

## POTENTIAL SOURCES OF NEW GENETIC VARIABILITY IN MUTANT AND MUTANT/ RECOMBINANT WHEAT DH-LINES

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### ABSTRACT

Mutation breeding is today considered a powerful tool for broadening the genetic variability and to improve important agronomic characteristics. Then, evaluation of mutant forms becomes necessary to speed up the advancement of mutant lines carrying superior traits. This paper reports the results of evaluation over three season environments for plant height, thousand kernel weight (TKW), test weight (TW), protein concentration and other quality indices in a set of 307 mutant and mutant/recombinant wheat DH lines.

**Key words:** mutant/ recombinant DH-lines, stability, quality.

### INTRODUCTION

Wheat is one of the most important cereal crops in the world, providing 30-40% of the food for human population. Population growth and increasing living standards impose the creation of varieties that provide high productivity with high quality indices in order to sustain the nutrients and necessary calories. The present genotypes must be improved for features needed to counteract the effect of biotic and abiotic stress factors.

Classical breeding techniques, like cross or recombinant breeding followed by selection cycles have led to declining variability and agronomic potential in the context of climate change pressure. Besides spontaneous mutations that sustained the plants evolution, the use of induced mutagenesis became an effective and rapid way to induce new variability.

Mutagenesis is the process in which heritable changes may occur in the genetic information of the organism that are not caused by genetic segregation or recombination, being induced by chemical, physical or biological agents (Kharkwal et al., 2012; Roychowdhury et al., 2013).

The use of mutagenesis in breeding programs is based on selecting biological

material that carries mutant genes of interest, and also the mutagenic agent and the dose of the mutagen that achieves the optimum mutation frequency with the least possible unintended damage (Mba et al., 2010).

After applying the mutagenesis, very important in the identifying of the new variability sources is the selection of individual forms from a large mutated population that carry the needed traits, followed by the reevaluation of the selected forms under a controlled environment, in large samples (Forster et al., 2012).

Globally, as results of many breeding programs that include mutagenesis, more than 3200 mutants were released as new cultivars, in around 200 cultivated species (<http://mvgs.iaea.org>)

After applying the mutagenesis the M1 mutant plants are heterozygous, because only one allele per plant is affected by one mutation during event.

By using DH-technology it became possible to attain a complete homozygosity and to make a rapid and easier selection for specific traits and particularly for those controlled through recessive alleles.

The objective of this study was to evaluate the variability for several traits in a set of 307 mutant and mutant/recombinant DH-lines and to identify the genotypes with genetic stability

and superior agronomic traits that may represent an interesting breeding material.

## MATERIAL AND METHODS

The mutant and mutant/recombinant wheat DH lines were obtained at NARDI Fundulea by using a specific mutagenic protocol including two modern wheat genotypes, applications of two irradiation cycles, hybridization and DH technology (Giura, 2013).

The lines were sown in the field in October, along with parental genotypes, in pairs of rows of 1 m in length with a distance of 25 cm between the rows and 50 cm between the pairs of rows. The different climatic conditions of the three experimental seasons, with variations for temperature and recorded precipitations, might have influenced the genotype response and plant development.

We measured the plant height at maturity stage with a linear graduated meter, to identify the semi-dwarf lines that can be considered an important breeding material for preventing lodging.

The protein concentration, together with other quality parameters like Zeleny

sedimentation values and wet gluten content are very important in bakery. Therefore, the lines with a higher quality, not associated with low productivity are an ideal material for sustaining food needs.

We determined protein content and also Zeleny sedimentation values and wet gluten content, with FOSS INFRATEC 1241, analyzer using near-infrared transmittance technology.

The thousand kernel weight (TKW) was determined by counting grains with Contador Seed Counter and weighing the mass with electronic balance. For test weight (TW) we used a graduated cylinder of 100 ml volume and the volume weighing was done with electronic balance.

Statistical analysis was performed using the RStudio programmer available at: <https://www.rstudio.com>.

For a better visualization and delimitation of the observation data were divided by quartiles (Table 1), in order to make the selection of the interesting material easier. If for the TKW, TW, protein concentration, Zeleny sedimentation values and wet gluten content the interest quartile was the highest ( $\geq Q3$ ), for the plant height we selected the inferior quartile ( $\leq Q1$ ).

Table 1. The quartiles for the studied parameters in each year

Parameter	Year	Minimum	Q1	Q2	Q3	Maximum
Plant height (cm)	2015	55	80	84	*/**87	100
	2016	63	94	*100	**103	116
	2017	61	82	85	**90	*109
TKW (g)	2015	28.9	46.9	**49.6	*51.9	60.5
	2016	25.6	36.2	*/**39.9	44.4	54.4
	2017	28.3	42.8	**46.7	*49.7	58.8
TW (g/0.1dm <sup>3</sup> )	2015	759.2	835.0	*/**846.3	857.5	891.6
	2016	703.7	**785.0	*810.2	830.4	872.8
	2017	668.7	787.0	803.7	*/**817.5	862.5
Protein (%)	2015	11.3	*/**13.9	14.4	15.3	19.4
	2016	9.6	11.8	12.4	*/**13.3	17
	2017	11.1	13.07	**13.7	*14.4	18
Zeleny sedimentation values (ml)	2015	33.2	*/**48.7	53.7	58.9	79.2
	2016	9.1	*29.2	**34.6	40.8	67.1
	2017	25.8	**36.9	40.9	47.5	*66.9
Wet gluten content (%)	2015	25.4	**33.7	*35.7	38.2	50.6
	2016	17.5	26.3	28.6	*/**31.4	42.7
	2017	24.2	30.6	32.8	35.3	*/**46.22

The marks for the location of the parental genotypes (\* - IZVOR, \*\* - F00628G-34) were placed in the cells with the smallest value higher than parent value.

The lines that registered lower values ( $\leq Q1$ ) for plant height and higher values ( $\geq Q3$ ) for the other analyzed parameters, were reported to the lower, respectively higher values for calculate how they are proportionally located in the sorted lists compared to minimum (MinInd) or maximum (MaxInd).

$$\text{MinInd} = 100 - (\text{Height}_{\text{Current genotype}} - \text{Height}_{\text{Shortest genotype}}) * 100 / \text{Height}_{\text{Shortest genotype}}$$

$$\text{MaxInd} = \text{Value}_{\text{Current genotype}} * 100 / \text{Value}_{\text{Genotype with MAXIMUM value}}$$

In this way, by reporting the MinInd and MaxInd averages to the standard deviation, we identified the lines that in all three experimental years ranked in zone with values  $\leq Q1$  for plant height, respectively zone with values  $\geq Q3$  for TKW, TW, protein concentration, Zeleny sedimentation values and wet gluten content.

From the Figure 1 one can see that after ranking the lines according to the number of years in which they recorded  $h \leq Q1$ , a significant number of semi-dwarf lines were identified, remaining relatively stable in all three experimental seasons.

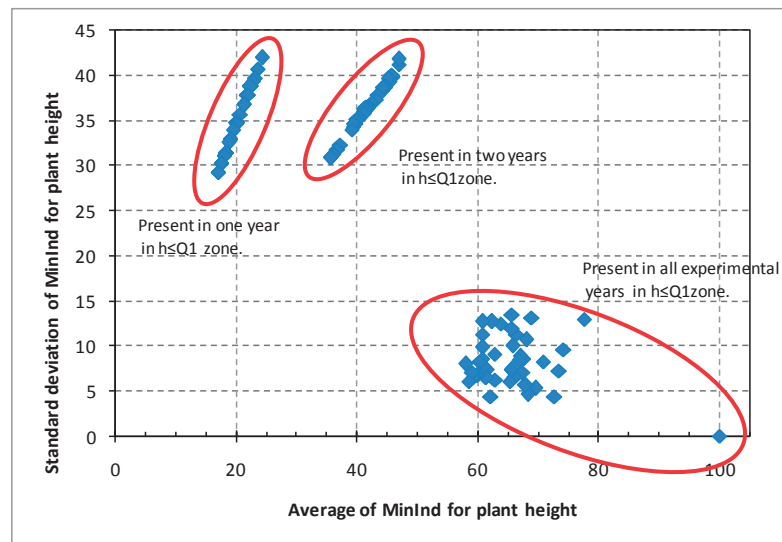


Figure 1. Average of minimization index for plant height (genotypes with values  $\leq Q3$ )

For TKW, TW, protein concentration, Zeleny sedimentation values and wet gluten content, the lines that recorded higher values,

( $\geq Q3$ ) in all three years were identified (Figures 2-6).

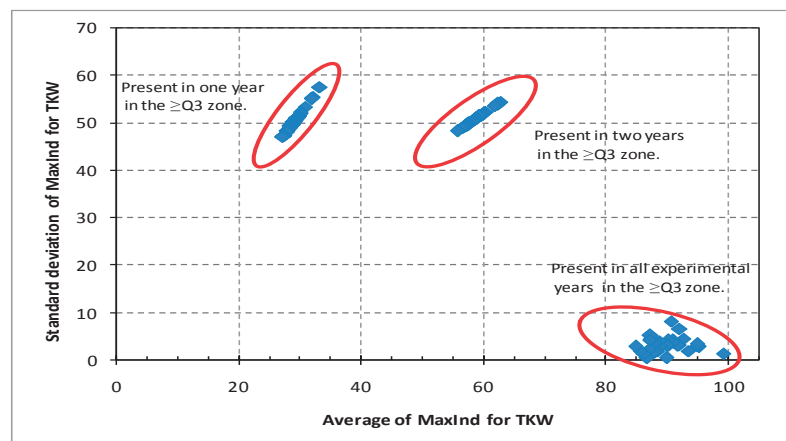


Figure 2. Average of maximization index for TKW (genotypes with values  $\geq Q3$ )

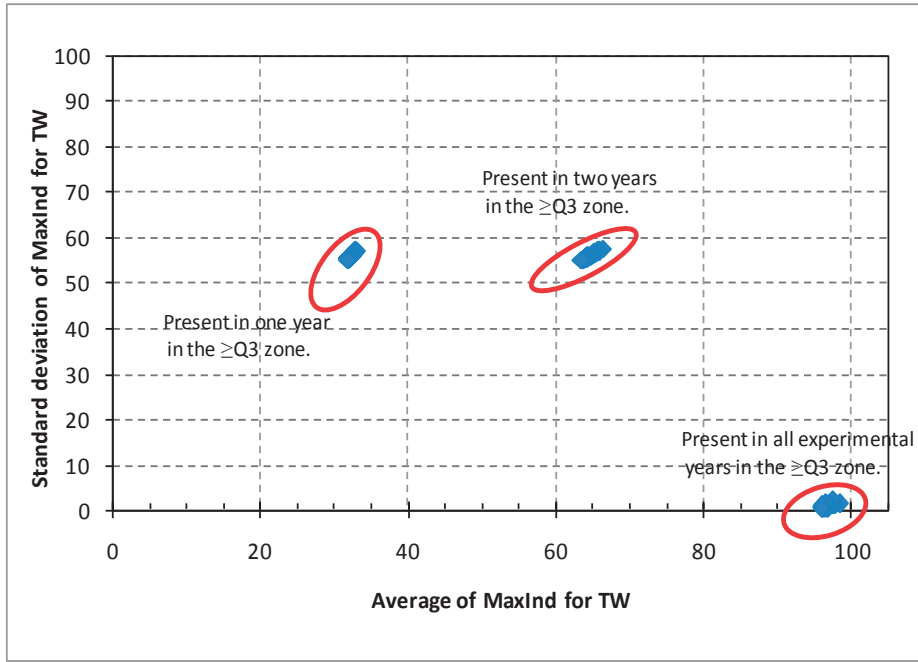


Figure 3. Average of maximization index for TW (genotypes with values  $\geq Q3$ )

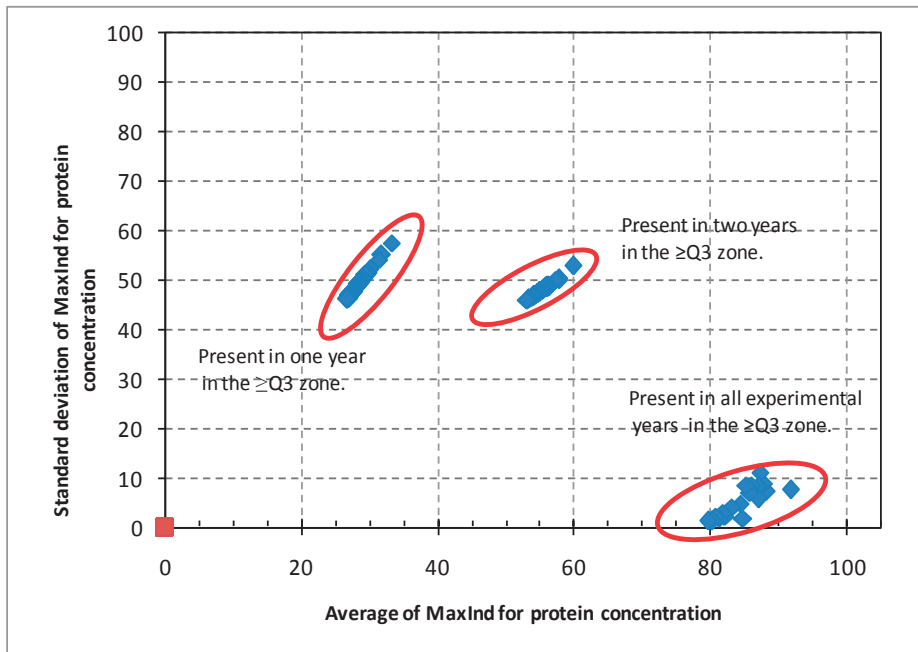


Figure 4. Average of maximization index for protein concentration (genotypes with values  $\geq Q3$ )

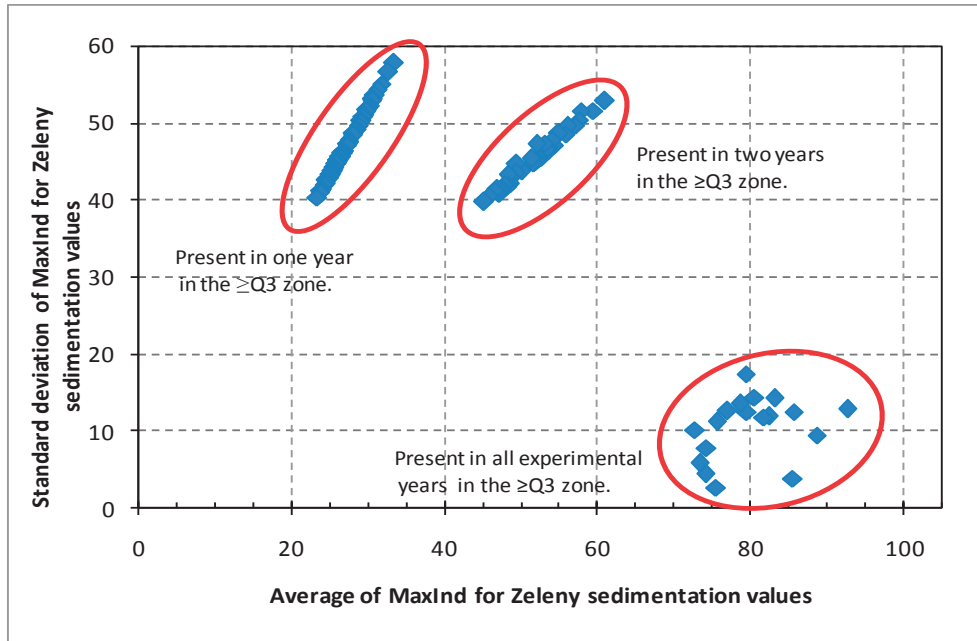


Figure 5. Average of maximization index for Zeleny sedimentation values (genotypes with values  $\geq Q3$ )

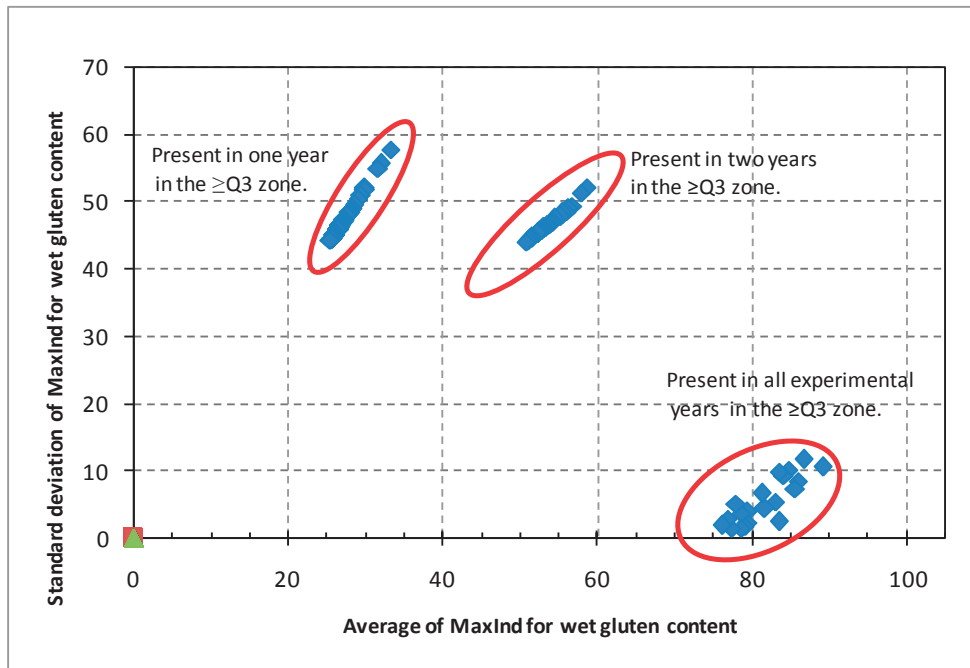


Figure 6. Average of maximization index for wet gluten content (genotypes with values  $\geq Q3$ )

## RESULTS AND DISCUSSION

After identifying the most stable lines that registered lower values for plant height respectively higher for the other analyzed parameters, the genotypes that were highlighted for more than two characters

were selected for further characterization.

According to the data presented in Table 2, there are lines that accumulate two characters, but also lines that accumulate three, four or five characters, all of them being significantly different from the two parental genotypes.

Table 2. Selected lines that were highlighted for more than two analyzed parameters

Genotype	TKW (g)	TW (g/0.1dm <sup>3</sup> )	Plant height (cm)	Protein (%)	Zeleny sedimentation values (ml)	Wet gluten content (%)
Ai I 65	51.4	856.4	83.3			
Ai I 73	57.4	847.1				
Bi I 36				15.7	59.1	
Bi I 44				16.7	66.1	41.7
Ai II 14	50.3	841.2				
Ai II 19		844.7	83.0			
Ai II 20	51.1	842.8		15.5	60.7	39.0
Ai II 34	52.2	853.4				
Ai II 45	50.8		80.3	15.4	60.7	38.7
Ai II 47				15.6	57.9	37.7
Ai II 51				14.7	53.7	36.7
Ai II 175	52.3	849.8				37.0
Ai II 202				14.6	52.9	35.9
Ai II 225				14.4	52.7	
Ai II 232				15.3		38.7
Ai II 238					56.4	38.9
Ai II 248			78.7			36.9
Ai II 259	54.1		80.3			
Bi II 63	50.0			15.8	58.9	40.2
Bi II 71					57.9	39.7
Bi II 79						35.5
Bi II 96						40.0
Bi II 107					52.3	36.5
Bi II 142					52.0	36.6
Bi II 147			73.3		62.6	39.8
<i>Izvor (Control)</i>	45.7	831.1	91.7	13.5	44.3	32.1
<i>F00628G-34 (Control)</i>	43.2	799.7	93.0	12.6	36.2	29.5

Most of the selected lines presented a good quality (Bi I 44, Ai II 20, Ai II 45, Ai II 47, Ai II 51, Ai II 202, Bi II 63), based on protein concentration and the other two quality indicators, Zeleny sedimentation values and wet gluten content.

According to literature (Oury et al., 2003; Taulemesse F. et al., 2016), the quality and productivity are often negatively correlated, but this association is often caused by the influence of environmental conditions.

So, in this terms we identified three mutant/recombinant wheat DH lines (Ai II 20, Ai II 45 and Bi II 63), that registered both quality and elements of productivity, expressed by protein concentration, Zeleny sedimentation values, wet gluten content and TKW, superior to parental genotypes.

The line Ai II 20 recorded stable and high values for five of analyzed parameters, in all three years, and in a previous study it was also mentioned as heaving good results for TKW, TW and protein concentration (Dobre et al., 2016).

The genotypes Ai II 225 and Ai II 232 revealed a high protein concentration in an experiment performed in 2014-2015 (Dobre, 2016).

The present study confirmed the genetic potential for quality of this two wheat mutant/recombinant DH lines.

The selected semi-dwarf lines (Ai I 65, Ai II 19, Ai II 45, Ai II 259, Bi II 147) were also highlighted for quality (protein concentration, Zeleny sedimentation values, wet gluten content) and productivity (TKW and TW) indicators, the Bi II 147 line being remarked in another study as having a long coleoptile, a desired character in the breeding programs (Barbu et al., 2017).

All the selected mutant/ recombinant wheat DH lines exceeded the parental forms (already in use in the breeding programs). These mutant/recombinant DH lines will be forwarded to the wheat breeding department of NARDI Fundulea, for further investigations.

## CONCLUSIONS

From the 307 mutant/ recombinant wheat DH lines taken into study, 25 lines that proved a stable and high genetic potential for all the analyzed parameters, significantly superior to the two parents, were selected, being considered an interesting material for breeding programs.

The useful variability resulted by applying both mutagenesis and DH-technology, proved to be an important tool for releasing of new genes sources.

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