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NOTES AND COMMENTS

PREFERENTIAL SURVIVAL OF WHEAT HAPLOIDS OVER HYBRIDS IN A WHEAT×BARLEY CROSS

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1. Introduction

Barclay (1975) obtained only wheat haploids from the cross, *Triticum aestivum* × *Hordeum bulbosum* (2*x* and 4*x*), but other Triticum–Hordeum crosses yielded only hybrids (Kruse, 1973; Kimber and Sallee, 1976; Cauderon *et al.*, 1978; Martin and Sanchez-Monge Laguna, 1980*a*, 1980*b*; Thomas *et al.*, 1977; Finch and Bennett, 1980) or mixed progenies of hybrids and up to 15 per cent of haploids (Islam *et al.*, 1981). The present paper reports several Triticum–Hordeum crosses including a combination in which 80 per cent of progeny were wheat haploids and presents evidence that these haploids arose after chromosome elimination from hybrids.

2. Materials and methods

Table 1 gives genotypes, chromosome numbers and sources of parents. The wheats were crossable with rye and *H. bulbosum* (Snape *et al.*, 1979). *H. vulgare* cv. Paavo line P-4 is homozygous for the *tri* gene (Ahokas, 1977; Finch and Bennett, 1979). Tetraploid P-4 arose spontaneously from a 2*x* doubled haploid line and was also synthesized from diploid P-4 by colchicine treatment at the Plant Breeding Institute (PBI), Cambridge. *H. vulgare* line Tuleen 346 is like its parent variety, Bonus, but is homozygous for interchanges T1-5v, T2-6y and T3-7d obtained by irradiation of Bonus (Bennett, 1982). Germinated seeds were potted in John Innes No. 2 compost or (*H. vulgare* line 4*x* P-4 only) soilless compost (Finch and Bennett, 1981). Plants were grown at the PBI in glasshouses which were unlit in spring and summer and lit at other times continuously for 18 hrs each day by 400 W high pressure sodium lamps. Attempts were made to control temperatures at 20°C by day and 15°C by night but temperatures varied from about 10°C to about 50°C.

Plants were emasculated 1–5 days before pollination and a slim translucent polythene bag was placed over the spike. In crosses of *T. aestivum* line TH3929 with *H. vulgare* lines 4*x* P-4 and Tuleen 346, TH3929 was isolated from all dehiscent spikes but those of the male parent whenever pollination bags were removed. One day after pollination, spikes were sprayed to run-off with aqueous gibberellic acid solution (75 ppm GA) made from Berelex tablets (ICI Plant Protection Ltd., Fernhurst, Haslemere, Surrey, England) and the polythene bag was replaced by a slim brown paper bag. Embryos were excised in sterile conditions two to three weeks after pollination and cultured singly in the dark at 20°C in vials of

TABLE 1

Parental genotypes, chromosome numbers (2n) and sources

Genotype	2*n*	Source
T. aestivum L. line 3929	42	N. Darvey*
cv. Chinese Spring	42	PBI
H. vulgare L. cv. Paavo	14	H. Ahokas†
cv. Paavo line P-4	14	H. Ahokas†
cv. Paavo line P-4	27–29	PBI
cv. Bonus line Tuleen 346	14	N. A. Tuleen‡
cv. Sultan	14	PBI
cv. Luke	14	PBI
H. bogdanii Wilensky line H312	14	R. von Bothmer§
H. murinum L. ssp. *glaucum* (Steudel) Tzvelev lines 83 and 150	14	W. Lange‖
H. brevisubulatum (Trin.) Link ssp. *violaceum* (Boiss. & Hohen.) Tzvelev line H315	14	R. von Bothmer§
H. lechleri (Steudel) Schenk line 177	42	W. Lange‖
H. procerum Nevski line 119	42	W. Lange‖

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4 ml of 29 g/l Bacto Orchid Agar (Difco Laboratories, Detroit, Michigan, U.S.A.). When shoots about 1 cm long had developed, vials were moved into diffuse light at 20°C. Most plantlets were potted in soilless compost and grown in glasshouses with protection from desiccation for the first few days. Some of the inviable-looking plantlets were taken from agar and grown hydroponically (Finch and Bennett, 1981) but all died.

For chromosome counts, excised roots were pretreated for 4 hrs in fresh saturated 1-bromonaphthalene at 20°C or for 24 hrs in ice-water. Water used in pretreatment was aerated for five mins or longer a few minutes before use. Pretreated roots were fixed in Carnoy's fluid or fresh 1 : 3 acetic ethanol for at least 2 h, hydrolysed in 1 mol/1 HCl for 12 mins at 60°C, stained in Feulgen for 2 h and squashed in 45 per cent v/v aqueous acetic acid (often mixed with propionic orcein).

Embryo sac development in pollinated florets was checked by fixing spikes 1–4 days after pollination, staining excised ovaries in Feulgen and excising ovules and teasing out the embryo sacs on to a slide for microscopic examination. Plants used for this purpose were brought into a growth room at 20°C lit by cool white fluorescent tubes giving 500 cd sr m^{-2} at plant level for a few days before pollination and kept there till spikes were fixed.

3. RESULTS

Table 2 gives the numbers of spikes and florets crossed in each combination and shows the numbers of embryos and plants in the progeny. All seeds were small and seemed to lack endosperm except for the seed that

TABLE 2

Numbers of spikes and florets crossed and embryos 0·1 mm or more long at excision, wheat-like plants, hybrids and inviable plantlets obtained from Triticum–Hordeum *crosses*

♀ parent	♂ parent	Spikes	Florets	Embryos	Wheat-like plants	Hybrids	Inviable plantlets
TH3929	2*x* P-4	25	555	24	8($2n=21$)		2
	4*x* P-4	13	316	40	1($2n=42$)	1($2=22$)	4
	Tuleen 346	14	305	5	1($2n=21$)		
	Sultan	11	261	0			
	Paavo	5	97	0			
Chinese Spring	Sultan	21	491	4			
	2*x* P-4	4	91	0			
	Luke	8	155	0			
	H. bogdanii	18	344	0			
	H. brevisubulatum ssp. *violaceum*	4	74	0			
	H. lechleri	12	243	0			
	H. procerum	5	93	0			
Sultan	Chinese Spring	18	312	0			
H. murinum ssp. *glaucum*	Chinese Spring	4	62	0			

gave the wheat-like plant ($2n=42$) in TH3929 × 4*x* P-4, which looked like a selfed seed, and one from the same cross which had a small endosperm and no embryo. Embryos 0·1 mm or more long at excision were obtained only in crosses of *T. aestivum* line TH3929 with *H. vulgare* cv. Paavo lines 2*x* and 4*x* P-4 and line Tuleen 346, which yielded ten and six plants and one plant, respectively and of *T. aestivum* cv. Chinese Spring with *H. vulgare* cv. Sultan, which did not yield plants. The percentages of florets yielding a plant in crosses of TH3929 with 2*x* P-4, 4*x* P-4 and Tuleen 346 were 1·8, 1·9 and 0·3, respectively.

The cross, TH3929 × 2*x* P-4 gave eight vigorous TH3929 haploids ($2n=21$) indistinguishable from TH3929, except for their slightly smaller size and complete sterility, plus two inviable plantlets. The cross, TH3929 × 4*x* P-4, gave one hybrid ($2n=22$) plus four inviable plantlets and a fully fertile diploid plant ($2n=42$) indistinguishable from TH3929, which probably arose from pollination by wheat pollen. An apomictic origin is unlikely as the seed that produced this plant had a normal endosperm. The hybrid that reached maturity grew slowly, tillered profusely, was dwarf and had deformed spikes and indehiscent anthers. Inviable plantlets lived for up to 102 days after pollination and were green, but less than five cm tall with few tillers and short, thin, often deformed leaves. A sample of ten such plantlets growing on agar 54–64 days after pollination had root tip chromosome numbers of from 21 to 35 with variable numbers in the same root in five plants (table 3) showing that chromosome elimination was occurring. By contrast, each plant classified as wheat-like in TH3929 × 2*x* and 4*x* P-4 grew vigorously to maturity, was not deformed and did not vary in chromosome number in any of the two or more roots sampled from it.

The cross TH3929 × Tuleen 346 gave one plant ($2n=21$ in 6 roots) which is probably a haploid TH3929. This plant grew slowly for 42 days until transferred to fresh agar when it grew vigorously. At the time of

TABLE 3

Chromosome numbers in single roots of inviable progeny of the cross T. aestivum *line TH3929* × H. vulgare *cv. Paavo line 4x P-4*

Plant No.	*Chromosome numbers(s) in root*
1	21
2	23, 28, 30
3	25, 32, 34
4	26
5	30
6	30, 31, 32, 34, 35
7	31, 35
8	34, 35
9	35
10	35

writing, its juvenile morphology is like that of haploids from TH3929 × 2*x* P-4 and its karyotype is similar if not identical to theirs. Of 65 florets from 11 spikes of TH3929 × Tuleen 346 checked 1–4 days after pollination, 7 (10·8 per cent) were fertilized. At a maximum, 40 per cent of florets were fertilized and ten per cent of florets gave an embryo 0·1 mm or more long at excision in any one spike in this cross. Micronuclei were seen in embryo and endosperm tissue and in one floret fixed three days after pollination, all dividing endosperm nuclei had about 42 chromosomes. Chromosome elimination was evidently occurring in this cross.

4. DISCUSSION

Table 4 shows the percentages of Triticum haploids in other studies of Triticum–Hordeum crosses. The highest frequency of haploids reported in *T. aestivum* × *H. vulgare* progeny is 15 per cent and only *T. aestivum* × 4*x* and 2*x H. bulbosum* gave such high frequencies as TH3929 × 2*x* P-4. The yield of plants in the present work was too low for practical use in wheat haploid production, however. Thus, on average in the cross TH3929 × Tuleen 346, 10·8 per cent of ovules were fertilized but only 1·6 per cent of florets contained an embryo big enough to be worth culturing 2–3 weeks after pollination and only 20 per cent of these embryos developed into a plant. There was evidence of chromosome elimination in embryo and endosperm tissue in this cross 1–4 days after pollination and chromosome number varied within roots of inviable plantlets of TH3929 × 4*x* P-4 at 54–64 days. The low percentages of florets yielding viable plants in wheat × barley crosses is therefore probably due both to fertilization failure and to the production by chromosome instability of chromosome combinations in the cells of many hybrids that lead to the death of the young seed before the embryo is big enough to culture in our conditions. This may also be true of those crosses in table 2 that apparently yielded no embryos, since minute embryos could have been present but overlooked. Only 6·3 per cent of plants from crosses of TH3929 with 2*x* and 4*x* P-4 were viable hybrids whereas 50 per cent were viable haploids and the remainder inviable plantlets, probably unstable hybrids. This indicates that rapid complete elimination may enhance survival in our culture conditions.

TABLE 4

Numbers of plants and percentages of Triticum haploids in progenies of Triticum–Hordeum crosses

Female parent	Male parent	Plants	Per cent haploids	Reference
T. aestivum 6*x*	*H. vulgare* 2*x*	2	0·0	Islam *et al.*, 1976
		5	0·0	Fedak. 1980
		20	15·0	Islam *et al.*, 1981
		33	9·1	
T. aestivum 6*x*	*H. bulbosum* 2*x*	11	100·0	Barclay, 1975
	H. bulbosum 4*x*	59	100·0	Barclay, 1975
	H. bulbosum 4*x*+2*x*	223	99·1	Miller and Chapman, 1976
		293	99·3	
T. durum 4*x*	*H. vulgare* 2*x*	2	100·0	Shepherd and Islam, 1981
T. timopheevi 4*x*	*H. bogdanii* 2*x*	1	0·0	Kimber and Sallee, 1976
H. vulgare 2*x*	*T. aestivum* 6*x*	75	0·0	Clauss, 1980
		67	0·0	Islam *et al.*, 1976
		21	0·0	Kruse, 1973
		8	0·0	Mujeeb-Kazi and Rodriguez, 1980
		1	0·0	Thomas *et al.*, 1977
		15	6·7	Fedak, 1980
		187	0·1	
H. vulgare 2*x*	*T. durum* 4*x*	6	0·0	Thomas *et al.*, 1977
	T. timopheevi 4*x*	1	0·0	Cauderon *et al.*, 1978
	T. dicoccum 4*x*	6	0·0	Kruse, 1973
	T. monococcum 2*x*	1	0·0	Kruse, 1973
H. chilense 2*x*	*T. aestivum* 6*x*	16	0·0	Martin and S.-M. Laguna, 1980*a*
	T. durum 4*x*	11	0·0	Martin and S.-M. Laguna, 1980*b*
H. pusillum 2*x*	*T. aestivum* 6*x*	2	0·0	Finch and Bennett, 1980

TH3929 and genotypes crossed with it were pure lines and it is unclear why in some combinations there was so much variability between florets in the development of embryos from genetically identical zygotes. Such variability was also found by Islam *et al.* (1981), who reported up to 1·3 per cent seedset in Chinese Spring × *H. vulgare* and obtained progeny with 2*n* chromosome numbers ranging from 21 to 36 including haploids and plants with duplication and deficiency of some wheat and barley chromosomes. Shepherd and Islam (1981) suggested that abnormal disjunction at mitosis in young seeds might result in complete elimination of barley chromosomes or in duplication or deficiency of some wheat and barley chromosomes. Detailed chromosome constitutions of viable progeny have not been fully described. It would be interesting to know if only certain hybrid combinations were viable and whether these and the sequence of changes leading to them were correlated with the intragenomic ordering of chromosomes reported by Bennett (1982). Such possibilities may provide a more plausible explanation of why different florets on the same spike with genetically identical zygotes gave such different seeds than the possibility of significant environmental differences between florets.

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