Volume 17(4), 114- 118, 2013
JOURNAL of Horticulture, Forestry and Biotechnology
www.journal-hfb.usab-tm.ro

Wheat mutagenesis by combining recurrent irradiation, hibridization and DH-technology

Giura A.[1*]

[1]National Agricultural Research and Development Institute Fundulea; Institutul Naţional de Cercetare şi Dezvoltare Agricolă Fundulea

*Corresponding author. Email: agiura@ricic.ro

Abstract The well-known potential of induced mutagenesis process to generate genetic variability can be now better exploited in both genetics and crop improvement programs due to advancements and widespread interest in molecular genetics and biotechnology. With the aid of DH (doubled haploid) technology, it is presently possible to fix in only one generation any variability generated by either physical or chemical mutagens application and to search for the new allelic variants at DNA level, using molecular techniques. Similarly, any recombinant/mutagenic events generated through hybridization in association with mutagenic treatment(s) could be also fixed and expressed in phenotype, in homozygous condition without resorting to selection cycles in heterozygous populations.
The paper reports some results obtained using a specific mutagenic protocol including two modern wheat genotypes, two irradiation cycles application, hybridization and DH technology using the *Zea* system. Morphological modifications, resistance/sensibility to brown rust in circumstances of severe epidemics occurred in 2011 and other traits evidenced in a field survey of 338 wheat mutated/recombinant DH- lines are briefly described using selected examples.

Key words

recurrent irradiation, doubled haploids, *Zea* system, mutated/recombinant DH-lines

In Romania, wheat is the most important crop, after maize, with an annual acreage around two millions hectares. Several wheat cultivars released by NARDI-Fundulea are growing on major part of this area. However, the present genotypes lack features needed to counteract the predicted climate changes, to face with abiotic and biotic variable stress factors and particularly, to high mutability rate of foliar pathogens.
As crop improvement is entirely based on genetic variability exploitation, new and different genes or allelic variants are continuously and imperatively needed. In this respect, mutagenesis has become an important supplementary approach to classically plant breeding for the improvement of crop plant contributing substantially in providing new and different genetic variability. Because artificial mutagenesis is not essentially different from that naturally one – except the rapid mode of production- its inclusion in many worldwide breeding programs has been resulted in released of more than 3000 varieties that have been derived either as direct mutants or from their progenies, in around 170 cultivated species (12). Other numerous mutants could have been used directly in cross breeding programs as source of new variability but, their not specified traces were more often neglected along breeding cycles.

In plant breeding, induction mutation through irradiation has proven to be more effective, the majority of mutated released cultivars in major cereal species were obtained using gamma rays (12). Ionizing radiation as well as chemical mutagen can cause genetic changes in DNA structure and modify linkages offering promise to the improvement of crop plants. Through gamma irradiation process, besides the mutants possessing large deletion, more of which are not transmittable to the next generation, it is also possible to obtain small deletion (1 or 4 bp) which are normally transmissible (13). Moreover, it was proved that position and the size of deletion in the same loci have the capacity to alter the phenotype of resulted mutants through the process of transcription and translation, resulting forms with different characteristics: some act as dominant or recessive genes. To days, mutants harboring induced subtle genome alteration, usually single base pair changes, are becoming commonly used in gene discovery and function studies through the association of changes in their DNA sequences to modified phenotypic expressions. In addition, the usefulness of mutagenesis to influence crossover and recombination was clearly evidenced in barley and undoubtedly similar effects are of common evidence in other species (5;12). Successful application of mutagenesis could be enhanced by combining various methods. So, it was proved that irradiation of hybrid seed increased the mutation frequency, fostered genetic recombination and widened the mutation spectrum; hence more mutants could be provided for selection and genetic analysis too (8;15;18). To the same effect, recurrent irradiation can generate an greater range of genetic variability that a single mutagen treatment and therefore was proposed as a method of accumulating and expanding genetic variability (3). Consequently, any methodology aiming to improve the efficiency of mutation and certainly, the accumulation of further experience in using doubled haploid techniques will be instrumental in making more beneficial the application of mutagenesis in plant breeding and genetics. The special advantages of induced mutation are that variants with desired traits can often be produced in high frequencies, in a short time, in a chosen genetic background without disrupting the original genetic constitution of the crop.

On the other hand, as the cost of production greatly depends on the number of generation required for mutants evaluation, by using DH technology it become possible to perform a rapid and easier selection for desired plant type and, particularly for traits controlled through recessive alleles that constitute the greatest majority of genetic variability produced by irradiation with gamma rays. Consequently, it is expected a noteworthy diminution of overall expenses in selection procedures.

In this respect, our wheat mutagenesis program was based on specific protocol including two genotypes, two irradiation cycles application, hybridization and DH (doubled haploid) technology using *Zea* system. Starting material was represented by two modern Romanian wheat genotypes: cultivar Izvor released in 2009 and advanced breeding line F00628-34, each having valuable but some contrasting agronomic traits. Cultivar Izvor proved to be the most drought tolerant genotype created at NARDI-Fundulea with high yield ability in droughty years, carrying "*or*" recessive allele (controlling osmotic adjustment) on 7A chromosome (1, 2). The advanced breeding line F00628-34 manifested a good resistance to foliar pathogens, higher yielding potential in areas without water stress and carries 1A/1R translocation (16).

Mutagenic treatments with gamma rays consisted of two irradiation cycles were performed by Seibersdorf Centre-IAEA. For the first cycle, seed of each genotype (50 g) was treated with 200 gamma rays (200Gy). In the second cycle, the treatments were done with differing doses on the hybrid seeds resulted from direct (100Gy) and reciprocal crosses (200Gy) of M1 plants derived from the first cycle. Then, the four types of M1 plants were crossed by maize under greenhouse conditions, haploid plants were regenerated "*in vitro*", colchicine treatment applied on plantlets at three-five tillers stage and finally DH0 seed harvested. Details of the protocol were presented elsewhere (6).

Material and Methods

Following seed multiplication a number of 338 mutated/recombinant DH lines derived from the second irradiation cycle and their parents were sown in a field trial in 2010-2011 season, in pair of rows, 1m long, with 25 cm between rows and spaced apart 50 cm between pairs. The lines were thoroughly screened at all stages in the field and selection for various agronomic traits was made.

The lines expressed a complex of valuable agronomic characteristics are shortly presented below.

Results

Plant height

Plant height is one of the main morphological characteristics that can be induced with high frequency by irradiation and can be easily detected in field experiment. Reduction of plant height in mutagenic experiments is a general trend while highest plants are less frequently noticed. In wheat more than 20 genes were described for reducing plant height from which the *Rht1* was used prevalently in wheat breeding at NARDI- Fundulea. In field experiment with DH mutated/recombinant lines, dispersion for plant height stretches 50 cm between 60 cm the shortest line to 110 cm of the tallest line, the majority of lines being grouped in the classes of 85 - 90 cm, in comparison with 99 cm of parent F00628G-34 and respectively 100 cm of Izvor. From a total of 338 lines, 306 were shorter in comparison with both parental forms and only 27 were taller. Relations between plant height and other morphological parameters are in due course. There are special interests in selection, if possible, of variants with short straw but longer coleoptile that may represent useful source for wheat breeding: a deeper sowing and increasing initial autumnal growth rate as well as spring crop development.

Ear emergences

A range of 12^{th} days from the early to late heading was noticed in DH lines population. Part of this variability- a matter of hormone controlling differentiation- could have been generated by mutagenic treatment alone since similar results were also evidenced, although on smaller degree, in DH line resulted from parental M1`s after first irradiation cycle application with 200Gy (data not presented). However, in DH lines with two irradiation cycles, on hybrid materials, heading variation may resulted by combinatory effects of both parental genes recombination and the two mutagenic treatments affecting earliness "per se" genes, photoperiod responsive genes and vernalization requirement ones. However, the data accumulated for heading variation up to now, for individual lines, could partially be explained as different reaction to photoperiod and/or vernalization requirements.

Flag 1eaf dimensions

Scores of flag leaf dimension evidenced a marked modification regarding both the length and width (Fig1 and 2). As for plant high, the dispersion for flag leave length and flag width dimensions exceed the leaf dimensions of both parental forms. The shorter flag leave of 15 cm was recorded in one line and the longest of 29 cm were measured for four lines. The majority of DH lines were grouped in classes of 19.1-23 cm and only 12 lines exhibited a flag leave longer than the parent Izvor. As regard flag width, the majority of lines showed similar measure as the parents. Nevertheless, were observed leaf sizes up to 28 mm long and less width till 10.2 mm. Several lines exceed by flag leave dimensions. Different leaf dimensions mean diverse areas, and probably there are some differences in stomata number on both side of the flag leaf associated with drought resistance and productivity. The grain in modern wheat is more dependent on the leaves than the spike for photo-assimilate, as well as on greater remobilization of assimilate from stems. Some of the mutants with marked modification regarding leaf area may offer plant physiologist and geneticists more efficient probes for investigating wheat plant physiological processes.

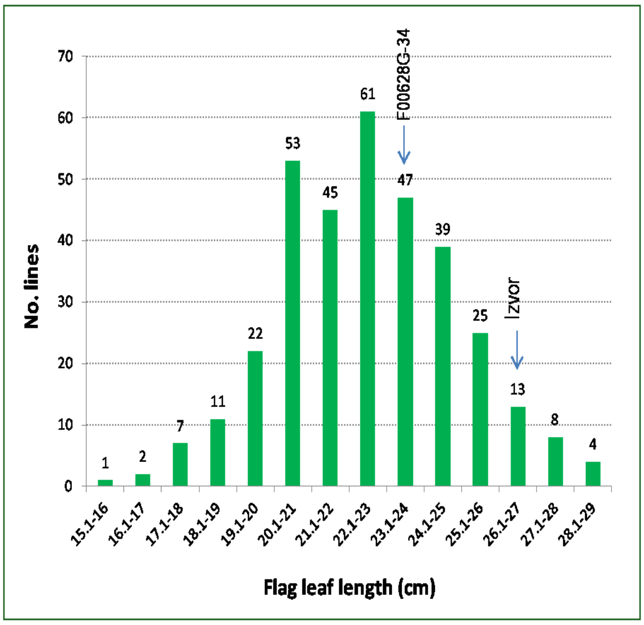


Fig 1 Dispersion for flag leaf length in 338 mutated/recombinant DH lines

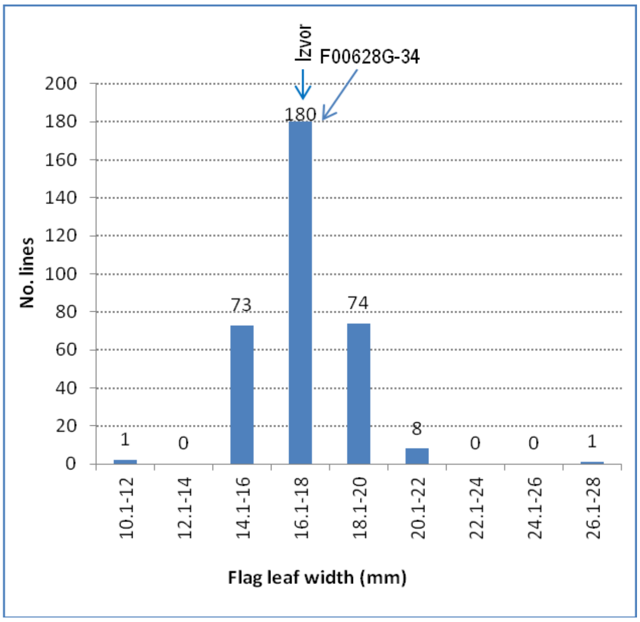


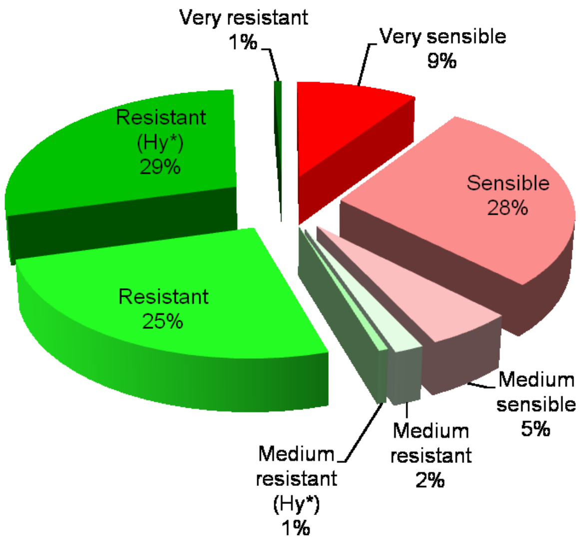
Fig 2 Dispersion for flag leaf width in 338 mutated/recombinant DH lines

Leaf rust

Leaf rust is one of the most important diseases seriously affecting wheat yield in Romania and therefore, is essential to develop cultivars with an acceptable level of resistance. In addition to conventional breeding methods induced mutation have been used several times as an alternative tool for developing useful new gene source against this pathogen (7).

Both wheat genotypes used in our study as starting material were previously quoted as having an acceptable level of resistance and therefore the initial objectives were not directed to improve leaf rust resistance. However, in 2011 due to an uncommon epiphytotic conditions, only cultivar Izvor carrying *Lr34* gene still expressed the typical “slow” rusting level of resistance while the *Lr* unknow resistant gene of breeding line F00628G-34, probably located on the short rye arm of translocated chromosome 1A/1R, have been overcome. Among of the 71 genes involved in leaf rust resistance, *Lr34* gene was described as a significant contributor to durable leaf rust resistance. *Lr34* gene located on chromosome 7D (4) has remained durable, and no evolution of increased virulence towards *Lr34* has been observed for more than 50 years. It has been incorporated into more than 50% of wheat cultivars around the world (11). Durable, by definition, refers to a situation where a cultivar which has been grown for many years over a considerable acreage and range of condition, maintained its resistance even when other cultivars have been severely infected in epidemics. *Lr34* increases the latent period and reduces uredinial size and receptivity (14;17). This is in contrast to many other rust resistance genes, the so called gene-for-gene class, that provide resistance to some but not all strains of a rust species. Interestingly, in our population of DH mutated/recombinant lines the whole spectrum of symptoms as response to fungus, from high susceptibility to complete immunity, was noticed (Fig. 3). The most relevant was the manifestation of hypersensitive like-phenotype (HPL) exhibited hypersensitive response (HR) in several DH-lines that conferred an additional resistance to the already healthy performance displayed by Izvor parent at adult plant stage.

Mutants exhibiting hypersensitive response that results in localized cell death at the site of pathogen infection have been identified in several species including common wheat (9). Introgression of the HLP mutation into different wheat genetic stocks conferred heightened level of resistance to leaf rust and no detrimental pleiotropic effects that affected the agronomic performance (10). Mutation induced by radiation can modify the host parasite interaction by alteration of relevant gene sequence and thus enhance resistance, but keeping intact the original plant type. It is worth to mention that ear productivity parameters of our resistant lines, including kernel weight, were higher compared to parental forms or to susceptible/medium susceptible DH lines. In a preliminary field test (D2) some lines exceeded the best performer parent Izvor with up 12% yield.



	No. Linii DH	%
Very sensible	32	9.47
Sensible	95	28.11
Medium sensible	18	5.33
Medium resistant	6	1.78
Medium resistant (Hy*)	2	0.59
Resistant	86	25.44
Resistant (Hy*)	97	28.70
Very resistant	2	0.59
	338	100.00

Fig.3 Dispersion for leaf rust resistance/sensibility in 338 mutated/recombinant DH lines

Conclusion

The results showed that mutational experiments by combining recurrent irradiation, hybridization and DH technology can be successfully used to generate new variability regarding a whole spectrum of plant morphological traits (plant height, leaf dimensions, leaf flag position, albedou appearance, ear productivity parameters), ear emergence, maturity time, and also disease resistance/sensibility etc.

Some promising DH- lines were already selected and passed to breeders for further field plots evaluation.

The genetic stock of mutated/recombinant DH-lines may offer new opportunities in approaches toward the long term goals of structural and functional genetics studies in wheat.

References

1. Banică Constantina., Ciucă Matilda, Giura A. – 2008 – Pollen grain expression of osmotic adjustment in Romanian winter wheat. In: EWAC Newsletter (Proc 14th Int.EWAC Conf. 2007), Istanbul,Turkey: 100-102.

2. Bănică Constantina, Petcu Elena, Giura A., Săulescu N. – 2008 – Relationship between genetic differences in capacity of osmotic adjustment and other physiological measures of drought resistance in winter wheat (*Triticum aestivum* L.). Romanian Agricultural Research

3. Data S.K. – 2009 – Role of classical mutagenesis for development of new ornamental varieties. In: Induced Plant Mutation in Genomic Era (Ed. Q.Y.Shu), IAEA, Vienna, 2009: 300-302.

4. Dick P.L. – 1987 – The association of a gene for leaf rust resistance with the chromosome 7D

suppressor of stem rust resistance in common wheat. Genome, 29; 467-469.

5. Filev K. ─ 1991─ Successful use of mutation breeding in durum wheat. In: Plant Mutation Breeding for Crop Improvement (Poc. Symp. Vienna, 1990), IAEA, Vienna 1991:295-299.

6. Giura A. ─ 2011─ Includerea tehnologiei DH într-un protocol de mutageneză la grâu- rezultate preliminare. An. INCDA-Fundulea, vol. LXXVIII, nr. 1: 1-10.

7. Jorgensen J.H. ─ 1991─ Mutation studies on cereal disease resistance. In: Plant Mutation Breeding for Crop Improvement, vol. 2 (Proc Symp., Vienna, 1990), IAEA, Vienna, 1991: 81-91.

8. Kajjidoni S.T., Roopalaksmi K., Revanappa S., Nagaral I. ─ 2009 ─ An inovative way of developing and improved variety utilizing both gamma ray induced and recombinational variability in blackgram (Vinga mungo L. (Hepper). In: Induced Plant Mutation in Genomic Era (Ed.Q.Y.Shu),IAEA, Vienna, 2009: 336-337.

9. Kamlofski C. A., Antonelli E., Bender C., Jaskelioff M., Danna C.H., Ugalde R., Acevedo A. ─ 2007 ─ A lesion-mimic mutant of wheat with enhanced resistance to leaf rust. Plant Pathol, 56 (1): 46-54.

10. Kamlofski C. A., Acevedo A. ─ 2010 ─ The HLP mutation confers enhanced resistance to leaf- rust in different wheat genetic backgrounds. Agricultural Sciences vol. 1, no 2: 56-61.

11. Krattinger S.G., Lagudah E.S., Spielmeyer W., Sing R.P., Huerta-Espinosa J., CFaden H., Bossolini E., Selter L.L., Keller B. ─ 2009 ─ A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323: 1360-1363

12. Lagoda P.J.L. ─ 2009 ─ Networking and fostering of cooperation in plant mutation genetics and breeding: Role of the Joint FAO /IAEA Division. In: Induced Plant Mutation in Genomic Era (Ed. Q.Y.Shu), IAEA, Vienna 2009: 27-30.

12. Nilan R.A. ─ 1966 ─ Barley cytogenetics and breeding: A progress report. In: Mutation in Plant Breeding (Proc. Panel Vienna, 1966), IAEA, Vienna, 1966: 177-185.

13. Nakagawa H. ─ 2009 ─ Induced mutation in plant breeding and biological researces in Japan. In: Induced Plant Mutation in Genomic Era (Ed. Q.Y.Shu), IAEA, Vienna, 2009: 48-54.

14. Rubiales D., Niks R. E. ─ 1995 ─ Characterization of Lr34, a major gene conferring nonhypersensitive resistance to wheat leaf rust. Plant Dis 79: 1208-1212.

15. Savov P.G. ─ 1991 ─ Use of radiation mutagenesis in the breeding of new wheat lines and cultivars. In: Plant Mutation Breeding for Crop Improvement, vol 1 (Proc Symp., Vienna, 1990), IAEA, Vienna, 1991: 289-293.

16. Săulescu N.N., Ittu G., Ciucă Matilda, Ittu Mariana, Mustăţea P. ─ 2011─ Transferring useful Rye genes to wheat, using Triticale as a bridge. Czech. J.Genet, Plant Breed. 47 (Special Issue): S56- S62.

17.Singh R.P., Huerta-Espino J. ─ 2003 ─ Effect of leaf rust resistance gene Lr34 on Components of slow rusting at seven growth stages in wheat. Euphytica, 129: 371-376.

18. Wang L.Q. ─ 1991 ─ Induced mutation for crop improvement in China. In: Plant Mutation Breeding for Crop Improvement, vol.1 (Proc Symp.,Vienna, 1990), IAEA, Vienna, 1991: 9-32.