J. Genet. & Breed. 49: 353-358 (1995)

Genotype effect and histocytological events in relation to embryo formation after intergeneric crosses between durum wheat (*Triticum turgidum* Desf. var *durum*) and maize (*Zea mays* L.) or teosinte (*Zea mays* L. *ssp mexicana*)

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Received July 11, 1995

ABSTRACT

Eleven commercial durum wheat varieties were crossed with four maize pollen sources and one teosinte pollen source. Pollen tube growth, egg divisions, development of fruit-like structures (swollen ovaries) and embryo occurrence were observed. Female genotype effects were found for all traits analysed. Cv. Primadur was found to be superior for frequency of fertile spikes, whereas cvs Arcour and Ardente durum were found inferior for this trait. Moreover, cvs Lloyd and Primadur showed high numbers of fruit-like structures in fertile spikes as compared with cv. Armet. Cvs. Primadur, Villemur and Aristan ranked first for embryo occurrence in fruit-like structure. Pollen genotype effects were less evident except for a strong positive effect of teosinte pollen on embryo occurrence in fruit-like structures. These results are discussed in comparison with other published experiments.

Key words: Fruit-set, Gynogenesis, Haploid, *In vitro* culture

INTRODUCTION

Haploidization is a powerful biotechnological tool widely used in plant genetics and breeding. Besides rapid creation of pure lines for genetic or breeding purposes after diploidization (De Buyser and Henry, 1987), haploid cells and tissues can serve as targets for *in vitro* selection (Swanson *et al.*, 1988) and genetic transformation (Neuhaus *et al.*, 1987). Anther culture has been used for more than 15 years for the routine production of new inbred lines in hexaploid bread wheat (Picard and De Buyser, 1977). Unfortunately, tetraploid durum wheat has so far been more recalcitrant to this technique (Sharma *et al.*, 1982): in addition to poor embryo recovery from anther culture, more than 95% of developed plantlets were albinos (Hadwiger and Heberle-Bors, 1986).

A second way of producing haploid plants is to use female gametes. Bread wheat ovule and embryo sac culture were obtained by Zhu and Liu (1981) and Yang and Zhou (1982). However, the efficiency of the method was found to be very low.

The third way of producing haploid plant relies on intergeneric or interspecific crosses. The gynogenesis technique induced with foreign interspecific pollen was first introduced in cereals for the production of haploid barley plants with *Hordeum bulbosum* pollen (Kasha and Kao, 1970). This technique has been adapted to bread wheat *Triticum aestivum* castrated flowers (Sitch and Snape 1986, Koba *et al.*, 1991); however, it was found to be ineffective in wheat genotypes with some *Kr* alleles (Koba *et al.*, 1991; Sitch and Snape, 1987a; Sitch and Snape, 1987b).

In crosses involving wheat as the female parent and maize as the pollen parent, rapid elimination of the male genome was observed by Laurie and Bennett (1988), allowing the development of haploid embryos under controlled conditions. Maize pollen and male gametes from other species such as pearl millet (Ahmad and Comeau, 1990), teosinte (Ushiyama *et al.*, 1991) and sorghum (Ohkawa *et al.*, 1992) were used to fertilize *T. aestivum* flowers for breeding uses under special circumstances.

Crosses between *Triticum turgidum* var. *dur-*

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um and *Zea mays* have also been performed: fertilization potential was assessed by O'DONOUGHUE and BENNETT (1988), and green haploid plants recovered (COUMANS *et al.*, 1992; RIERA-LIZARAZU *et al.*, 1992; AMRANI *et al.*, 1993; SARRAFI *et al.*, 1994).

This paper describes the occurrence of some cytological events during the fertilization of durum wheat by maize and teosinte pollen. The haploid status of plantlets recovered after embryo culture and the effects of female and male genotypes on fertilization and embryo occurrence are discussed here.

MATERIALS AND METHODS

Plant material

This paper deals with the results we obtained during the 1992-1993 season in Mauguio (INRA) and Montpellier University laboratories.

Female parents. On each parent, more than nine spikes (i.e. more than 340 castrated flowers) were studied. The 11 parents are cultivars of durum wheat registered in the French official catalog (GEVES, 1993) and are, or have been, grown extensively in this country during the past ten years (Table 1). They were all bred by French laboratories except cv. Lloyd which was released by North Dakota University.

Male parents. Each pollen donor was used on more than 14 emasculated spikes. The maize material was CM 139 a line from Agriculture Canada, Kiwase, a Japanese population, Zapalote, a Mexican population and Liza, a commercial hybrid from Pioneer. Teosinte was a *Z. mays* spp *mexicana* population maintained at INRA Montpellier, France.

Emasculation and pollination

Emasculation and pollination were done according to LAURIE and BENNETT (1986). About 3 days before anthesis, in order to facilitate the castration process the spikelets of the basal third of the spike as well as each central flower of the remaining spikelets, were removed. The apical third of the spike was cut out. The remaining flowers were emasculated after cutting out the apical part of glumes, lemmas and paleas. After emasculation, spikes were protected by a light paperbag in order to keep a sufficient moisture level (SUENAGA and NAKAJIMA, 1989). The flowers were pollinated 3 days after.

Immediately after pollination, the culms were cut at 30 cm from the top, maintained in the growth chamber in a solution described by KATO *et al.* (1990), modified by USHIYAMA *et al.* (1991) under 16 h light photoperiod and 25° C constant temperature. One day later, a solution of 0.75 mg/L of gibberellic acid (GA 3) was sprayed on the spike.

Histological observations

Pollen viability was assessed using fluoresceine diacetate (HESLOP-HARRISON and HESLOP-HARRISON, 1970) and Alexander blue (ALEXANDER, 1969). Pollen germination on stigma was observed after staining with decolorized aniline blue under fluorescence microscopy (O'DONOUGHUE and BENNETT, 1994);. Ovaries were imbedded in histological resine GMA (GERRITS and SMID, 1983) after glutaraldehyde fixation. Thin sections were stained with periodic Schiff-naphtol blue black (CHUANG YING CHAO, 1979), or Groat hematoxyline (MARTOJA and MARTOJA, 1967).

Ten days after pollination fruit-like structures from some swollen ovaries were dissected and the occurrence of embryos surrounded by liquid tissue replacing endosperm was checked. Dissection of swollen ovaries was done after disinfection with 6% calcium hypochlorite and washing with sterile distilled water in a sterile laminar flow environment. Embryos were transferred on to B5 medium (GAMBORG *et al.*, 1968) under 16h photoperiod at 25° C. Plantlets were tranferred in potting mixture in the greenhouse. The haploid status was checked using flow cell apparatus PARTEC (Software DPAC Version 2.0 Data pool).

Statistical analysis

The Chi^2 test was used to compare female and male genotypes for fruit setting on pollinated flowers and embryo occurrence on fruits, each genotype contrasting with the others. Some data were evaluated using Fisher's exact test for small samples.

RESULTS AND DISCUSSION

Chronological events

Pollen tube observations showed that maize pollen from the four genotypes analysed germinated and entered the silks within less than 30 min

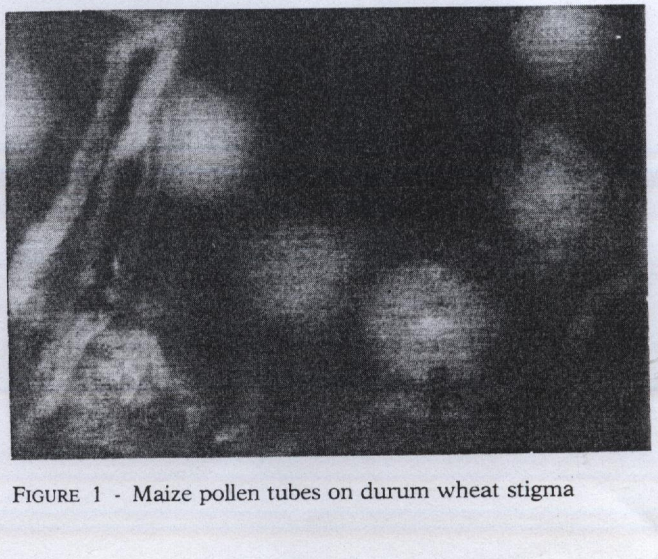

FIGURE 1 - Maize pollen tubes on durum wheat stigma

in all observed pollinated flowers. The first pollen tubes reached the style after 2 h and the ovule after 4 h (Fig. 1). In wheat genotypes such as cvs Acalou and Mondur, which showed high fruit-setting, pollen tubes were visible at the ovule level in all pollinated flowers. However, the aniline blue technique did not allow clear visualization of the fertilization event. Similar results were obtained in durum wheat by O'DONOUGHUE and BENNETT (1993).

Thin sections of ovules embedded 48 to 72 h after pollination showed first divisions of the zygote as well as of the surrounding endosperm cells (Fig. 2 and 3). Proembryos developed in some fruits and aborted in others (Fig. 4).

The next obvious event was the swelling of ovaries (Fig. 5), which developed typical fruit-like structures where endosperm was replaced by liquid tissue. Ten days after pollination the embryos were easily recovered and could develop green plants after growing on B5 medium. The haploid status of the regenerated small and sterile plants was confirmed by flow cytometry.

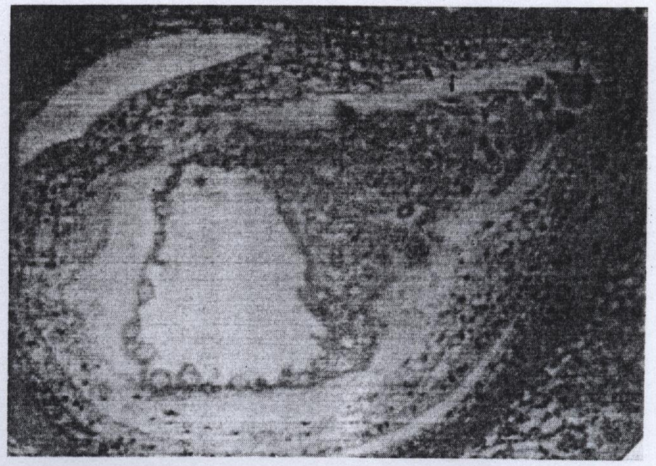

FIGURE 4 - Endosperm degeneration

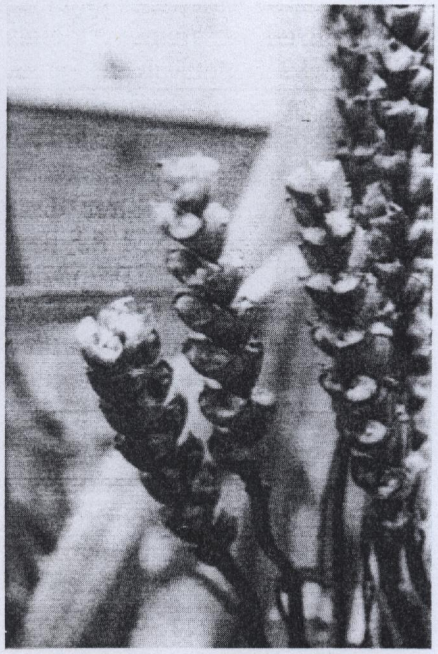

FIGURE 5 - Fruit-like structures in swollen ovaries

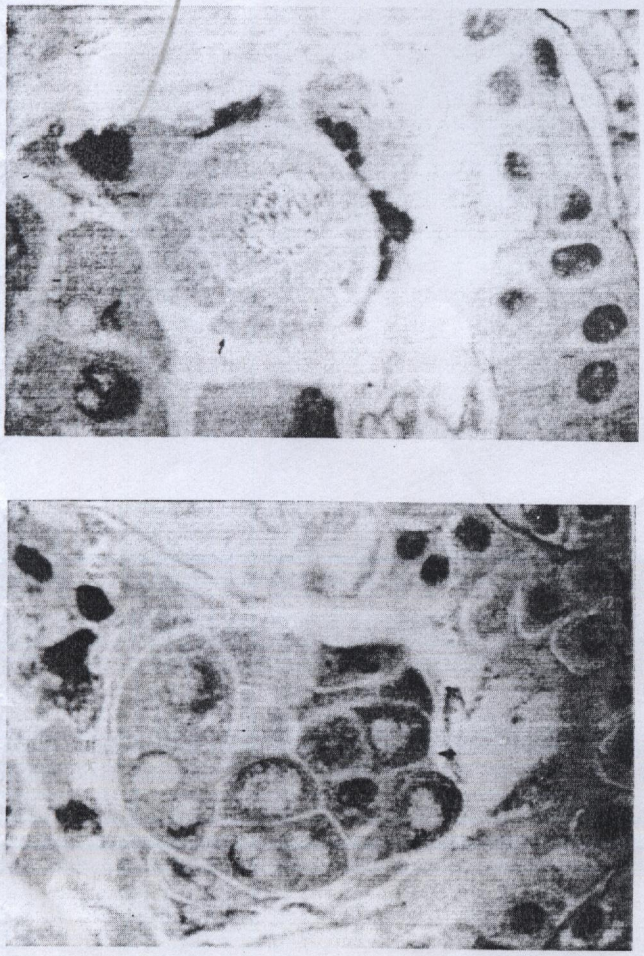

FIGURE 2 and 3 - Divisions of zygote and endosperm cells

Female parent effects

Ovary swelling and fruit-like structures did not occur in all pollinated spikes (Table 1). Spikes with reactive ovaries occurred less frequently in cvs Arcour and Ardente, and more frequently in cv. Primadur.

Pollinated flowers on fertile spikes did not always develop into swollen ovaries and fruit-like structures (Table 2). Cvs Acalou, Aristan, Armet and Mondur were found to be inferior for ovary swelling and fruit-like structures, whereas cvs Ardente, Lloyd and Primadur were shown to be superior.

TABLE 1

Occurrence of ovary swelling and fruit-like structures in durum wheat spikes

Female genotype	Number of spikes	Number of Spikes with ovary swelling and fruit-like structures	% Fruitful spikes	Probability[a]
Acalou	14	11	78.6	0.259
Ambral	25	16	64.0	1.000
Arcour	17	3	17.7	0.000
Ardente	21	6	28.6	0.001
Aristan	10	6	60.0	1.000
Armet	11	9	81.2	0.213
Lloyd	16	11	68.8	0.790
Mondur	24	19	62.5	1.000
Néodur	11	4	36.4	0.107
Primadur	45	37	82.2	0.002
Villemur	20	15	75.0	0.238
Mean			64.0	

[a] Fisher's exact test (2-tail)

The presence of embryos after dissection of fruit-like structures was checked for the different females (Table 3). Cvs Aristan, Primadur and Villemur yielded more embryos than cvs Arcour and Armet. Cvs Lloyd and Néodur did not develop any embryo.

A comparison of the different steps leading to embryo formation did not indicate any particular coherence except for cv. Primadur, which was significantly better for embryo occurrence on fruit

TABLE 2

Fruit-like structures (ovary swelling) in fertile spikes of durum wheat

Female genotype	Number of flowers on fertile spikes	Number of fruit-like structures	Fruits/100 flowers	χ^2	χ^2 Probability
Acalou	409	194	47.4	6.34	0.012
Ambral	577	306	53.0	0.03	0.852
Arcour	103	56	54.4	0.04	0.842
Ardente	195	122	62.6	6.84	0.009
Aristan	209	90	43.1	9.34	0.002
Armet	390	146	37.4	43.17	0.000
Lloyd	458	302	65.3	31.74	0.000
Mondur	789	392	49.7	5.15	0.023
Néodur	151	73	48.3	1.59	0.206
Primadur	1385	814	58.8	21.92	0.000
Villemur	529	279	52.7	0.10	0.749
Mean			53.4		

TABLE 3

Embryo occurrence on fruit-like structures in durum wheat genotypes

Female genotype	Number of fruit-like structures	Number of embryos	Embryos/ 100 fruits	Probability[a]
Acalou	194	15	7.7	0.226
Ambral	306	22	7.2	0.227
Arcour	56	1	1.8	0.026
Ardente	122	17	13.9	0.229
Aristan	90	18	20.0	0.008
Armet	146	8	5.5	0.004
Lloyd	302	0	0.0	0.000
Mondur	392	32	8.2	0.093
Néodur	73	0	0.0	0.000
Primadur	814	124	15.2	0.000
Villemur	279	57	20.4	0.000
Mean			10.6	

[a] Fisher's exact test (2-tail)

(Table 4). The case of cv. Lloyd with good ovary swelling and production of fruit-like structures without any embryo, is noticeable.

Pollen parent effects

No significant effect of the pollen parent upon the number of spikes with ovary swelling and fruit-like structures was observed (Table 5).

TABLE 4

Caracterization of durum wheat genotypes with respect to intergeneric crosses

Female genotype	Spike reaction	Ovary swelling and fruit-like structures on fertile Spike	Embryo occurrence
Acalou	n.s.	inferior	n.s.
Ambral	n.s.	n.s.	n.s.
Arcour	inferior	n.s.	inferior
Ardente	inferior	superior	n.s.
Aristan	n.s.	inferior	superior
Armet	n.s.	inferior	inferior
Lloyd	n.s.	superior	none
Mondur	n.s.	inferior	n.s.
Néodur	n.s.	n.s.	none
Primadur	superior	superior	superior
Villemur	n.s.	n.s.	superior

n.s. not significant: $p > 0.05$
superior/inferior: $p < 0.05$

TABLE 5

Occurrence of durum wheat spikes with ovary swelling and fruit-like structures in intergeneric crosses with different maize or teosinte pollen donors

Male genotype	Number of spikes	Number of Spikes with ovary swelling and fruit-like structures	% Fruitful spikes	χ^2	χ^2 Probability
CM 139	23	11	47.8	2.19	0.13
Kiwase	123	77	62.3	0.02	0.87
Liza	15	11	73.3	0.85	0.35
Teosinte	61	44	72.1	3.41	0.06
Zapalote	34	16	47.1	3.77	0.05

The proportion of ovary swelling and fruit-like structures in 100 flowers varied from 54.0 to 60.0 according to the male genotype (Table 6). Kiwase was found to be significantly inferior, and Zapalote significantly superior for this trait.

The proportion of embryo varied from 6.1 to 13.9 *per* 100 fruit-like structures, teosinte pollen being markedly superior for this trait (Table 7).

TABLE 6

Ovary swelling and fruit-like structures in flowers from on fertile spikes pollinated with different *Zea sp.* genotypes

Male genotype	Number of flowers on fruitful Spikes	Number of fruit-like structures	Fruit-like structures 100 flowers	χ^2	χ^2 Probability
CM 139	389	215	55.3	0.05	0.817
Kiwase	3071	1657	54.0	8.85	0.003
Liza	402	239	59.5	2.28	0.131
Teosinte	1694	966	57.0	1.34	0.246
Zapalote	537	325	60.0	5.24	0.023
Mean			55.8		

TABLE 7

Embryo occurrence in wheat fruits parent after pollination with different *Zea sp.* genotypes

Male genotype	Number of fruit-like structures	Number of embryos	Embryos/100 fruits	χ^2	χ^2 Probability
CM 139	215	13	6.1	3.13	0.07
Kiwase	1657	137	8.3	5.40	0.02
Liza	239	16	6.7	2.30	0.12
Teosinte	966	134	13.9	30.57	0.00
Zapalote	325	22	6.8	3.04	0.08
Mean			9.5		

Comparison of these results with those obtained in early experiments involving a range of durum genotypes pollinated with maize (Table 8), indicates some agreement for fertility as determined by frequency of developed ovaries or fruit-like structures. However, the relatively low embryo occurrence in our material as compared with those of O'Donoughue and Bennett (1994) and Riera-Lizarazu *et al.* (1992), could be due to the excessive temperature (25° C) in the growth chamber.

The striking result was the relative high frequency of embryo in spikes pollinated with Teosinte as previously observed by Ushiyama (1991) in *T. aestivum* female parent. Kiwase pollen parent was found to be somewhat inferior for ovary swelling and fruit-like structures.

The influence of genotype on the different steps leading to embryo formation was marked in our material, particularly with the female cv. Lloyd, which was good in ovary swelling and fruit-like structures and poor in embryo formation. Surprisingly, the teosinte pollen showed a strong effect on embryo occurrence but not on spike fertility, ovary swelling and development of fruit-like structures.

TABLE 8

Range of fertility and embryo occurrence in different durum wheat parents pollinated with *Zea sp.* in five experiments

Reference	Number of female durum parent lines	Fruit-like structures/100 pollinated flowers on fertile spikes: minimum	Fruit-like structures/100 pollinated flowers on fertile spikes: maximum	Embryos/100 pollinated flowers on fertile spikes: minimum	Embryos/100 pollinated flowers on fertile spikes: maximum
Amrani *et al.*, 1993	11	28	60	2	12
O'Donoughue & Bennett, 1994	10	43	67	30	62
Riera-Lizarazu *et al.*, 1992	5	—	—	12	22
Sarrafi *et al.*, 1994	6	74	77	2	14
Present results	11	37	65	0	11

The relationship between the presence of the pollen tube at the ovule micropyle and the induction of zygotic divisions is currently being investigated.

ACKNOWLEDGEMENTS

We are grateful to CIRAD-BIOTROP laboratory for histocytological preparations.

REFERENCES

AHMAD, F.and COMEAU, A., 1990. Wheat x pearl millet hybridization: consequence and potential *Euphytica*, **50:** 181-190.

ALEXANDER, M.P., 1969. *Stain Technol.*, **44:** 117-122.

AMRANI, N., SARRAFI, A. and ALIBERT, G., 1993. Genetic variability for haploid produciton in crosses between tetraploid and hexaploid wheats with maize. *Plant Breeding*, **110:** 123-128.

COUMANS, M.P., BOUTOUCHENT, F., DUSAUTOIR J.C. and KAAN, F., 1992. Obtention d'embryons par croisements interspécifiques entre le blé dur et d'autres céréales. In: P. MONNEVEUX and M. BEN SALEM (eds) *Colloque INRA N° 64: Tolérance à la sécheresse des céréales en zone méditerranéenne, diversité génétique et amélioration variétale.* INRA Paris pp. 375-381.

CHUANG YING CHAO, 1979. Histochemical study of a PAS substance in the ovules of Paspalum orbiculare and Paspalum longifolium. *Phytomorphology*, **4:** 381-387.

DE BUYSER J. and HENRY, Y., 1987. Utilisation des haploïdes doublés en sélection. *Bull. Soc. Bot. France Actual. Bot.*, **133:** 51-57.

GAMBORG, O.L., MILLER, L.A. and OJIMA K., 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.*, **50:** 151-158.

GERRITS, P.O. and SMID, L., 1983. A new, less toxic polymerization system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. *J. of Microscopy*, **132:** 81-85.

GEVES, 1993. *Bulletin des variétés. Céréales à Paille.* GEVES Guyancourt.

HADWIGER, M.A. and HEBERLE-BORS, E., 1986. Pollen plant production in *Triticum turgidum* L *ssp durum*. In: *Proc. Int. Symp. on Nucl. Techn. and in vitro Cult. for Plant Improvement.* SM 282/17P IAEA Vienna, pp. 213-220.

HESLOP-HARRISON, J., HESLOP-HARRISON, Y., 1970. *Stain Technology*, **45:** 115-120.

KASHA, K.J. and KAO, K.N., 1970. High frequency haploid production in barley (*Hordeum vulgare* L.). *Nature*, **225:** 874-876.

KATO, K., TOMO, S., YAMAZAKI S. and HAYASHI, K., 1990. Simplified culture method of detached ears and its application to vernalization in wheat. *Euphytica*, **49:** 161-168.

KOBA, T., HANDA T. and SHAMADA, T., 1991. Efficient production of wheat-barley hybrids and preferential elimination of barley chromosomes. *Theor. Appl. Genet.*, **81:** 285-292.

LAURIE, D.A. and BENNETT, M.D., 1986. Wheat x maize hybridization. *Can. J Genet. Cytol.*, **28:** 313-316.

LAURIE, D.A. and BENNETT, M.D., 1988. The production of haploid wheat plants from wheat x maize crosses. *Theor. Appl. Genet.*, **76:** 393-397.

MARTOJA, R. and MARTOJA, M., 1967. *Initiation aux techniques de l'histologie animale.* Masson eds, Paris.

NEUHAUS, G., SPANGENBERG, G., MITTELSTEN SCHEID O. and SCHWEIGER, H.G., 1987. Transgenic rapessed plants obtained by the microinjection of DNA into microscope-derived embryoids. *Theor. Appl. Genet.*, **75:** 30-36.

O'DONOUGHUE, L.S., 1990. Chromosome behaviour and reproductive physiology in cereal wide crosses. *Ph. D.* Dissertation, University of Cambridge, Downing College, July 1990.

O'DONOUGHUE, L.S. and BENNETT, M.D., 1988. Wide hybridization between relatives of bread wheat and maize. *VIIth Intern Wheat Genet. Symp.*. IPSR. Cambridge. pp. 397-402.

O'DONOUGHUE, L.S. and BENNETT, M.D., 1994. Comparative responses of tetraploid wheats pollinated with *Zea mays* L and *Hordeum bulbosum* L. *Theor. Appl. Gen.*, **88:** 673-680.

OHKAWA, Y., SUENAGA K. and OGAWA, T., 1992. Production of haploid wheat plants through pollination of sorghum pollen. *Japan J. Breed*, **42:** 891-894.

PICARD, E. and DE BUYSER,J., 1977. High production of embryoids in anther culture of pollent derived homozygous spring wheats. *Ann. Amélior. Plantes*, **27:** 483-488.

RIERA-LIZARAZU O., MUJEEB-KAZI, A. and WILLIAM M.D.H.M., 1992. Maize (*Zea mays* L) mediated polyhaploid production in some Triticeae using a detached tiller method. *J. Genet. & Breed*, **46:** 335-346.

SARRAFI, A., AMRANI, N. and ALIBERT, G., 1994. Genetic analysis of embryo formation and haploid production in crosses between tetraploid wheats with maize. *Genome*, **37:** 176-178.

SHARMA, G.C., WANG, W.C. and SAPRA, V.T., 1982. Effect of genotype, media and temperature pretreatment on callus initiation in triticale, wheat, and rye anther cultures. *Cereal Res. Commun.*, **10:** 143-150.

SITCH, L.A. and SNAPE J.W., 1986. Doubled haploid production in winter wheat and triticale genotypes using the Hordeum bulbosum system. *Euphytica*, **35:** 1045-1051.

SITCH, L.A. and SNAPE J.W., 1987a. Factors affecting haploid production in wheat using the Hordeum bulbosum system. 1 Genotypic and environmental effects on pollen grain germination, pollen tube growth and frequency of fertilization. *Euphytica*, **36:** 483-496.

SITCH, L.A. and SNAPE J.W., 1987b. Factors affectin haploid production in wheat using the *Hordeum bulbosum* system. 3) Post fertilization effects on embryo survival. *Euphytica*, **36:** 763-776.

SUENAGA, K. and NAKAJIMA, K., 1989. Efficient production of haploid wheat (*Triticum aestivum*) through crosses between Japanese wheat and maize (*Zea mays*). *Plant Cell Reports*, **8:** 263-266.

SWANSON, E.B., COUMANS, M.C., BROWN, G.L., PATEL, J.D. and BEVERSDORF, W.D., 1988. The caracterization of herbicide tolerant plants in *Brassica napus* after in vitro selection of microspores and protoplasts. *Plant Cell Reports*, **7:** 83-87.

USHIYAMA, T., SHIMIZU, T. and KUBAWARA, T., 1991. High frequency of haploid production of wheat through intergeneric cross with teosinte. *Japan J. Breed*, **41:** 353-357.

YANG, H.Y. and ZHOU, C., 1982. In vitro induction of haploid plant from unpollinated ovaries and ovules. *Theor. Appl. Genet.*, **63:** 97-104.

ZHU, Z. and LIU, A., 1981. Introduction of haploid plant from unpollinated ovaries of *Triticum aestivum* culture *in vitro*. *Acta Genetica Sinica*, **8:** 586-594.

J. Genet. & Breed. 49: 359-364 (1995)

Influence of genotype on the induction of haploidy in *Cucumis melo* L. by using irradiated pollen

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Received July 20, 1995

ABSTRACT

The influence of gamma ray doses (0.6, 1.2 and 2.4 KGy) and of the genotype on the induction of haploidy by irradiated pollen was studied in three genotypes of *Cucumis melo* var. *reticulatus* and two of var. *inodorus* employed as both male or female parents in ten cross-combinations. Haploid plants of melon were obtained at 0.6 and 1.2 KGy, haploid production being strongly influenced by the genotype and the dose used. Among the cross-combinations, the highest frequency of haploids was obtained when inbred line L. 7-82 and cv. Isabelle were employed as female and male parents, respectively. Pollen irradiated at 0.6 KGy showed the highest frequency of parthenogenetical response. Thirty-seven haploid and 74 diploid embryos were extracted from 4-week-old fruits and cultured *in vitro*. All the plants obtained exhibited normal phenotype.

Key words: ^{60}Co γ-rays, melon, parthenogenesis.

INTRODUCTION

The establishment of an efficient system to produce haploids *in vitro* represents a real tool for breeding progress in melon (*Cucumis melo* L.) because spontaneous haploidy occurs rarely in this species (Savin *et al.*, 1988). The first haploids, at a very low production rate, were obtained by Dumas de Vaulx (1979) through interspecific crosses between *C. melo* (2n) and *C. ficifolius* (4n), whereas attempts to produce haploids by *in vitro* androgenesis and gynogenesis were unsuccessful (Dryanovska and Ilieva, 1983, 1985). Few authors obtained gynogenic embryos through parthenogenesis induced *in situ* by pollen denatured with high doses of gamma rays (Sauton and Dumas de Vaulx, 1987; Cuny *et al.*, 1993; Ficcadenti *et al.*, 1993). This technique proved to be effective for haploid production but the embryo yield was found to be highly influenced by different factors such as genotype, environmental conditions and dose of gamma-rays utilized (Sauton, 1988; Cuny *et al.*, 1991; 1993).

In order to evaluate the response in haploid production in relation to the doses of gamma rays, in the present work, the irradiation pollen technique was applied to genotypes of *Cucumis melo* L. belonging to *reticulatus* or *inodorus* botanical varieties.

MATERIALS AND METHODS

Plant material

Cvs Isabelle and Vedrantais and inbred line L. 7-82 of *C. melo* var. *reticulatus* as well as cvs Purceddu and Giallino di Paceco of *C. melo* var. *inodorus* were used. Plants were grown in open field from April to August 1993. Ten plants of each genotype were employed as either male or female parent in a cross-combination scheme. The average temperature was 13.7 °C at night and 27.4 °C during the day.

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