25	18.60	48.35	2.50	42.99	5847.25	79.10	12.60	12.95	73.45
Ai- II 152	91.70	11.80	9.55	18.50	48.95	2.37	41.53	6757.25	79.20	13.15	12.40	73.40
Ai- II 172	83.20	9.80	8.80	20.15	62.85	2.865	38.625	5714.5	78.25	12.85	13.10	72.40
Ai- II 183	82.60	10.75	10.50	21.10	69.10	3.30	41.98	5633.75	77.65	12.70	12.20	72.95
Ai- II 193	83.70	10.85	9.95	20.05	65.05	3.10	42.69	6724	79.30	13.25	12.60	73.10
Ai- II 201	78.35	12.10	10.20	19.90	62.75	3.005	40.755	5770.5	77.10	12.60	11.90	72.85
Ai- II 223	82.70	10.55	10.15	19.70	45.60	2.465	44.925	6392.5	78.00	12.50	11.80	73.45
Izvor	*87.90*	*10.70*	*10.10*	*20.00*	*53.15*	*2.42*	*42.755*	*5955*	*79.75*	*12.40*	*12.80*	*71.95*
F00628-34	*88.50*	*9.90*	*9.75*	*17.85*	*55.90*	*3.015*	*43.625*	*6687.5*	*77.00*	*12.95*	*12.75*	*73.90*
Average of experience	**86.35**	**11.00**	**10.00**	**19.96**	**58.02**	**2.83**	**42.16**	**6177.78**	**77.80**	**12.79**	**12.55**	**73.01**
Std. dev.	**9.09**	**1.21**	**1.32**	**1.26**	**7.40**	**0.37**	**5.61**	**1776.08**	**2.22**	**0.60**	**0.70**	**1.24**
Min.	**74.70**	**8.00**	**6.00**	**17.60**	**44.60**	**2.21**	**33.29**	**3150.00**	**70.80**	**10.50**	**10.50**	**70.40**
Max.	**108.00**	**13.80**	**12.40**	**22.60**	**69.60**	**3.52**	**52.18**	**9520.00**	**81.40**	**14.00**	**13.70**	**75.30**
Amp. var.	**33.30**	**5.80**	**6.40**	**5.00**	**25.00**	**1.31**	**18.89**	**6370.00**	**10.60**	**3.50**	**3.20**	**4.90**

Ai- II 152 was the most productive line (6757.25 Kg/ha), outrun both parental forms as concern grain yield (5955 Kg/ha - Izvor, respectively 6687.5 Kg/ha - F00628-34). Grain yield varied from 3150.00 to 9520.00 Kg/ha with an average of 6177.78 Kg/ha. The least productive line was Ai- II 47 (5566.25 Kg/ha). This character varied much so that amplitude variation was higher (6370 Kg/ha). This high variation is due to environmental conditions from the experimentation area. Same high values for the genotype-environment interaction identified [2] in an experience with a material represented by 25 winter wheat cultivars in three locations from west part Romania.

[6] sustain that better behavior of some mutant/recombinant lines such as A i- II 107, A i-I 77, A i- II 123 and A i- I 75 lines as concern yield, demonstrate a good adaptation in the experimentation area which means these are best-suited for cultivation in different locations from south part of Romania.

Registered values for HW in the mutant/recombinant lines are close to those presented by parental forms, variation amplitude being of 10.60 Kg. Humidity at harvest also presented close values.

Wheat occupy an important role in human feeding, the improvement of his nutritive value must constitute a constant concern of breeding. The most important researches of breeding were conducted to the increase of grains protein content. The results obtained for this character indicate few lines with values over 13% (Ai- I 75 - 13.55%, Ai- II 126 - 13.35%, Ai- II 172 - 13.10%, Ai- II 47 - 13.05%) which outrun parental forms (12.80% - Izvor, respectively 12.75% - F00628-34) and experience average (12.55%).

Similar results as concern protein content found [3]. The variation of the mutant/recombinant lines experimented by this researcher was of 12-14%, while for Izvor and F00628-34 line, the registered values were higher.

Starch is another important component in wheat crop. It is the largest component of the mature cereal grain. Values from this characteristic ranged from 70.40% to 75.30% with an average of 73.01% and a variance amplitude of 4.90%. There were few lines which differ significantly comparative with parental forms such as: Ai- I 69 (74.50%), Ai- II 131 and Ai- II 223 (73.45%), Ai- II 152 (75.40%), Ai- I 27 (74.35%), respectively Izvor (71.95%) and F00628-34 (73.90%).

[9] reported a protein content of the grains on dry weight basis between 13.12- 19.41% and for starch content from grain samples determined by the polarimetric method values between 60.14 and 66.93% in 20 wheat genotypes which are widely used in the agriculture and breeding in Romania and Hungary.

From this experiment it can emphasize line Ai- II 55 which presented average height, the biggest number of fertile tillers and of spikelets/spike and TKW; it also presented values under average length of spike, number of grains/spike and weight/spike, but with higher values of yield than average.

There were established the correlation coefficient between the analyzed characteristics which indicate some positive relations between grains weight/spike and number of grains/spike (0.853**), yield and stem length (0.704*), starch content and yield (0.700*, $P \leq 0.05\%=0,630$). Negative correlation was also identified between TKW and stem length (-0.683), yield and TKW (-0.835), starch and TKW (-0.680).

[15] estimated positive association between grain yield and number of tillers per plant. In this case it was registered a value of 0.335 of the correlation coefficient. Also, [12] found strong links between the no. of seeds/spike and the seeds weight/spike, between the no. of seeds/spike and the high of the strain, between seeds weight/spike and the high of the strain and between the no. of emerged plants/m^2 and the no. of spikes/m^2 in a study made on isogenic lines of wheat.

Number of grains/spike had positive direct effect upon stem length (0.590) and for number of spikelets (0.533). The correlation coefficient between yield and yield components, generally demonstrate a compound sequence of interacting association [16].

The variability of the morphological, productive and qualitative characteristics of the experimented mutant/recombinant lines is given by the strong dependence with the meteorological conditions. In the south part of Romania, drought is more and more present, especially in critical phases of plants development. Also, this variability could be related to the origin of *Zea* method or parental forms used to hybridization.

Conclusion

As a result of this experiment a number of lines can be selected for diverse characteristics which were superior comparative with parental forms.

Data indicate that few DH mutant/recombinant lines can be used in wheat crop improvement programs and it can develop DH lines that could be used directly as cultivars as these lines performed better than the parental forms.

Further efforts to create and characterize DH lines of wheat with superior agronomic or quality characteristics will be made.

Table 2

Correlation between the analyzed characters

	Stem lenght	No. of fertile tillers	Spike length	No. of spikelets	No. of grains/spike	Grains weight/spike	TKW	Yield	HM	U%	P%
No. of fertile tillers	0.005										
Spike lenght	-0.098	0.226									
No. of spikelets	0.135	0.292	0.133								
No. of grains/spike	0.590	0.136	0.141	0.533							
Grains weight/spike	0.029	0.071	0.207	0.329	**0.853****						
TKW	**-0.683**	0.071	0.419	-0.055	-0.082	-0.075					
Yield	**0.704***	0.335	-0.362	0.041	-0.066	-0.031	**-0.835**				
HW	-0.406	-0.175	0.188	-0.197	-0.097	-0.037	**0.629**	-0.477			
U%	0.368	0.437	0.053	-0.040	-0.046	-0.036	-0.050	0.227	-0.004		
Protein	0.167	-0.044	-0.402	0.125	0.199	0.142	-0.172	0.232	0.088	0.025	
Starch	0.491	-0.374	-0.198	-0.206	-0.212	-0.044	**-0.680**	**0.700***	-0.406	0.185	-0.252

P≤0.05%=0,630

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