

SHORT PAPER

Production of disomic wheat–barley chromosome addition lines using *Hordeum bulbosum* crosses

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SUMMARY

The possibility of using *Hordeum bulbosum* crosses to facilitate production of disomic wheat–barley addition lines from monosomic additions was investigated. Aneuhaploids with 22 chromosomes were obtained in the expected gametic frequencies after crossing monosomic, disomic and monotelodisomic addition lines, involving four different barley chromosomes, as the female parent with tetraploid *H. bulbosum*. Thus the added barley chromosomes were not eliminated when preferential elimination of the *bulbosum* chromosomes took place in the hybrid embryos. Disomic addition lines were obtained after treating the aneuhaploids with colchicine. This method could have wider application in the production of other wheat–alien chromosome disomic addition lines, especially where the transmission frequency of the alien chromosome through the pollen is very low, but its use will depend on the wheat parent being crossable with *H. bulbosum* and the alien chromosome being retained during the elimination of *bulbosum* chromosomes.

1. INTRODUCTION

After O'Mara (1940) described a systematic procedure for adding individual pairs of rye chromosomes to wheat, many other workers have used a similar approach to add chromosomes of several other alien species to wheat. These addition lines have had many uses including assigning genes in the alien species to particular chromosomes, determining the genetic similarity (homoeology) between alien chromosomes and particular wheat chromosomes, and also for transferring desirable characters such as disease resistance from the alien species to wheat.

In producing addition lines, it has usually been relatively easy to produce 43-chromosome monosomic addition lines from a 49-chromosome heptaploid, but it has often been difficult to isolate the disomic addition lines from these monosomics. This difficulty stems from the fact that in monosomic additions, although the transmission rate of the extra chromosome in female gametes is expected to be approximately 25%, the 22-chromosome pollen bearing the alien chromosome usually competes very poorly with 21-chromosome euploid wheat pollen in effecting fertilization of the egg. For example, Sears (1956) obtained only 1.3% disomics from among the progeny of a selfed monosomic addition of an *Aegilops umbellulata* chromosome (6C^u) to hexaploid wheat. Furthermore, Mochizuki (1963) could not recover any disomic addition plants when screening 1055 progeny of a selfed monosomic addition line having an *Agropyron elongatum* chromosome added to tetraploid wheat.

There are some exceptions to this pattern, and Knott (1964) and Cauderon (1966) reported a transmission rate of 25% and 20% for a telocentric and a univalent chromo-

some of *Ag. intermedium* when present as monotelosomic or monosomic additions to wheat, respectively. Also, there are other examples where a specific *Aegilops* chromosome has almost 100% transmission through the pollen in monosomic addition lines involving particular chromosomes of *Ae. triuncialis* (Endo & Tsunewaki, 1975), *Ae. longissima* and *Ae. sharonensis* (Maan, 1975) and *Ae. caudata* (Endo, 1978; Endo & Katayama, 1978) added to common wheat. The preferential transmission of the *Aegilops* chromosome in both the male and female gametes results from inviability of all gametes not bearing the alien chromosomes.

In those numerous examples where it is difficult to obtain disomics from selfed monosomics, Chang *et al.* (1973) suggested that if pollen of the monosomic could be cultured to give haploid plants, then many of these would be 22-chromosome aneuhaploids which could provide disomic addition lines upon chromosome doubling. Subsequently, Barclay (1975) demonstrated that when Chinese Spring wheat is pollinated with diploid and tetraploid *Hordeum bulbosum*, the *bulbosum* chromosomes are selectively eliminated during embryo development giving wheat haploids, and this suggested to the present authors another possible way of producing disomic addition lines without having to rely on transmission of the alien chromosome through the pollen. Thus if the 22-chromosome eggs in monosomic additions could be fertilized with *H. bulbosum* and there was preferential elimination of the *bulbosum* chromosomes, 22-chromosome aneuhaploids would result and these could give disomic additions after chromosome doubling.

In the present study disomic, monotelo-disomic and monosomic wheat-barley addition lines have been hybridized with *H. bulbosum* to test the feasibility of this procedure for obtaining disomic lines. We were particularly interested to test the efficiency of this method for producing disomic wheat-barley addition line 3, since in earlier cytological screening only one disomic was found in 100 progeny from the monotelo-disomic ($21'' + 1t''$) addition line.

2. MATERIALS AND METHODS

The plant material used as the pistillate parent in crosses with *H. bulbosum* included monosomic, monotelo-disomic and disomic addition lines having chromosomes of Betzes barley added to Chinese Spring wheat, which had been produced in this laboratory (Islam, Shepherd & Sparrow, 1975, 1978, 1981). Tetraploid *H. bulbosum* ($2n = 28$) (accession CPI 14804 from the Plant Introduction Centre, Division of Plant Industry, C.S.I.R.O., Canberra City, A.C.T., Australia), was used as the pollen parent.

The plant material used in crosses was grown in potting compost in 30 cm pots under optimal conditions in a glasshouse in spring and early summer. The procedures used for emasculation, pollination and embryo culture were similar to those used earlier in making wheat \times barley crosses (Islam *et al.* 1975, 1981). However, because anthesis in *H. bulbosum* commences early in the morning, the *bulbosum* pollen used in crosses did not always come from freshly dehisced anthers. Sometimes pollen from partly dehisced older anthers was used.

The somatic chromosome constitution of the parent and progeny plants was determined from root tips which were taken from potted plants and pretreated in α -bromonaphthalene, fixed in glacial acetic acid and then stained by the Feulgen method. Meiotic preparations were made from anthers fixed in freshly made 1:3 acetic acid-alcohol and stained by the same Feulgen method.

To induce chromosome doubling, seedlings with four to five tillers were treated for up to 72 h with 0.1% colchicine in 2% dimethyl sulphoxide using Bell's (1950) tiller-capping method.

For isozyme studies, the methods of Tang & Hart (1975) and Kahler & Allard (1970) were used.

3. RESULTS AND DISCUSSION

The seed set obtained after crossing the addition lines with tetraploid *H. bulbosum* pollen is shown in Table 1. The crosses involving disomic addition lines 2 and 4 and monosomic addition line 6 were all carried out within a three-week period in late spring and the percentage seed set was similar with each line. However, the crosses with monotelodisomic addition 3 were made in early spring, so it is not clear whether the lower rate of success is due to genotypic or environmental differences. With all addition lines, there was much variation in the degree of seed set on individual spikes just as observed by Miller & Chapman (1976) with crosses between wheat monosomics and *H. bulbosum*.

Table 1. Progeny obtained after pollinating disomic and monosomic wheat-barley addition lines with *H. bulbosum* ($2n = 28$)

Pistillate parent	MI configurations in PMCs	No. florets pollinated	Seeds obtained		Embryos cultured		No. plants obtained
			No.	(%)	No.	(%)	
Disomic addition							
2	22"	141	41	(29)	12	(29)	7
4	22"	140	31	(22)	12	(38)	2
3*	21" + 1t"	196	25	(13)	14	(56)	10
Monosomic addition							
6	21" + 1'	256	70	(27)	50	(71)	30

* = Monotelodisomic addition.

When pollen from freshly dehisced anthers was used in crosses, the frequency of seeds with embryos was greater than when older pollen was used, since the latter pollen often induced ovary development only. The percentage of embryos recovered varied with the different addition lines, but since separate batches of pollen were used to fertilize these lines it cannot be decided whether the differences are genotypic or due to batch differences in the pollen. Monosomic addition 6 appeared to be a better parent than the disomics used, since a higher proportion of its seeds possessed embryos and, furthermore, the embryos tended to be larger than those obtained from the disomics. However, in contrast to the usual response with disomics, a single spike of disomic addition 2 gave eight seeds with larger-sized embryos, suggesting that the chromosome constitution of the embryos may not be the most important factor in determining their development.

The chromosome constitution of the progeny obtained from these crosses is given in Table 2. As 22-chromosome aneuploids were recovered from each of the disomic and monotelodisomic addition lines tested, it is evident that the barley chromosomes are recognized separately from *H. bulbosum* chromosomes when elimination of the latter chromosomes takes place in the developing embryos. The occurrence of a 21-chromosome plant among the progeny of monotelodisomic addition 3 seems to be inconsistent with this conclusion, but this plant could have originated from an egg cell which lacked the barley chromosome because of asynapsis or non-disjunction in the megaspore mother cell.

None of the 41 progeny examined had more than 22 chromosomes in its root tips and hence the elimination of *bulbosum* chromosomes must have been complete. Similarly, Miller & Chapman (1976) observed complete elimination of *bulbosum* chromosomes in 134 out of 136 progeny from monosomics of wheat crossed with *H. bulbosum*. They suspected that at least one *bulbosum* chromosome might have been retained in the other two plants.

The recovery of 22-chromosome plants from monosomic addition line 6 with a frequency of 19% was of special interest since it showed that there was no strong selection against embryos with 22 chromosomes in this monosomic at least. However, Miller & Chapman (1976) in their study of crosses between monosomic wheat and *H. bulbosum* found that there was some selection against the 20-chromosome aneuploid embryos, compared to the normal 21-chromosome euploid embryos.

Approximately 20% of the embryos obtained from crosses of monosomic and disomic addition lines with *H. bulbosum* pollen had two shoot apices, and one other seed contained a twin embryo, but the cause of these developmental abnormalities is not known.

Table 2. *Chromosome constitution of progeny from crosses between wheat-barley addition lines and H. bulbosum (2n = 28)*

Addition line	MI configurations in PMCs of parent	No. of progeny with indicated chromosome constitution		
		21	21 + t	22
Disomic 2	22''	0	0	6
Disomic 4	22''	0	0	2
Monotelo-disomic 3	21'' + 1t''	1	3	3
Monosomic 6	21' + 1'	21	0	5

At meiosis the progeny plants showed mostly univalents (21', 21' + t', 22'), but some pairing was observed in occasional PMCs similar to that occurring in normal Chinese Spring haploids. Doubled sectors were obtained in most of the plants treated with colchicine, and disomic and ditelosomic addition lines with 22'' and 21'' + t'' have been obtained. Proof that the required barley chromosomes were present in the disomic additions 3, 4 and 6 produced from these *H. bulbosum* crosses was obtained by detecting barley esterases, alcohol dehydrogenase and glutamic oxaloacetic transaminase isozymes, respectively, in these lines.

To summarize, the present work demonstrates a new method of producing alien disomic addition lines which does not depend on rare transmission of the alien univalent through the pollen of monosomic addition lines. Consequently, where the recovery of disomics from progeny of selfed monosomics is very low (Sears, 1956; Evans & Jenkins, 1960; Islam *et al.* 1981), this *bulbosum* method may be much more efficient than the conventional method of screening cytologically a large number of their progeny. This method will also be useful where an alien telocentric is transmitted preferentially over the complete chromosome. For example, Sears (1968) obtained only two substitution plants with 20'' + 1'' 2R among 41 progeny of a plant with 20'' + 1t'' 2R. A similar problem was encountered in our attempts to select a disomic addition in the conventional manner from among the progeny of monotelo-disomic addition of chromosome 3 of barley. Only one 22'' plant was recovered from among 100 of its progeny whereas with the *bulbosum* crosses three out of the seven embryos obtained had 22'.

An important question remaining is whether this method can be applied more widely to the production of disomic addition lines with other alien species. Obviously this method can only be used when the wheat parent is crossable with *H. bulbosum*, and so far such wheats are restricted to Chinese Spring and a few Australian cultivars (Snape *et al.* 1979). Furthermore, use of this procedure with other alien species will depend on finding whether the individual alien chromosomes to be added to wheat are retained during the elimination of the *bulbosum* chromosomes in developing embryos.

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