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Production of Haploid Green Plants by Intergeneric Crossing of *Triticum durum* Desf × *Zea mays* L.

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Abstract: The haploid regeneration capacity in intergeneric crossing (wheat x maize) of seven varieties of durum wheat by embryo rescue and effect of different factors used in this assay were submitted to study. Our results showed that the parental genotypes have a significant effect on the developed ovaries, embryogenesis and regeneration of haploid green plants. Oued Zenati and Bidi 17 varieties have a better productivity with regard to the haploid plants obtained. On the other hand, Djenah- khetifa variety did not produce haploid plants. The ears cut off and kept in a nutritive solution gave an amount of embryos and green plants that were higher than the ears that remained on the parental plant. The time required before the ears are separated and kept in a nutritive solution is an essential factor for obtaining better results. In our study, it was clearly that two or three days following the pollination were considered an optimal period for cutting the ears. Beyond this period, the results are closer to those found with using the ears *in situ*.

Key words: *Triticum durum* · *Zea mays* · Intergeneric · Haplodiploidization · Embryo rescue

INTRODUCTION

One of the methods currently used for improving wheat is haplodiploidization. This method arouses a highly vivid interest among the breeders because it allows shortening the time of selection leading to pure stocks comparing with the conventional methods of selection [1- 3]. Several techniques of haplodiploidization are used in the systems of selection of wheat [4]. Although it considered a resistant species to certain haplodiploidization techniques [5], some recent studies allowed overrunning this status using intergeneric crossing depending on genotypes [6-8]. These hybridizations by crossing wheat with maize and durum wheat with *Hordeum bulbosum* allowed obtaining wheat haploid plants with a high production of non-differentiated embryos [9]. Maize, (*Zea mays* L.), can fertilize durum wheat (*Triticum durum)* [6, 9, 10], The zygote obtained from the hybridization of wheat and maize consists of a combination of 14 chromosomes of wheat and 10 chromosomes of maize, the albumen being either absent, or highly abnormal [6,11]. The 10 maize chromosomes show centromeres which present a low affinity for the achromatic spindle, which causes their rapid elimination during the first three cellular divisions and the formation of a haploid embryo with chromosomes from the parental wheat [6, 11-13]. It is the same technique that we tried to use in order to obtain haploids from crossing between parents of durum wheat x maize.

The purpose of this study is to have a better understanding of the haplodiploidization technique identifying the effect of parental genotypes on the developed ovaries, embryo development, green plant regeneration and optimal period that lead this technique to be successful.

MATERIALS AND METHODS

Seven durum wheat varieties (Oued Zanati, Bidi 17, Hedba 03, Djenah-Khetifa, Belikhe 2, Haurani and Waha) were pollinated with two maize cultivars (Guich and Elbadia). The durum wheat grains were pre-germinated in Petri dishes at room temperature about 25°C in the dark, however maize grains were pre-germinated at heat and then in the dark. The seedlings obtained from wheat and maize were replanted gradually

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in pots of 5 kg containing a mixture of soil and sand (2/3:1/3 v/v). Pots were placed in the greenhouse and watered according to their retention capacity (0.52l) every three days with plain water.

Wheat flowers were castrated 1 to 4 days before anthesis and as soon as the stigmata became feathery, some freshly pollen grains were collected from maize which carefully deposited with a brush within 24 h. A solution of 2, 4-dichlorophenoxy acetic acid (100 mg/l) was injected in the last internode and a drop of the solution was deposited on each flower for two days after pollination. Gibberellic acid solution (75 mg/l) was sprayed on the cobs. Two processes were performed, whereas one required the presence of the ears on the parental plants (*in situ*) for 10 to 20 days after pollination and the other was based on the use of the ears which were cut off after 1 to 5 days of pollination and were kept in a solution of 2,4-D (100 mg/l), sucrose (40 g/l) and ethanol (10 ml/l) in a culture room with photoperiod 16 h and humidity 80% at 20°C. The formed ovaries were sterilized in sodium hypochlorite (3%) before cutting of the rescued embryos and their aseptic culture in medium B5 were added to indole acetic acid (IAA) (1 mg/l), Kinitine (0.25 mg /l) and sucrose (2%). The Petri dishes were kept in the dark until the germination of the embryos and then transferred to test tubes in the same medium. After a sufficient growth, the seedlings were weaned in sterile soil in pots and kept in the culture room.

The chromosome doubling was obtained by soaking the plants (stage 3 to 4 leaves) in colchicine solution at 0.1 % for 5 h followed by rinsing with tap water [14]. The haploid (n) and diploid (2n) chromosomes number were verified by chromosome counting using mitotic examination, where the root tips were hydrolyzed in 5N HCl for 10 minutes at room temperature then stained with feulgen stain squash technique according to Darlington and Lacour [15].

The analysis of variance was performed between different parameters of durum wheat × maize crosses via the statistical package MINITAB ver. 13.31.

RESULTS

Effect of the Parental Genotypes upon Different Traits: The studied traits of seven durum wheat varieties were crossed with two maize cultivars (Guich and Elbadia). were represented in Table 1. The influence of wheat genotypes on the developed ovaries percentage was statistically significant. These results were forcibly in correlation with those of the embryos (r = 0.759) even

Table 1: Effect of the different of durum wheat genotypes on some studied parameters

Hard wheat	NPF	DO %**	NE**	NE/DO%*	NGP**	NGP/DO%*
Oued Zenati	200	49c	19d	19.38e	10e	10.20e
Bidi 17	98	54d	22e	41.57g	8d	15.11d
Hedba3	220	70e	15c	9.74c	2b	1,3c
Djenah-khetifa	52	15a	2a	25.64f	0a	0a
Belikhe2	190	28 b	3a	13.04d	2b	3.75b
Haurani	188	42.5c	5b	6.25b	4c	5b
Waha	340	25b	1a	1.17a	1a	4.31b

The genotypes having the same letter were included in the same group at the confidence interval (α= 0.05) according to MINITAB analysis ver. 13.31.
*indicates that the differences were significant between the different genotypes at 0.05% and** at 0.001%
NPF: number of pollinated flowers DO %: developed ovaries percentage, NE: number of embryos, NE/DO: the ratio between the number of embryos formed and developed ovaries, NGP: number of green plants and NGP/DO: the ratio between the number of the green plants obtained and developed ova

Table 2: Intergeneric hybridization between durum wheat and maize genotypes

Maize	Durum wheat	NPF	DO%*	NE	NGP*
Guich	Oued Zenati	100	29.0	10	6
	Bidi 17	48	32.0	18	5
	Hedba 03	110	35.0	10	2
	Djenah-Khetifa	26	10.0	2	0
	Belikhe 2	95	18.0	2	2
	Haurani	94	32.5	5	3
	Waha	170	18.5	0	1
Elbadia	Oued Zenati	100	20.0	9	4
	Bidi 17	48	24.0	4	3
	Hedba 03	110	35.0	5	0
	Djenah-Khetifa	26	5.0	0	0
	Belikhe 2	95	10.0	1	0
	Haurani	94	10.0	0	1
	Waha	170	6.5	1	0

Fisher value is 0.013 for DO %, 0.086 for NE and 0.001 for NGP,
* means that the difference between genotypes is significant at 0.05%

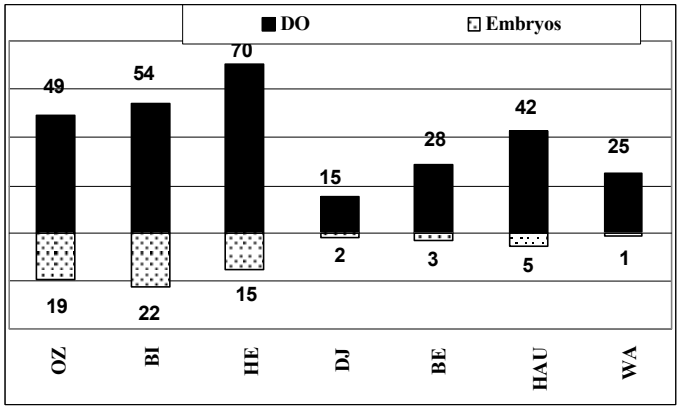

Fig. 1: Comparison between developed ovaries (DO) and embryos formation in the different studied varieties of durum wheat

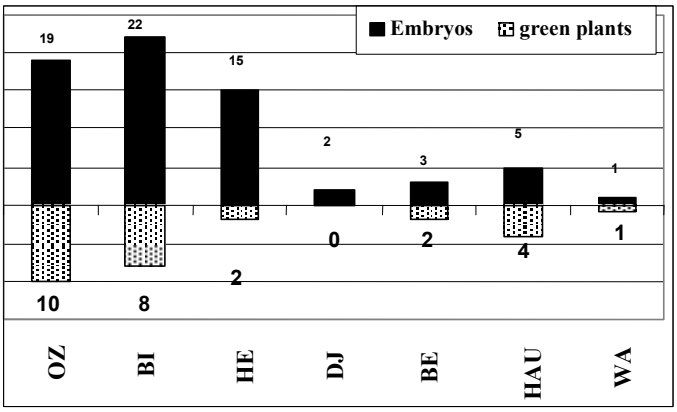

Fig. 2: Comparison between the number of embryos and the green plants in the different varieties of durum wheat

Fig. 3: The haploid green plant obtained by the hybridization of Hedba variety and maize

though an exception was noted in this study. For instance, Hedba variety showed a high developed ovary percentage (70 %), while the number of formed embryos was low with 15 embryos (Fig. 1). The differences between wheat genotypes in terms of formed embryos and the green plants obtained were also significant (Fig. 10). A positive correlation was found between the number of embryos and the green plants obtained (r = 0.797). In addition, the male parental maize effect was significant on the developed ovaries and on the green plants obtained (Table 2). Guich genotype was more producible in relation to Elbadia genotype concerning the embryogenesis and the formation of green plants; the differences between maize cultivars were studied. The germination of the embryos in green plants was obtained (Fig. 3) for Oued Zenati (10 plants), Hedba (2) and Bidi 17 (8 plants) crosses with the two maize cultivars on the other hand, no regeneration of the green plants for Djenah –khetifa was obtained. Generally, the 27 haploid green plants were formed consisting 14 chromosomes (Fig. 4).

Effect of the Growth Medium upon Different Traits: According to the variance analysis, it was shown that no significant effect for the developed ovaries between the ears that are kept on the parental plant and those are cut off and kept in a nutritive solution (Fig. 5).

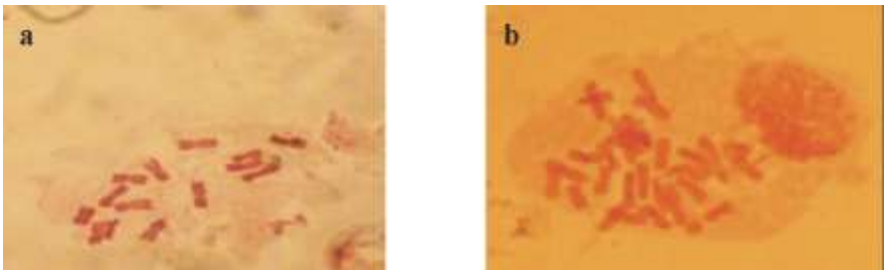

Fig. 4: (a) a haploid cell from the intergeneric crossing between Blikhe and maize, (b) a diploid cell of variety Blikhe from a chromosomal doubling by colchicine

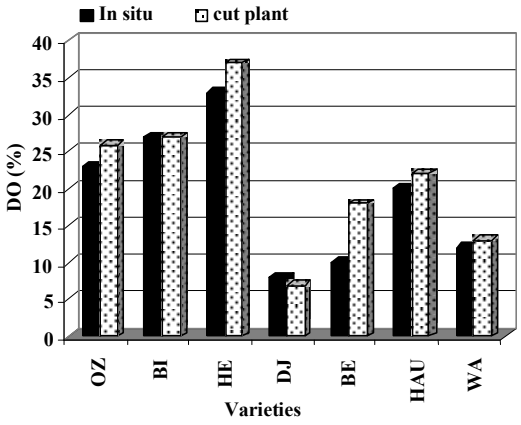

Fig. 5: Comparison between the developed ovaries rate for the ears that were kept *in situ* and that were cut for the different durum wheat varieties

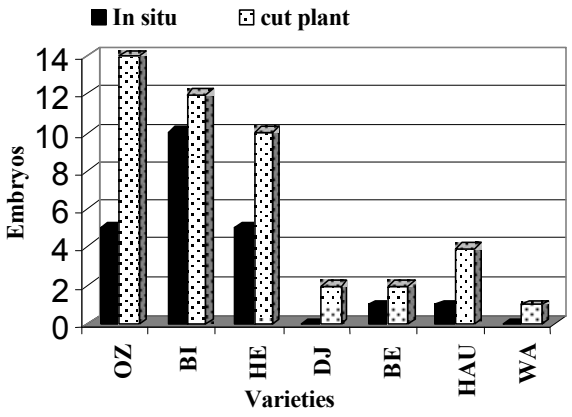

Fig. 6: Comparison between the embryo numbers for the ears that were kept *in situ* and that were cut for the different durum wheat varieties

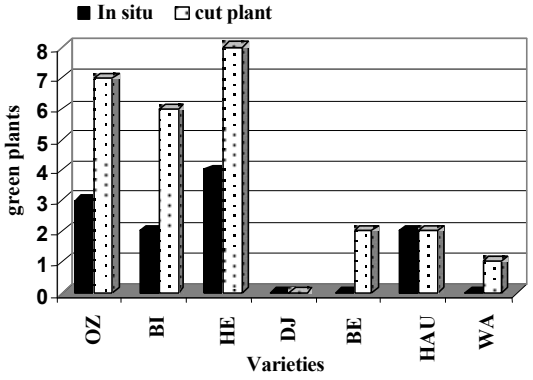

Fig. 7: Comparison between number of green plants for the ears that were kept *in situ* and that were cut off for the different varieties of durum wheat

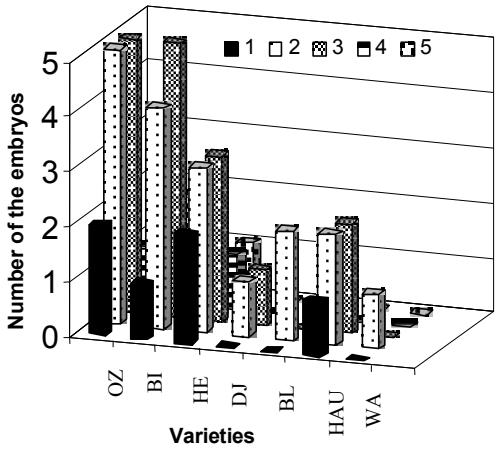

Fig. 8: Evaluation of the number of embryos in relation to the survival period of the ears on the parental plants in the different varieties.

Oppositely, a significant effect of the medium was observed (F=9.14) on the development of embryos (Fig. 6) and on obtaining green plants (Fig. 7) (F=8.59), the ears which are cut are proven to be the most producible compared with the ears which remained on the parental plant (*in situ*).

Optimal Survival Period on the Parental Plant Before Separating the Ears: The analysis of variance revealed a significant effect of the necessary survival period of the parental plants prior to the cutting of the ears, either for the embryogenesis (F=10.86) (Fig. 8), or for the regeneration of the green plants (F=7.67) (Fig. 9).

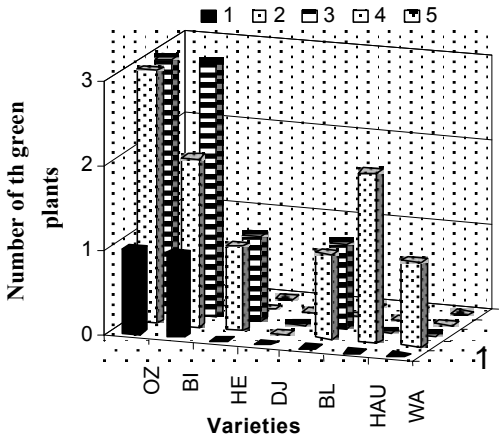

Fig. 9: Evaluation of the number of green plants in relation to the survival period of ears on the parental plants in the different varieties.

Individual CI at 95% (a)

DAYS	AVERAGE	0.00	1.00	2.00	3.00
D1	0.86		(------*-----)		
D2	2.57			(------*-----)	
D3	2.29			(------*-----)	
D4	0.57	(------*-----)			
D5	0.29	(------*-----)			

Individual CI at 95% (b)

DAYS	AVERAGE	0.00	0.60	1.20	1.80
D1	0.29		(--------*-------)		
D2	1.43			(--------*-------)	
D3	1.14			(-------*-------)	
D4	0.00	(--------*-------)			
D5	0.00	(--------*-------)			

Fig. 10: The confidence interval at 95 %, (a) of the embryos, (b) of the green plants.

According to the confidence interval table and regardless of the varieties studied, it was shown that the optimal survival period of the ears on the parental plant before the separation and keeping in a nutritive solution to obtain the embryos and regeneration of the green plants were two or three days after pollination.

DISCUSSION

Haplodiploidisation via intergeneric crossing of durum wheat × maize, the only technique used to date to overcome the problem of albinism that occurs with the androgenesis and gynogenesis along with the arrest of the pollen grain germination under the action of genes Kr1 Kr2. In the case of durum wheat × *Hordeum bulbosum* hybridisation, although the regeneration rate of green plants remains low, similar results were found by Chlyah and Savaskan [8, 16]. In our study, parental genotypes have a significant effect on the developed ovaries, embryo development and regeneration of the green plants. These results are in compliance with the results of Sarrafi [17] and Saidi [18], some reports found non-significant effect of parental genotypes [19], because they used a limited number of genotypes. The developed ovaries were not correlated with the development of the embryos in Hedba variety because the developed ovaries showed an inflation of the ovary that is caused by the application of the hormones during the pollination. A positive correlation was found between the grown embryos and the regenerated green plants. This result was corroborated by Matzak [13]. Among our results, a significant effect was observed on maize and on the regeneration of the green plants, this effect was little in the results of most researchers because they used only one male genotype [8]. The ears were cut and kept in a nutritive solution, which gives a number of embryos and green plants higher than that remained on the parental plant, this difference was due to the presence of 2, 4-D at high concentration in the solution which favours the survival and development of the embryos [3].

The time required before the ears separation and keeping them in nutritive solution is an important factor for finding better results since the pollen grain germination and the fertilization require a sufficient time. Among results, two or three days following the pollination were considered an optimal stage for cutting the ears. Beyond that time, the results are closer to those found through using the ears *in situ*.

CONCLUSION

This study shows that the intergeneric crossing allows obtaining chlorophyll haploid plants in durum wheat which grow regularly from perfectly formed embryos with no calluses. The durum wheat × maize hybridization is of a more global interest, since the choice of the maize cultivar allows optimizing the formation and regeneration rate of embryos. The percentage of green plants obtained is still relatively low due to the small size of some embryos at the time of their transfer *in vitro* conditions.

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