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Wide hybridization experiments in cereals

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Summary. Wide hybridization is a useful tool in plant breeding, but little is known about its possible range. For the cereals, wheat, barley and rye, this was tested with 15 different species of the *Poaceae* and *Panicoideae*. Embryo formation could be obtained with *Agropyron repens, Alopecurus agrestis, Dactylis glomerata, Festuca glauca, Hordeum bulbosum, Lolium perenne, Pennisetum americanum,* and *Zea mays.* As well, haploid as diploid embryos occurred. New embryo culture techniques should enable these embryos to grow to plants.

Key words: Intergeneric hybrids – Cereals – Grasses – Embryo formation

Introduction

Wide hybridization is considered to be a useful tool in plant breeding for creating new species, gene transfer or induction of haploids. In vitro techniques, mainly embryo culture methods provide new opportunities for realizing such work.

In cereals the most important new species is *Triticale,* first described in 1875 by Wilson as a sterile hybrid and produced as a fertile one by Rimpau in 1888 (Müntzing 1979). Gene transfer to wheat from related species *Aegilops* and *Agropyron* has been done intensively by Sears, Riley and Wienhues-Ohlendorf (Röbbelen and Sharp 1978). The induction of haploids via hybridization is common in barley by crossing *Hordeum vulgare* × *H. bulbosum,* a system which was discovered by Symko in 1969 (Nitzsche und Wenzel 1977).

Problems in hybridization work result mainly from genotype. In wheat the function of special crossability genes Kr_1 and Kr_2 are known (Lein 1943). Similar genes occur in barley (Elbern 1981). When open pollination species are used as pollinators, the results are usually better than in reciprocal crosses (Elbern 1981). After crossing self-incompatible species (SI) and self-compatible species (SC) the barriers to crossing are stronger in the direction SI × SC than vice versa (Matzk 1980).

Pollen tube development is said to be reduced in interspecific and intergeneric hybridization. To overcome this handicap a large variety of chemicals are successfully used, e.g. α-amino caproic acid, chloramphenicol, acriflavine, salicylic acid, or gentisic acid (Bates and Deyoe 1973; Bates et al. 1974). Similar experiments, however, of other authors have failed (Elbern 1981).

Generally, no prognosis can be made on which combination will result in certain pre-determined consequences. Therefore, a very large number of intergeneric combinations between cereals and very different gramineae have to be tested. In our experiment, barley, rye and wheat were used as mother plants and, as pollinators, grasses were selected with different characteristics: low and high chromosome number, good pollen producers and apomicts, both closely and distantly related species. As a measure of the results, endosperm and embryo formation were investigated.

Material and methods

Three cultivars and various lines of rye (*Secale cereale* L.), 44 cultivars of wheat (*Triticum aestivum* L.), and two cultivars and several lines of barley (*Hordeum vulgare* L.) were used as the female parents. The males consisted of 15 different species of *Gramineae* (Table 1). Most of the plants were grown in the greenhouse, and only a few females and endemic males were grown outside.

Three to five days after emasculation the stigmas were pollinated with freshly harvested pollen. Pollen germination and pollen tube growth were performed as described by D'Souza (1972). Fertilization and embryo development were investigated by microtome sectioning. Pistils were fixed in 1:3 acetic acid:ethanol at various times after pollination and transferred via ethanol and xylene into paraplast. Longitudinal, dorsiventral 10–15 µm thick sections were stained with crystal violet and orange G counter stain.

Twohundred and thirty-six enlarged ovules of rye, 181 of barley and 122 of wheat were analysed.

Results

The realized combinations and the results are summarized in Table 1.

It appeared that pollen germination on the stigmas depended more on the male donors then on the female receptors. However, other factors independent of species or sex influenced the germination ability and therefore no conclusions could be drawn form these results.

Grouping and number of the realized pollinations and of investigated pistils are not orthogonal, therefore results are presented without statistics. Pollen tubes were found in the embryo sacs of many different combinations and their remnants occurred at a considerably high frequency (Figs. 1 A, B and 2 F). Pollen tubes were detected in the ovules of *Secale cereale* × *Dactylis glomerata*, *S. cereale* × *Festuca glauca*, *S. cereale* × *Zea mays*, *S. cereale* × *Hordeum bulbosum*, *Hordeum vulgare* × *Alopecurus agrestis*, and *Triticum aestivum* × *Zea mays*.

The fertilization process (fusion of sperm and egg or embryo sac nuclei) was not visualized as the pistils were prepared and fixed at least 24 h after pollination, a time too late to observe the fusion process. Single or double fertilization was presumed to occur since micronuclei or aneuploid chromosome numbers could be observed in embryos or the endosperm, and also in haploid embryos and diploid endosperm (Figs. 1 C, E).

Embryos were present in the embryo sacs of *S. cereale* pollinated with: *Dactylis glomerata* (Fig. 1 E), *Festuca glauca* (Fig. 1 F), *Hordeum bulbosum* (Figs. 1 C, D), and *Zea mays* (Figs. 1 A, B); *Hordeum vulgare* pollinated with *Agropyron repens* (Fig. 2 E), *Alopecurus agrestis* (Fig. 2 A), *Lolium perenne* (Figs. 2 B, D), and *Pennisetum americanum* (Fig. 2 C); and *Triticum aestivum* pollinated with *Alopecurus agrestis*, *Dactylis glomerata* (Fig. 2 F) and *Zea mays*. In all these cases globular embryos were formed, however, six to ten days after pollination they degenerated. Endosperm was very poorly, if at all, developed. In crosses of *Secale cereale* with *Hordeum bulbosum* or *Zea mays* endosperm development often appeared normal (Fig. 1 C), sometimes without embryos. In all cases, however, the endosperm development collapsed before the tenth day after pollination. In *Hordeum* and *Triticum* the endosperm development occurred rarely, the only exception was the hybrid *Hordeum vulgare* × *Agropyron repens*.

Table 1. Number of pollinated ears of *Hordeum vulgare*, *Secale cereale* and *Triticum aestivum* with different species of *Gramineae*

Male species	2n	Female species		
		Hordeum vulgare	*Secale cereale*	*Triticum aestivum*
Agropyron repens	28	17/+	0	0
Agropyron scabrum[a]	42	0	5/−	9/−
Alopecurus agrestis	14	78/+	0	1/−
Alopecurus pratensis	42	0	17/−	33/+
Avena sempervirens	14	4/−	14/−	4/−
Avena sterilis	42	0	0	25/−
Dactylis glomerata	28	4/−	11/+	26/+
Festuca glauca	28	0	2/+	2/−
Hordeum bulbosum	14	0	122/+	48/−
Lolium perenne	14	76/+	9/−	17/−
Pennisetum americanum	28	45/+	22/−	0
Phalaris arundinacea	28	0	0	5/−
Phleum pratense	42	0	0	27/−
Zea mays	20	34/−	62/+	52/+
Zingeria biebersteiniana	4	4/−	3/−	19/−
Total		262	267	268

+ = development of globular embryos
− = globular embryos not observed
[a] apomictic

Discussion

In the present survey some unexpected results were obtained. All the cereals used belong to the tribes *Hordeae*, and successful hybridization could be expected with other species of this tribe. These are *Agropyron repens*, *A. scabrum* and *Hordeum bulbosum*. Two of the species gave rise to embryos, while *A. scabrum* did not. This may dependent on the fact that this species is apomictic and a very bad pollen producer. Pollen fertility determined in Lugol's solution was only about 30% and hybridization experiments of other authors with this species also failed (Matzk 1982). In the present survey, however, the negative results could also depend on the low number of pollinated ears.

From the other species of *Poaceae*, embryo formation could be obtained with *Alopecurus agrestis*, *Dactylis glomerata*, *Festuca glauca* and *Lolium perenne*. While the latter three belong to the tribe *Pooideae*, which is closely related to the *Hordeae*, *Alopecurus* belongs to the more distantly related *Agrosteae*. However, *Alopecurus agrestis* gives excellent pollen germination in vitro, often nearly 100%, in contrast to most other grass species (Nitzsche, unpublished). There may be a correlation between pollen germination in vitro and behavior in interspecific hybridization.

Of all the species mentioned, the majority are in the subfamily *Poaceae* whereas only two pollinators, *Pennisetum americanum* and *Zea mays*, belong to the subfamily *Panicoideae*. Nevertheless, successful embryo

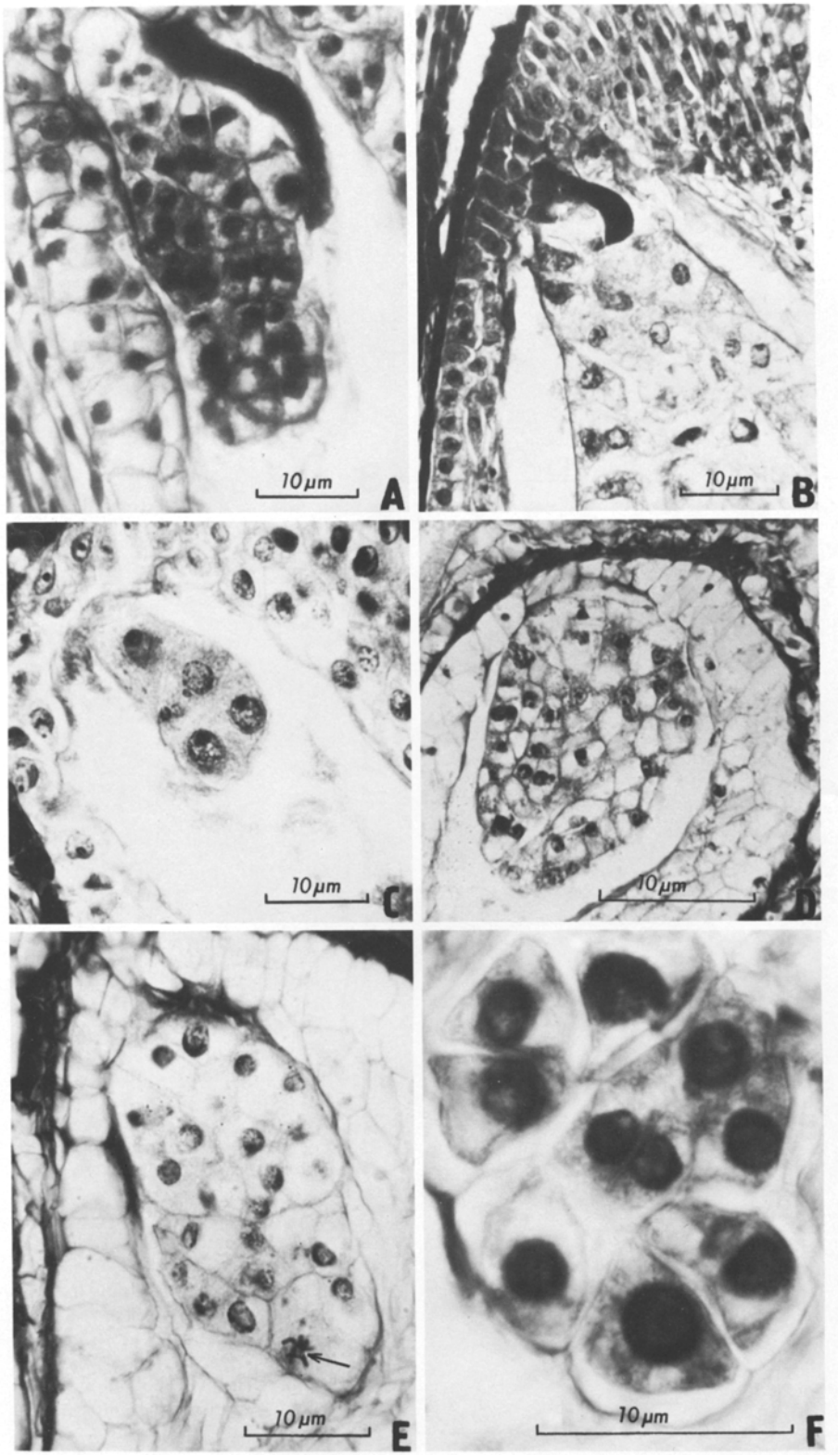

Fig. 1A–F. *Secale cereale* cv. 'Kustro' × *Zea mays.* **A** Globular embryo and pollen tube inside the embryo sac, 8 days after pollination (d.a.p.); **B** Pollen tube and endosperm inside the embryo sac, 6 d.a.p.; **C–D** *S. cereale* cv. 'Kustro' × *Hordeum bulbosum;* **C** Globular embryo inside the embryo sac, 3 d.a.p.; **D** Degenerating globular embryo inside the embryo sac, 9 d.a.p.; **E** *S. cereale* (line 152) × *Dactylis glomerata;* globular embryo inside the embryo sac, 3 d.a.p., in one cell (*arrow*) a haploid number ($n=7$) of chromosomes; **F** *S. cereale* cv. 'Kustro' × *Festuca glauca;* globular embryo 9 d.a.p.

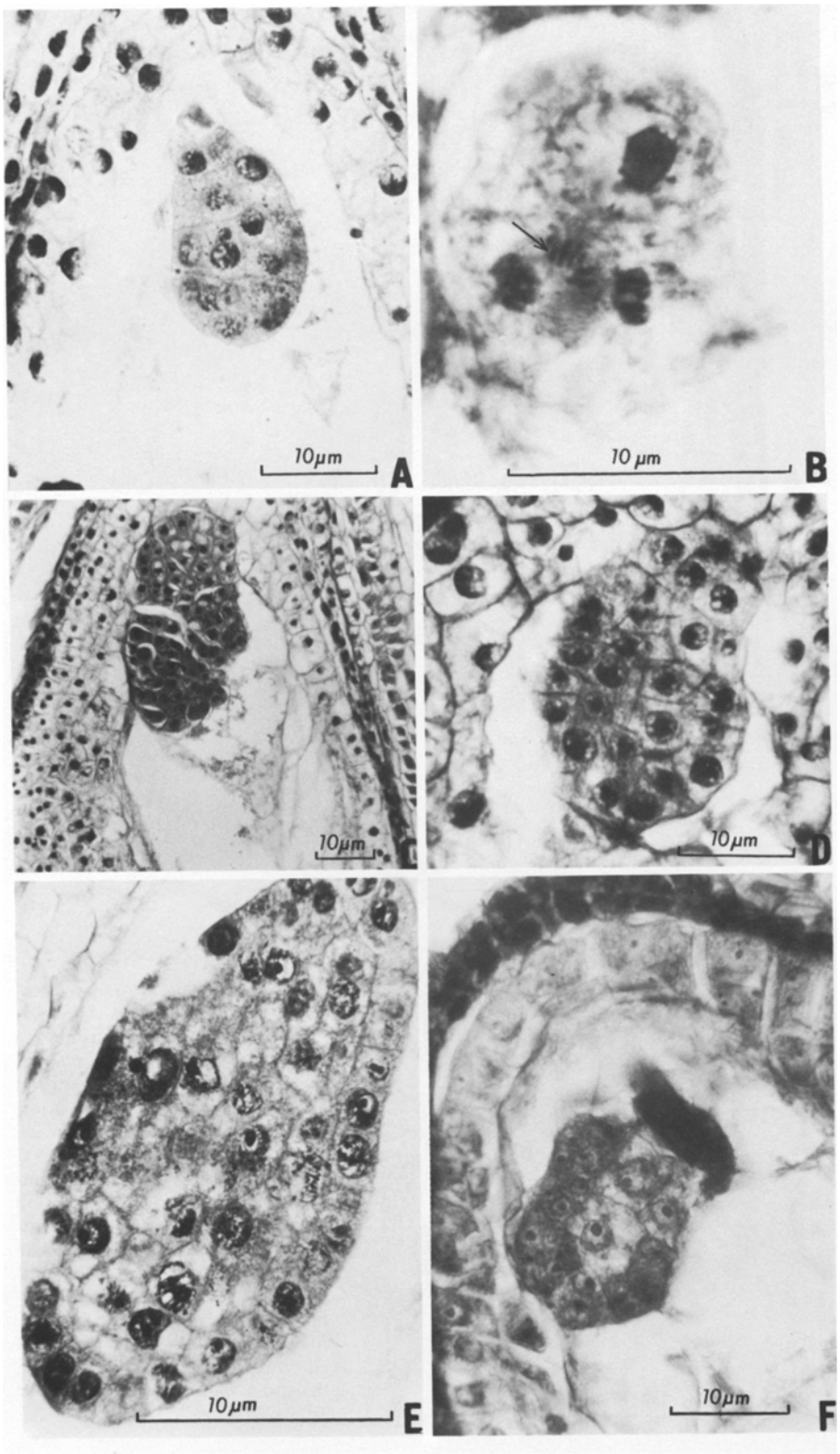

Fig. 2. A *Hordeum vulgare* (line MS 6283 b) × *Alopecurus agrestis;* globular embryo inside the embryo sac, 4 d.a.p.; **B** *H. vulgare* (line MS 6283 b) × *Lolium perenne;* mitosis (7 chromosomes-*arrow*) in globular embryo situated inside the embryo sac 3 d.a.p.; **C** *H. vulgare* (line MS 6283 b) × *Pennisetum americanum,* globular embryo inside the embryo sac, 10 d.a.p.; **D** *H. vulgare* (line 119) × *Lolium perenne;* globular embryo inside the embryo sac, 6 d.a.p.; **E** *H. vulgare* (line MS 7683 b) × *Agropyron repens;* globular embryo inside the embryo sac, 13 d.a.p.; **F** *Triticum aestivum* cv. 'Kobold' × *Dactylis glomerata;* globular embryo and remnants of pollen tube inside the embryo sac, 6 d.a.p.

development also could be obtained. This implies that very wide hybridization may be possible.

On the female side, embryo formation was more frequently observed in *Hordeum* and *Secale* than in *Triticum*. This could have been determined by genotype, in particular, by the crossability genes, as previously mentioned. But it may also be dependent on the inorthogonal hybridization pattern, which resulted from practical reasons.

From all the investigated crosses not one seems to fulfill the conditions which must be kept for haploid production. Independent of the detected haploid cells, the frequency of haploid embryo formation is too low for practical application.

On the other hand, the formation of hybrid embryos offers new possibilities for intergeneric hybridization and gene transfer. Problems surely exist in embryo culture techniques for extremely young embryos, but this problem should be able to be overcome in the near future.

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