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Production of Trigeneric (Barley × Wheat) × Rye Hybrids*

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Summary. Rye (*Secale cereale* cv. Prolific 2n = 14 and 2n = 14 + 2B was crossed onto hybrids between barley (*Hordeum vulgare* 2n = 14) and wheat (*Triticum aestivum* 2n = 42). Pollinated florets were injected with GA_3 to promote fertilization and hybrid embryo development. At 16 days after pollination the watery caryopses were removed, embryos dissected and cultured on a modified B_5 medium. Approximately 20% of the cultured embryos produced both roots and coleoptile and developed into viable seedlings. Viable seeds were also obtained at a low frequency from the same cross combinations. The hybrids were wheat-like except for the hairy neck characteristic of rye. There were 35 chromosomes in somatic tissue; 21 wheat, 7 barley and 7 rye. The rye chromosomes were distinguishable by their larger size and terminal C-bands. A lower seed set was obtained using pollen from rye plants with 2n = 14 + 2B chromosomes than from plants without B chromosomes.

Key words: Intergeneric hybrids – Embryo cultures – Giemsa banding – Crossability – B chromosomes

Introduction

Considerable progress has been made in the last few years in intercrossing cereal genera. Viable hybrids between barley and wheat were first reported by Kruse (1973) from crossing *Hordeum vulgare* L. with *Triticum aestivum* L., *Triticum dicoccum* Shrank, and *Triticum monococcum* L. This was followed by reports of hybrids between Betzes barley and Chinese Spring wheat (Islam et al. 1975; Fedak 1977b; Cauderon et al. 1978) and between barley with tetraploid wheats (Mujeeb et al. 1978). In all cases a low frequency of hybrids was obtained only when pollination was followed by application of GA_3 to pollinated florets and followed by culturing of immature embryos on an artificial medium.

Initial reports of pollination of barley × rye showed that a large proportion of ovules became fertilized but endosperm development failed and embryo growth ceased after 5 days (Quinke 1940). The treatment of pollinated barley florets with GA_3 promoted embryo development (Kruse 1967) and postponed degeneration. A small proportion of the embryos when excised and cultured on an artificial medium have developed into hybrid plants (Kruse 1967; Cooper et al. 1978; Fedak 1979a) or haploids of barley (Kruse 1967; Fedak 1977b).

Trigeneric hybrids between *Hordeum, Triticum* and *Secale* were obtained by crossing Prolific rye onto (Betzes × Chinese Spring) hybrids (Fedak 1978, 1979a). *Secale cereale* L. was also crossed onto a *Triticum timopheevi* Zhuk. × *Hordeum bogdanii* Wil. to give a hybrid combining different species from the same three genera (Kimber and Sallee 1979). This report describes the production of trigeneric hybrids by pollinating a number of barley × wheat hybrids with rye and reveals cultivar differences in cross compatibility.

Material and Methods

All of the barley × wheat hybrids employed in this study were produced by the same procedures as used to produce Betzes (*H. vulgare* L.) × Chinese Spring (*T. aestivum* L.) hybrids in an earlier study (Fedak 1977b). In addition to the Betzes × Chinese Spring hybrid, other barley × wheat hybrids were obtained from *Hordeum* cultivars Emir, Bonus and Betzes *des 8k*, a desynaptic mutant crossed with *T. aestivum* cultivars Chinese Spring and Koga as listed in Table 1. The cultivars of *S. cereale* employed in this study were Prolific (a spring rye with and without B chromosomes), Sangaste and Puma which are winter types and the species *Secale vavilovii*.

The parental materials, barley × wheat hybrids, and the rye cultivars were grown in 5 inch pots containing a soil-proroot mix.

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The growth room was maintained at day/night temperatures of 20/15°C, a relative humidity of 70%, a 16 hr photoperiod and average illumination of 800 microeinsteins m^{-2}sec^{-1} provided by a combination of fluorescent and incandescent lamps.

All of the barley × wheat hybrid plants were male sterile so emasculation was not necessary. To facilitate pollination, the outer glumes, lemmas and paleas of the maternal plants were clipped at the time when the stigma was receptive. Pollen was collected from the rye parents into petri dishes and immediately applied to the clipped florets with a fine brush. At 24 and 48 hours following pollination, GA$_3$ at a concentration of 75 ppm was applied to each floret with a hypodermic syringe. A second method of pollination involved leaving the florets intact but brushing the pollen onto extruded stigmas. This was followed by application of GA$_3$ to intact spikes till run-off by means of an atomizer.

At 16 days following pollination, differences in caryopsis development were noted. Those that had a firmly filled endosperm were allowed to continue their development to eventually form mature seeds. Those caryopses with a watery consistency were removed, the embryos excised and cultured on a modified B$_5$ medium (Fedak 1977b). The culture vials were placed in darkened containers in an incubator maintained at 25°C and 90% R.H. After root and coleoptile growth was initiated, the vials were removed from darkened containers and placed under a weak fluorescent light (5 microinsteins m^{-2}sec^{-1}). Regenerated plantlets showing good root and shoot development were transplanted to a soil-peat-moss mix and transferred to the growth chamber.

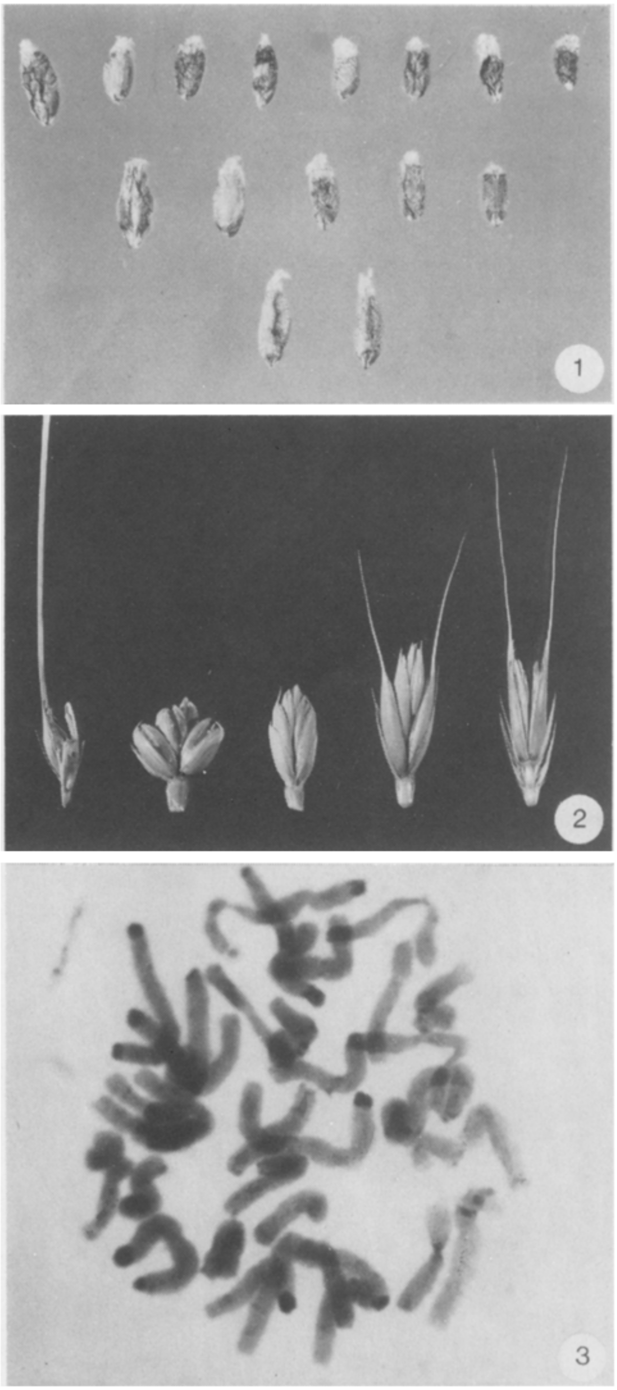
For Giemsa C-banding the fixed roots were washed in distilled water and then softened in an enzyme solution (% Driselase + 0.1% Cellulase 'Onozuka' w/v in distilled water and brought to pH 5-5.5 with 0.1N HCl) for 3 minutes. The root tips were squashed in aceto-carmine and the coverslip removed by the dry ice method. The slides were then air-dired for 2 hours, dehydrated in absolute ethanol for 2 hours and then stored in a dessicator until used within one or two days. The subsequent steps in the procedure were essentially the same as Jones (1978) except that demineralized water was used in place of tap water.

Results

The caryopses that developed on clipped florets had turned to a brown color by the 20th day following pollination. They were plump liquid-filled, and contained embryos that varied in length from 0.5 to 1.8 mm. The morphology of the embryos were often distorted. The development of these embryos on the modified B$_5$ medium was variable but generally very poor. Some merely proliferated into a mass of callus, others produced only roots or shoots. Those embryos that produced simultaneous root and shoot growth survived to produce viable seedlings.

A sample of the seeds obtained from crossing Prolific rye onto barley × wheat hybrids is shown in Figure 1. There was considerable variation among seeds for size and morphology. Some variants were more wheat-like and others more rye-like in phenotype. Most of the seeds were plump and well filled but some were badly shrivelled and inviable when mature. Many of the plump seeds did not germinate. Nevertheless the majority of the trigeneric hybrids obtained in these studies were derived by means of seeds rather than embryos.

The frequency with which seeds were obtained from barley × wheat hybrids when pollinated with Prolific rye is shown in Table 1. When Prolific 2n = 14 was used as the



Figs. 1-3. 1 Seeds obtained from pollinating (Betzes × C.S.) × Prolific 2n = 14; 2 Spikelet structure of Betzes, Chinese Spring, Betzes × C.S. hybrid, (Betzes × C.S.) × Profilic hybrid, Profilic rye; 3 Somatic chromosomes of (Betzes × C.S.) × Prolific hybrid. Terminal bands identify the rye chromosomes

Table 1. Frequencies of seeds obtained from pollinating six barley X wheat hybrids with Prolific rye, with and without B chromosomes

Barley-wheat parent	Rye parent	No. of spikes pollinated	No. of florets pollinated	No. of seeds obtained	Seeds as % of pollinated florets
Emir X Chinese Spring	Prolific $2\underline{n} = 14$	36	2392	96	4.0
	" $2\underline{n} = 14 + 2B$	4	288	7	2.4
Betzes X Chinese Spring	Prolific $2\underline{n} = 14$	48	4048	59	1.5
	" $2\underline{n} = 14 + 2B$	53	4040	17	0.42
Betzes **des 8k** X Chinese Spring	Prolific $2\underline{n} = 14$	18	1488	11	0.74
	" $2\underline{n} = 14 + 2B$	12	949	9	0.95
Betzes X Koga	Prolific $2\underline{n} = 14$	8	568	–	–
Bonus X Koga	Prolific $2\underline{n} = 14$	7	528	–	–
Bonus X Chinese Spring	Prolific $2\underline{n} = 14$	3	160	–	–
Overall Totals		189	14461	199	1.36
Total with Prolific $2\underline{n} = 14$		120	9184	166	1.81
Total with Prolific $2\underline{n} = 14 + 2B$		69	5277	33	0.63

pollen source the Emir x Chinese Spring (C.S.) combination gave the highest seed set of 4.0% followed by the Betzes x C.S. and Betzes *des 8k* x C.S. at frequencies of 1.5 and 0.74%, respectively. No seed was obtained on the Betzes x Koga, Bonus x Koga and Bonus x C.S. combinations. The failure of the latter crosses may be attributed to the small number of florets pollinated and/or a low crossability. Although not shown in Table 1, other rye cultivars such as Sangaste and Puma and *S. vavilovii* failed to induce any seed on any of the barley-wheat hybrids.

It has been demonstrated that rye pollen having two B chromosomes shows a higher rate of germination and growth in vitro (Puertas and Carmona 1976) as well as in vivo (unpublished) than pollen from 2n = 14 plants. In the present study, however, pollen from rye plants with 2B chromosomes was not as effective in fertilizing barley x wheat hybrids as pollen from 2n = 14 plants. The Emir x C.S. and Betzes x C.S. hybrids when pollinated with 2B pollen yielded seed at one-half the frequency realized from using 2n = 14 rye pollen. The relative seed set induced by the two types of pollen in the Betzes *des 8k* x C.S. hybrid seems to be an exception to the above, but smaller numbers of florets were pollinated in this combination and the differences were not meaningful. A comparison of the effectiveness of the two types of rye pollen in inducing seed set in three combinations of barley-wheat hybrids showed that 14 chromosome pollen induced seed set at a frequency of 1.81% of pollinated florets while only 0.63% was realized from using 2B pollen.

The trigeneric hybrids showed vigorous vegetative growth. The anthers appeared from normal to somewhat shrunken and spikes were completely self-sterile. Wheat characteristics predominated in spike morphology. A hairy neck was indicative of the presence of the rye genome. Spikelet structure was also predominantly wheat-like containing three florets (Fig. 2). Slightly awnletted lemmas on the florets were possible indicators of the presence of alien chromatin.

Most of the hybrid plants had a somatic chromosome number of 35, consisting of 21 wheat, 7 barley and 7 rye chromosomes. Trigeneric hybrids with chromosome numbers of 36, 37 and 38 were also obtained. The barley and wheat chromosomes could not be distinguished because of similarities in length, but the rye chromosomes could be distinguished in most somatic preparations on the basis of greater length. The identity of the rye chromosomes was verified by a Giemsa C-banding procedure that revealed the terminal bands (Fig. 3).

Discussion

This study demonstrates that unpredictable differences exist in the crossability of cultivars from different genera. For example, the original barley-wheat hybrids were obtained at different frequencies. The Betzes x C.S. combination was produced more readily than the Emir x C.S. combination at relative frequencies of pollinated florets of 1.41% and 0.60%, respectively (unpublished). However, when the two hybrids were pollinated by Prolific rye the frequency of trigeneric hybrids obtained from the Emir x C.S. combination was twice as high as that from the Betzes x C.S. combination. In fact, the latter crosses gave a higher frequency of progeny than the original barley x wheat hybridization. Other barley-wheat hybrids such as Betzes x Koga, Bonus x Koga and Bonus x C.S. were obtained at very low frequencies (unpublished) and yielded no progeny when pollinated with Prolific rye.

There was also differences between rye strains in their crossability with other species. It has been shown that

Prolific rye induces haploids when crossed onto certain barley cultivars (Fedak 1977a), while viable hybrids were obtained from pollinating other barley strains with Petkus rye (Cooper et al. 1978; Fedak 1979a). The pollination of a number of barley cultivars with Sangaste rye yielded numerous hybrid embryos that did not develop beyond the seedling stage (Fedak 1977c). In the present study only Prolific rye was effective in stimulating hybridization with barley × wheat hybrids and no success was realized with the rye cultivars Sangaste, Puma or the rye species of *S. vavilovii.*

The wheat phenotype predominated in the trigeneric hybrids in the same way as in wheat-rye and barley-wheat hybrids. Whether this is due to the polyploid nature of wheat and multiplicity of loci or whether the wheat genome tends to suppress the expression of homoeologous chromosomes of other species has not been established.

The occurrence of trigeneric hybrids with chromosome numbers greater than 35 can probably be explained by events occurring at meiosis of Betzes × C.S. hybrids. A low frequency of PMC's with more than 28 chromosomes were detected in Betzes × C.S. hybrids (Fedak 1979b) which probably arose through abnormalities at premeiotic mitosis (Fedak 1979b) or through abnormal movements of univalents at metaphase. These events would lead to the production of hyperploid gametes in the staminate tissue and probably also in the pistillate tissue. Progeny with chromosome numbers greater than the expected have been obtained from backcrossing Chinese Spring onto Betzes × C.S. hybrids (Fedak 1979c) which appears to be a similar phenomenon to the hyperploid trigeneric hybrids reported here. It follows that the duplicated chromosomes would be those of barley or wheat.

The production of hybrids including the three major cereal crops genera, introduces the exciting potential of transferring genetic material between them for purposes of crop improvement. Whether this can be done through the production of amphiploids, production of intergeneric chromosomal addition or substitution lines followed by the manipulation of meiotic pairing control genes will be the subject of future studies.

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