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RESULTS OF USING *ZEA* METHOD FOR DOUBLED HAPLOID PRODUCTION IN WHEAT BREEDING AT NARDI FUNDULEA - ROMANIA

Nicolae N. Săulescu, Gheorghe Ittu, Aurel Giura, Pompiliu Mustăţea, and Mariana Ittu

National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania.
*Corresponding author. E-mail: n.n.saulescu@gmail.com

ABSTRACT

The paper reports some results obtained in the wheat breeding program of the National Agricultural Research & Development Institute Fundulea, (NARDI) Romania, by using *Triticum x Zea* crosses for producing doubled haploid (DH) lines.

Four out of eight bread wheat cultivars, and one out of two winter durum cultivars, released from the breeding program of N.A.R.D.I. Fundulea during the last ten years, were doubled haploids, obtained using the *Zea* method. Yield trial results obtained so far suggest that no yield or adaptation penalty was associated with the homozygosity or to the narrower genetic basis of these cultivars. The DH cultivar Glosa has been rapidly adopted by farmers, being already grown on over 300,000 ha (16% of total wheat area in Romania), only five years after its registration and 14 years after the final cross. A hypothetical cultivar obtained by the pedigree system from the same cross could have reached the same area only three years later, so that during these three years the area grown with Glosa cumulated over one million hectares.

The DH line that became Glosa was already used in crosses in 2001, and new cultivars were selected from these crosses and registered in 2010 and 2011, six years earlier than could have been possible if the conventional pedigree system was used. The increase in the speed of obtaining new cultivars can cumulate with each further cycle of breeding.

Further improvement of DH system efficiency is expected to be achieved by combining it with marker assisted selection at the haploid level.

Key words: doubled haploid, bread-wheat, durum wheat, breeding, *Zea* system.

INTRODUCTION

Acceleration of genetic progress is essential for increasing the contribution of wheat breeding to the required doubling of agricultural production by 2050.

One of the ways to accelerate genetic progress is to save time in obtaining recombinant inbred lines ready for yield evaluation, by producing doubled haploids (DH) that can shorten the time needed to reach homozygosity.

A doubled haploid wheat variety 'Florin' was developed by the anther culture method and was released as early as 1987 (de Buyser et al., 1987).

However, the application of anther culture method in wheat breeding had several disadvantages, among which the fact that there were large genotypic differences in the response to anther culture.

A different approach in producing wheat haploids is based on sexual hybridisation of wheat by maize and spontaneous elimination of maize chromosomes in the early cell division cycles of hybrid zygote. Development of haploid embryos in formed karyopses lacking endosperm can be successfully obtained by *in vivo* treatments with plant growth regulators on pollinated spikes (Suenaga and Nakajima, 1989; Giura, 1993, 1994, 2007a). This opened new possibilities of integrating DH system in wheat breeding programs (Laurie and Bennett 1986, 1988; Laurie and O'Donoughue, 1994; Inagaki and Tahir, 1992; Suenaga, 1994). Non-responsive genotypes for callus induction and plantlet formation in the anther culture method proved to be good parental material in wheat x maize crosses (Kisana et al., 1993). All the wheat varieties examined by Suenaga (1994) produced haploid embryos with the *Zea*

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system, which was considered to be superior to other DH systems, like anther culture and the *Hordeum bulbosum* system.

By 2003, 21 cultivars, obtained using the DH technology, had been released in China, Germany, Brazil, Canada, France, Hungary, Sweden and U.K. (Thomas et al., 2003), and probably many more have followed.

This paper reports the results obtained so far in using *Triticum x Zea* crosses for producing DH lines, in the wheat breeding program of the National Agricultural Research & Development Institute Fundulea (NARDI), Romania.

MATERIAL AND METHODS

About 3 to 5% of the crosses made each year in the wheat breeding program of NARDI Fundulea have been included in the special program to obtain doubled haploid lines. These crosses have been selected based on a subjective estimation of the expected frequency of superior segregants. Crosses between related lines that complement each other, and incomplete backcrosses have been preferred.

The protocol for producing doubled haploid lines was constantly improved and adapted to our working conditions (Giura and Mihăilescu, 2000), focusing on the many factors that influence haploid production, such as: physiological state and vigour of parental plants at pollination time; environmental conditions inside and outside greenhouse during crossing period (especially temperature and relative humidity variation); timing of the crossing period and selection of best pollen sources (Mihăilescu and Giura, 1998; Verma et al., 1999). Special attention was paid to wheat genotype responses to the plant growth regulators combinations and/or specific components (Giura, 1997); the number of days interval from pollination to embryo rescue time (Giura and Bănică, 2004); artificial media used for *in vitro* regeneration, etc. As a result of constant protocol improvements, the efficiency of haploids production increased from 1-3 embryos per spike at the beginning, to 4-6 embryos/spike or even more, lately.

A strong selection pressure was applied on the head rows on which doubled haploids were first grown in the field. Selected doubled haploid lines were included in preliminary yield trials on 2 replications differentiated by fertilization, planting date and/or water supply, then in yield trials performed in 8 environments, and the outstanding ones were promoted to official testing and finally were registered as cultivars and multiplied.

Yield data from 30 yield trials, performed during 2009-2011 in diverse environments in Southern Romania, were used to estimate the performance of DH cultivars, as compared with several conventionally obtained cultivars.

The area on which the doubled haploid cultivar Glosa was grown during the last years, estimated from the official data on share of the cultivar in seed production, was compared with the area on which a hypothetical cultivar produced from the same cross by conventional pedigree method could have been grown.

RESULTS

DH bread wheat cultivars registered and their performance

Out of 8 cultivars released from the bread wheat breeding program of NARDI Fundulea during the last ten years, four were doubled haploids, obtained using the *Zea* method (Table 1). This is more than expected based on the small proportion of crosses included in production of DH lines.

From Table 1, one can notice that crosses from which DH cultivars originated included more related parents, and therefore more restricted segregation than crosses used with the conventional pedigree method.

Nevertheless, a comparison of multi-location, multi-year data from yield trials, shows that cultivars obtained using the DH system were competitive with the ones obtained exploring a wider genetic variation by the conventional pedigree system (Table 2). In fact, the two best performers were both DH cultivars. The coefficients of variation of the yields obtained in contrasting environments, where the average yields of all tested cultivars varied from 1655 to 9517 kg ha^{-1} (data not shown), suggest that yield stability was similar, regardless of the applied breeding approach. This suggests that no yield or adaptation penalty was associated with the homozygosity or to the narrower genetic basis of these cultivars. The performance of DH cultivars is also illustrated by the rapid adoption by farmers of the cultivar GLOSA, which only five years after its registration in 2005 was already grown on 16% of total wheat area in Romania. In 2010, Glosa was already the third most grown cultivar and since, the trend has been to further extension.

Table 1. Cultivars released from NARDI Fundulea during 2002-2011

Cultivar	Year of release	Cross	Breeding system
Delabrad 2	2002	F308O2-20/DROPIA	Pedigree
Dor F	2002	F151M1-1/F2076W12-11// F4105W2-1	Pedigree
Faur F	2004	F307P2-1101/DELABRAD „S”	**DH *Zea***
Glosa	2005	DELABRAD”S”/F508U1-1// DELABRAD”S”	**DH *Zea***
Gruia	2005	AF93-2/DELABRAD „S”	Pedigree
Izvor	2008	KARL/F201R2-111//F508U1-1	Pedigree
Litera	2010	ERYT26221/F96869G1-1//GLOSA	**DH *Zea***
Miranda	2011	ERYT26221/F96869G1-1//GLOSA	**DH *Zea***

Table 2. Average yields of 30 yield trials in Southern Romania (2009-2011)

Cultivar	Breeding system	Grain yield (kg ha^{-1})	Coefficient of variation (CV%)
Miranda	**DH *Zea***	6019	26,3
Glosa	**DH *Zea***	5846	25,1
Gruia	Pedigree	5675	25,2
Izvor	Pedigree	5680	26,6
Litera	**DH *Zea***	5678	25,1
Faur F	**DH *Zea***	5517	27,0
Delabrad 2	Pedigree	5428	25,2
Boema 1	Pedigree	5322	26,6
Average of cultivars obtained by DH system		5765	25,87
Average of cultivars obtained by pedigree system		5526	25,90

On farm impact

Figure 1 describes the history of cultivar Glosa breeding, testing and extension, from the last cross made in 1997, to its registration in 2005, and to the extension on over 300,000 hectares in 2010.

The figure also includes the assumed history of a hypothetical cultivar, which could have been selected from the same cross, using the conventional pedigree system. Assuming the same number of years for testing and the same rate of extension, such a cultivar could have been registered in 2008 and could have reached the same growing area only in 2013.

Integrating the difference in area of extension from 2008 to 2013, between the DH and the pedigree obtained cultivars, we can deduce that Glosa was already grown on a cumulated area of over one million hectares, before a hypothetical cultivar obtained by the pedigree system from the same cross could have reached the same area on farms.

Having in mind the significant yield advantage of Glosa over previous cultivars, it is obvious that the use of the DH system was reflected in additional profit for farmers.

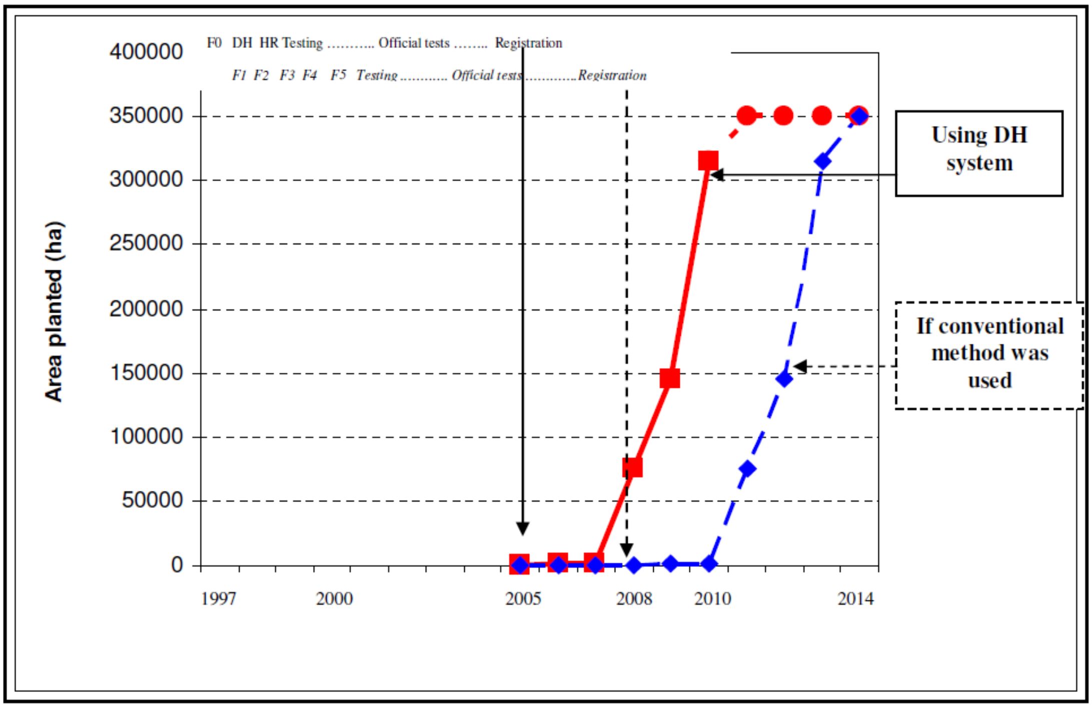

Figure 1. History of cultivar Glosa breeding, testing and extension, as compared with the assumed history of a hypothetical cultivar, which could have been selected from the same cross, using the conventional pedigree system

DH durum wheat cultivar registered

The suitability of Zea DH system for durum wheat was demonstrated first by O'Donoughue and Bennett (1994). Using specific modifications of the protocol, at NARDI a winter durum wheat cultivar Grandur, was obtained from a cross of two previously released NARDI cultivars (Pandur and Condurum), by using the Zea DH system (Giura, 2007b). This cultivar was officially registered in 2005, nine years after the cross was made. This is one of the few DH durum cultivars registered so far, and, as far as we know, the only DH winter durum cultivar obtained using this method.

Integration of DH system in a breeding program based on recurrent selection

Most breeding programs, including the ones in self pollinated crops, are based on the principles of recurrent selection, as they use best cultivars or lines selected in one selection cycle as parents for the next cycle of crossing. In this case, the effect of DH system on shortening the time needed to obtain homozygous lines can be greatly amplified. An example is the history of breeding the cultivar Miranda, which has Glosa as one of the parents (Table 3).

By using the DH system, the line that became Glosa was already used in crosses in 2001, and new cultivars were selected from these crosses and registered in 2010 and 2011. If the conventional pedigree system was used, Glosa could not have been used in crosses before 2004, and cultivars selected from such a cross and having the same selection history could only have been registered in 2016 and 2017, i.e. six years later than it was possible with the DH system.

Obviously the increase in the speed of obtaining new cultivars can cumulate with each further cycle of breeding, and this include using the DH system in pre-breeding programs, to provide new parents for the main breeding program.

DISCUSSION

Using the DH system can significantly shorten the time needed to reach the level of homozigosity needed for testing yield and other characters, as well as for using progenies as parents in further breeding cycles, in a breeding program. This means that finally farmers can get better cultivars sooner.

Table 3. Two scenarios of breeding wheat cultivars Glosa and Miranda

Year	Using DH system			If conventional method was used		
	Cycle 1	Cycle 2		*Cycle 1*	*Cycle 2*	
1997	Crossing			Crossing		
1998	Obtaining DH lines			*F1*		
1999	Head rows			*F2*		
2000	Plots 2 reps			*F3- Head rows*		
2001	Yield tests	**Crossing with GLOSA**		*F4 - Head rows*		
2002	Official tests	Obtaining DH lines		*F5 - Head rows*		
2003	Official tests	Head rows		*F6 - Plots 2 reps*		
2004	Official tests	Plots 2 reps		*F7 - Yield tests*	**Crossing with GLOSA**	
2005	**Registration GLOSA**	Yield tests		*Official tests*	*F1*	
2006		Yield tests		*Official tests*	*F2*	
2007		Official tests	Yield tests	*Official tests*	*F3- Head rows*	
2008		Official tests	Official tests	***Registration GLOSA***	*F4 - Head rows*	
2009		Official tests	Official tests		*F5 - Head rows*	
2010		**Registration LITERA**	Official tests		*F6 - Plots 2 reps*	
2011			**Registration MIRANDA**		*F7 - Yield tests*	
2012					*F8 - - Yield tests*	
2013					*Official tests*	*Yield tests*
2014					*Official tests*	*Official tests*
2015					*Official tests*	*Official tests*
2016					**Registration LITERA**	*Official tests*
2017						***Registration MIRANDA***

Cultivars obtained using the DH system are also expected to have better uniformity and stability, making easier to comply with the requirements for DUS (distinctness, uniformity, stability), imposed by UPOV (The International Union for The Protection of New Varieties of Plants).

On the other hand, some concerns have been expressed about the DH system, which have to be taken into consideration:

- There is a widespread belief that genotypic and phenotypic variability are positively associated with yield stability, and therefore homozygous cultivars obtained using the DH system might have lower yield stability over environments. Our data obtained so far suggest that, at least some DH cultivars might have good yield stability, similar with cultivars obtained through conventional pedigree system.

- In the conventional pedigree system selection is made on heterozygous progenies, and this increases chances of retaining valuable dominant or additive alleles before reaching homozygosity, and therefore a larger DH population is required to achieve the same level of genetic advance with the conventional method, in crosses containing greater genetic variation (Inagaki et al., 1998). Taking into consideration the small number of crosses and progenies used in our DH program, the proportion of DH released cultivars has been unexpectedly high. This suggests that the advantages of the DH system, particularly the advancement of newer, superior crosses, could counterbalance the limitations in the number of crosses and progenies. Further improvement of DH system efficiency is expected to be achieved by combining it with marker assisted selection at the haploid level.

A detailed economic analysis showed that the combination of MAS at the BC1F1 and haploid stage, not only increased genetic gain over the phenotypic alternative, but actually reduced the over all cost by 40% (Kuchel et al., 2005).

CONCLUSIONS

Using *Triticum x Zea* crosses for producing DH lines proved to be efficient in accelerating the release of new cultivars, in the wheat breeding program of NARDI Fundulea, Romania. The advantages of the DH system, particularly the advancement of newer, superior crosses, could counterbalance the limitations in the number of crosses and progenies.

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