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PLANT GROWTH REGULATORS A KEY FACTORS IN WHEAT – MAIZE CROSSES FOR HAPLOID PRODUCTION IN WHEAT

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Abstract

The paper highlights the role and importance of plant growth regulators (PGR) application before or post pollination with regard to embryo formation and haploid plant regeneration in wheat-maize hybridization. The efficiency of some PGR combinations (doses, procedures, time of application) and genetic influence on some parameters characterizing the efficiency of haploid production at NARDI Fundulea are briefly described. Taking into account the decreasing efficiency, in the last period, the need for new and different variants of PGR are discussed.

Key words: haploidy, doubled haploid, Zea system, PGR.

INTRODUCTION

Today, the haploidy is a topic of great interest in both genetics and breeding researches, and a very useful tool for developing of mapping populations and in shortening breeding programs by rapid homozygosity in a single generation.

Although, the haploid condition in wheat can be attained by using several procedures such as androgenesis, gynogenesis, microsporogenesis, the most effective ones is based on sexual hybridization of wheat by maize and spontaneous elimination of maize chromosomes in the early cycles of zygote cell division.

However, in postpollination events after elimination of male parent chromosomes the formed zygotes are immediately aborted due to the lack of endosperm formation. This conspicuous barrier was however overcome by using *in vitro* culture of pollinated spikelet or ovules on artificial medium that contains auxins (Laurie and Bennett, 1986, 1988; Comeau et al., 1988). Soon after that, Suenaga and Nakajima (1989) described a simplified method wich included a tiller injection with 2,4-D (2,4-dichlorophenoxyacetic acid) and Riera-Lizarazu and Mujeeb-Kazi (1990) tested a 2,4-D floret spray treatment and detached spike culture method. The regulatory effects of auxin treatments on the embryo formation by *in vivo* treatments were also proved using different pseudogamous species or lines of the *Poaceae* (Matzk, 1991). In barley, by using barley-Bulbosum hybridization system, Mihailescu et al (1994), Pickering and Walace (1994) have reported a positive influence on seed set, embryo formation and embryo differentiation after *in vivo* application of PGR solution containing GA3 (gibberellic acid) and 2,4-D.

These first reports emphasized that haploid embryos could be obtained and haploid plants certainly regenerated by *in vivo* treatments with auxins.

Further improvements and refined procedures concerning mode and time of application, PGR composition and doses, make the wheat - maize crosses the most efficient procedure for haploid production in common wheat.

At NARDI- Fundulea the wheat maize hybridization program was initiated in 1991, firstly by testing cross-compatibility between several maize early hybrids and some old and new released wheat cultivars (Giura, 1994).

Afterwards, working protocol was improved and particularly adapted to the relative limitative logistical supports, especially those for handling and growing parental plants in a greenhouse provided with heating facilities during winter time and supplemental illumination by incandescent bulbs. After several attempts have become obviously that in

such conditions wheat-maize crosses could be successfully done within a single crossing cycle of 30-45 days in March-April period when interaction of environmental factors especially temperature and atmospheric humidity proved to be more favorable.

During 1991- 1995 many PGR treatment variants were tested for their effects upon embryo formation, differentiation, germination and haploid plant recovery. The best ones were again tested in 1996 and two PGR variants (A_2 : 2,4-D 25 ppm, GA3 75 ppm and C_1: 2,4-D 18 ppm, Dicamba 9 ppm, BA 2 ppm) were noticed as having significant superior values for the parameters characterizing the *Zea* system efficiency in common wheat haploid production (Table1).

Table 1. The effects of hormonal treatment variants applied in vivo in cross breeding wheat x maize - Fundulea 1996-

Treatment	Parameters			
	QK/F	E/F	E/DK	E/Sp
A (control)	49.7	13.7	27.5	2.9
A1	49.7	13.8	27.8	3.2
A2	**60.0**	**21.4**	**35.6**	**5.3**
A3	62.5	12.1	19.4	3.2
B2	71.2	12.4	18.1	3.0
C	73.9	17.4	23.5	4.4
C1	**68.6**	**21.4**	**31.2**	**5.4**
C2	74.3	6.0	8.0	1.4

(After Giura and Mihailescu, 2000)

QK / F - quality kernels / 100 pollinated flowers
E / F - embryos formed / 100 pollinated flowers
E / DK - embryos / 100 dissected kernels
E / Sp - embryos / spike

Since then, the PGR variant A_2 was preferential used. Nevertheless, in the last years, in a new greenhouse, this PGR variant proved to be less efficient probably due to specific environmental conditions inside greenhouse provided with heating system based on methane gas turbo-blower that might decrease the humidity and increase CO_2 concentration.

Therefore it has become necessary a comparative reassessment of the PGR variants A_2 (2,4-D 25 ppm, GA3 75 ppm) and C_1 (2,4-D 18 ppm, Dicamba 9 ppm, BA 2 ppm).

MATERIALS AND METHODS

A number of 21 winter wheat F_1's genotypes from the wheat breeding program and sweet maize hybrid "Delicios" were cultivated in the same greenhouse compartment in 2014. A complex fertilizer (N:P:K; 20:15:10) was administrated by broadcasting before soil tillage. From the mid of December to the mid of February maize parent were sown in rows at several planting times, once per week to ensure a continuous pollen window during wheat - maize crosses in March – April. Wheat plantlets previously vernalized for 45 days were planted in large pots at the mid-January: three plantlets/pot. For tiller development and to reach on optimum physiological state at the time of pollination, wheat plants were supplementary watered before spike emergence with a complex nutritive solution of macro and micro nutrients.

Spike of wheat plants were hand emasculated leaving 18-20 flowers per spike. The upper spikelets and central florets of each spikelet were removed but one or two basal spikelets were left to prevent in some way spike desiccation. At the predicted day of anthesis, spikes previously emasculated were pollinated with freshly collected maize pollen. The hormonal treatments with A_2 (2,4-D 25 ppm, GA3 75 ppm) and respectively C_1 (2,4-D 18 ppm, Dicamba 9 ppm, BA 2 ppm) solutions (variants) were administrated by spraying on the spikes at 24 hours after pollination.

At 13-14 days after pollination the caryopses were extracted from the spikes and sterilized in 90% ethyl alcohol for a half minute, then in a 7% sodium hypochlorite solution for 5 minutes and rinsed subsequently 5 times in sterile water. The accidentally resulted self-pollinated seeds are very distinctly after size with solid endosperm and can be easily eliminated. The immature embryos were aseptically excised and cultured in vials containing modified B5 medium (Jensen, 1975) supplemented with 2% sucrose and 0,8% agar. The number of developed caryopses, cultured embryos and haploid plants regenerated were recorded for each spike and genotype.

RESULTS AND DISCUSSIONS

The two PGR variants A_2 and C_1 were applied after pollination on 1032 spikes. Following embryo rescue operation in aseptically condition under binocular microscope in a laminar flowhood a number of 1630 haploid embryos were transferred on artificial medium for regeneration.

Parameters haploid embryo/spike (He/Sp) and haploid plant regeneration capacity / cultured embryos (Hp/Ce) on each treatment variant were considered as the most appropriate to reevaluate the efficiency of the two PGR variants.

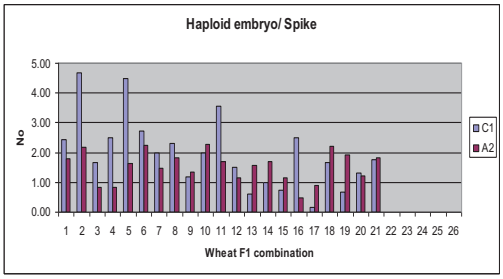

Figure 1: Haploid embryo/spike for C_1 and A_2 treatment variants

From the chart presented in Figure 1 it is obvious that under variant C_1 treatment the F_1's genotypes produced more embryos per spike compared to A_2 variant. However, some F_1's genotypes presented a higher average values for He/Sp when treated with A_2 variant. This fact could be explained if we be take into consideration a possible influence of genotype. A such phenomenon was also reported by Inagaki and Tahir (1990).Although the quality of embryos was not quantified, the regeneration capacity on B5-modified medium was in average higher with A_2 variant as compared to C_1 variant (Figure 2).

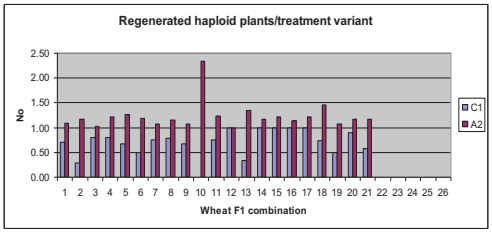

Figure 2: Haploid plants regenerated / cultured embryos for C_1 and A_2 treatment variant

Because the embryo development is a key point for *in vitro* culture response, the differences in embryo size might be closely related to the success of embryo growth response and plant regeneration ability (Giura, 1994). We could admit that A_2 treatment version might provide the necessary development conditions for a better regeneration rate. Moreover, the beneficial effects of A_2 variant on regeneration capacity were registered for all F_1's genotypes without any exception. Similar results were also registered in 1996 experiment when A_2 and C_1 variants over yielding significantly the control and other PGR variants by higher values, especially for haploid embryos per spike (He/Sp) parameter (Giura and Mihailescu, 2000).

However, when we evaluate and compare the results registered for 1996 and 2014 a decreasing efficiency of both PGR variants is evident (Table 2).

Table 2. Efficacy of growth hormone treatment expressed by the ratio embryos / spike

Year	Treatament A_2	Treatment C_1
1996	5.3	5.4
2014	1.5	2.0

Therefore, the influence of genotype could not be ignored, each year we received from breeders new and different series of F_1's genotypes, the experimental conditions inside greenhouse could remain an essential factor since haploid production efficiency is strongly influenced by temperature during the period of *in vivo* development up to the harvest caryopses. (Giura and Mihailescu, 2000).

CONCLUSIONS

Both growth hormone treatments gave satisfactory results, but it is however necessary to reevaluate the influence of different factors that operate inside greenhouse and to establish new combination/doses of growth hormones that can lead to better results.

It may be considered that new and different PGR variants may be further tested for increasing the haploid production efficiency mainly by sustain a better embryos development in the absence of endosperm as well as embryo regeneration capacity *in vitro* conditions.

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