Theor Appl Genet (1991) 81:285–292

# Efficient production of wheat-barley hybrids and preferential elimination of barley chromosomes

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Received July 19, 1990; Accepted September 5, 1990
Communicated by K. Tsunewaki

**Summary.** Intergeneric hybridization between four common wheat cultivars, *Triticum aestivum* L. cultivars Chinese Spring, Norin 12, Norin 61, and Shinchunaga, and cultivated barley, *Hordeum vulgare* L. cultivars Betzes, Nyugoruden, Harunanijou, and Kinai 5 were carried out in a greenhouse under 15–20 °C and long-day (15 h) photoperiod conditions. Two days prior to pollination, a 100 mg/l 2,4-D solution was injected into wheat stems. Among wheat cultivars, Norin 12, Norin 61, and Shinchunaga showed higher crossabilities than that of Chinese Spring, suggesting the presence of crossability gene(s) other than the *kr* system of Chinese Spring. Variation was also found among the barley cultivars as male parents. Betzes barley showed the highest crossability with wheat. Thus, the cross Norin 12 × Betzes showed the highest crossability (8.25%), followed by Norin 61 × Betzes (6.04%), Shinchunaga × Betzes (5.00%), and Shinchunaga × Kinai 5 (5.00%). The embryos were rescued by culture at 15–20 days after pollination. Seventy-four plants were obtained from 82 embryos. The morphology of the hybrid plants resembled that of wheat parents. Among 60 seedlings observed, 28 had 28 chromosomes, 8 had 21, 23 had aneuploid numbers of chromosomes (22–27), and 1 had 29 chromosomes. About half of the aneuploid hybrids showed mosaicism for chromosome number. By analyzing five isozyme markers of barley chromosomes, the chromosome constitutions of the aneuploid hybrids were determined. Barley chromosomes 1 and 5 were found to be preferentially eliminated in the hybrids, while chromosomes 2 and 4 were eliminated infrequently. The conditions and genetic factors for high crossability and the tendency of barley chromosome elimination are discussed.

**Key words:** Wheat – Barley – Crossability – Chromosome elimination

## Introduction

Intergeneric hybridization between cereal species is interesting from the viewpoints of cytogenetics, evolution, and practical breeding. Successful crosses between *Hordeum* and *Triticum* species were first made by Kruse (1973). He obtained intergeneric hybrids using *Hordeum vulgare* as female and *Triticum aestivum, T. dicoccum,* and *T. monococcum* as male parents. After his pioneering work, Islam et al. (1975) and Fedak (1977) obtained hybrids between the cultivated barley, Betzes, and the common wheat cultivar, Chinese Spring. Barley-wheat hybrids were also produced by other researchers (Chen et al. 1984; Shimada et al. 1987; Thomas et al. 1977). However, these hybrids were all self-sterile and colchicine treatment for diploidization was ineffective (Shimada et al. 1987). Plants in the backcrossed generations with wheat showed pistillody due to the interaction between the barley cytoplasm and the wheat nucleus (Islam et al. 1981; Shimada et al. 1987). Thus, crossing barley as female with wheat as male was found to be inadequate for obtaining fertile offspring. Only Xue et al. (1988) reported a self-fertile plant having 43 chromosomes in the third backcrossed generation with wheat from a barley-wheat hybrid.

In contrast, the reciprocal cross, wheat × barley, has been achieved by only a few workers (Islam et al. 1978; Fedak 1980; Sethi et al. 1986). By backcrossing wheat to the hybrid, Islam et al. (1981) produced chromosome addition lines of common wheat cultivar Chinese Spring having each of six chromosome pairs of barley cultivar Betzes. No other addition lines have been reported.

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Lein (1943) found two crossability genes, *Kr1* and *Kr2*, of wheat, where dominant *Kr1* and *Kr2* alleles reduced crossability with rye (*Secale cereale*). Riley and Chapman (1967) showed that these crossability genes were located on chromosomes *5B* and *5A*, respectively. Krowlow (1970) reported that chromosome *5D* also carried a crossability gene, *Kr3*. Strong correlations between the crossabilities of wheat with rye and *Hordeum bulbosum* were shown by Snape et al. (1979) and Falk and Kasha (1981). Fedak and Jui (1982) showed that homoeologous 5 chromosomes of common wheat had genetic factors responsible for crossability with cultivated barley when wheat was used as the male parent. It is probable that common wheat cultivars having high crossability with rye or *H. bulbosum* also have crossability with cultivated barley (*Hordeum vulgare*).

It was found in wheat-barley hybrids that the chromosome number varied continuously from the haploid wheat chromosome number (21) to the hybrid chromosome number (28). Hyperploid hybrids were also found (Islam et al. 1981; Sethi et al. 1986). The aneuploid hybrids were assumed to be produced by the elimination of barley chromosomes in the hybrid embryos, although the chromosome constitution of the aneuploid hybrids has not been studied. Linde-Laursen and von Bothmer (1988) showed, by using C-banding, that barley chromosomes 1, 5, 6, and 7 were preferentially eliminated in hybrids between hexaploid wild barley and diploid cultivated barley. Since isozyme polymorphism is well documented in wheat and barley (e.g. Benito et al. 1985; Hart et al. 1980), the chromosome constitutions of the aneuploid hybrids could be determined by using isozyme markers for each of the barley chromosomes.

In order to introduce the traits of early heading or early maturity of barley into wheat, we crossed wheat cultivars that were assumed to have *kr* alleles with cultivated barley having early heading or early maturing characteristics. In this paper, we report the results of crosses between wheat and barley cultivars and the preferential elimination of barley chromosomes, by analyzing aneuploid hybrids for the presence of isozyme markers for each of the barley chromosomes.

## Materials and methods

### *Plant materials*

Common wheat, *Triticum aestivum* L. ($2n=6x=42$) cultivars Chinese Spring, Norin 12, Norin 61, and Shinchunaga were used as the female parents. Cultivated barley, *Hordeum vulgare* L. ($2n=2x=14$) cultivars Betzes, Nyugoruden, Harunanijou, and Kinai 5 were used as the pollen parents.

Wheat and barley cultivars were kindly supplied by the following researchers: Norin 12 and Shinchunaga – H. Gocho, Kyushu Experiment Station; Norin 61 – S. Nonaka, Ishikawa Agricultural College; Betzes and Nyugoruden – M. Inagaki, National Agricultual Research Center, Tsukuba; Harunanijou – S. Takahashi, Sapporo Breweries Co.; and Kinai 5 – S. Yasuda, Research Institute of Bioresources, Okayama University.

Chinese Spring and Shinchunaga were classified as having *kr1kr2* alleles on the basis of their crossability with rye (Sasaki 1987). Since Norin 61 was derived from Shinchunaga, it was also through to have *kr* alleles. Norin 12 was occasionally found to be crossable with cultivated barley in a preliminary test (Koba et al. 1987).

Betzes barley is known to be crossable with wheat (Fedak 1980; Islam et al. 1981). Among the three Japanese barley cultivars used, Harunanijou and Kinai 5 are early heading or early maturing types. Kinai 5 is known to have the $ea_k$ gene (Yasuda 1977).

### *Crossing*

Seeds of wheat and barley were sown in the fall of 1987. After germination, seedlings were kept in a cold room (2 °C) for at least 1 month for vernalization treatment and adjustment of their flowering period. Seedlings were then transferred into a greenhouse at 2-week intervals. Temperature in the greenhouse was controlled between 15 °C and 25 °C during the night and day, respectively, under long-day (15 h) photoperiod conditions. Crosses were carried out by the first author from late December 1987 to February 1988. Wheat spikes were emasculated 2 or 3 days prior to anthesis, and 100 mg/l 2,4-D (2,4-dichlorophenoxyacetic acid) solution was injected by syringe into the first internode of wheat as described by Inagaki (1986).

### *Embryo rescue*

At 15–20 days after pollination, embryos were dissected from the seeds, put onto $N_6$ medium (Chu et al. 1975) supplemented with 0.5 mg/l IBA (indole-3-butyric acid), 0.5 mg/l $GA_3$ (gibberellic acid), and 400 mg/l casein hydrolysate, and kept at 20 °C in the dark until germination. Seedlings were then grown at 26 °C under 6,000 lx inflorescent light. When the seedlings became strong, they were transplanted into pots filled with vermiculite and kept for about 20 days in the same conditions. Thereafter, they were vernalized for about 1 month and then transplanted to soil in the greenhouse for backcrossing.

### *Cytology*

Somatic chromosome number was examined in root-tip cells of the seedlings. At least two root tips from each seedling were cut to a length of 1 cm, kept in distilled water at 0 °C for 24 h, and fixed with Farmer's solution (ethyl alcohol : glacial acetic acid = 3 : 1). Root tips were stained with 1% acetocarmine solution and chromosomes were observed after squashing.

### *Isozyme analyses*

Young leaves (0.1 g) of the hybrid seedlings were ground in liquid nitrogen with a mortar and pestle. The extraction medium consisted of 50 m*M* TRIS-HCl (pH 7.5), 1 m*M* EDTA, 0.2 M NaCl, 1% B-mercaptoethanol. Triton X-100 (1%) was added after the liquid nitrogen had all sublimed away. The homogenate was then centrifuged before it was used for electrophoresis.

After 7.5% polyacrylamide gel electrophoresis (PAGE), the gel was stained for 13 isozymes that have been located to particular barley chromosomes (Benito et al. 1985; Hart et al. 1980; Powling et al. 1981; Salinas et al. 1985), according to the method of Tsumura et al. (1989). Isozyme polymorphism between wheat and barley was observed in eight enzymes; endopeptidase (EP), glucose-6-phosphate dehydrogenase (G6PDH), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGDH), phosphoglucose isomerase (PGI), malate dehydrogenase (MDH), glutamate oxaloacetate transaminase (GOT), and

**Table 1.** Results of the crosses between wheat and barley[a]

| Wheat cv (female parent) | Barley cv (male parent) | | | | Total |
|---|---|---|---|---|---|
| | Betzes | Nyugoruden | Harunanijou | Kinai 5 | |
| Chinese Spring | 1/156 (0.64%) | 2/225 (0.89%) | 0/170 | 0/304 | 3/855 (0.35%) |
| Norin 12 | 17/206 (8.25%) | 1/212 (0.47%) | 1/204 (0.49%) | 0/136 | 19/785 (2.51%) |
| Norin 61 | 18/298 (6.04%) | 9/238 (3.78%) | 3/378 (0.79%) | 1/180 (0.56%) | 31/1094 (2.83%) |
| Shinchunaga | 10/200 (5.00%) | 4/182 (2.20%) | 10/656 (1.52%) | 5/100 (5.00%) | 29/1138 (2.55%) |
| Total | 46/860 (5.35%) | 16/857 (1.87%) | 14/1408 (0.99%) | 6/720 (0.83%) | 82/3845 (2.13%) |

[a] No. embryos produced/no. florets pollinated and the percentages in parentheses

**Table 2.** Analysis of variance of the crossabilities between wheat and barley after transforming percentages to angles

| Source of variation | *df* | SS | MS |
|---|---|---|---|
| Between wheat cultivars | 3 | 140.6705 | $46.8902^{NS}$ |
| Between barley cultivars | 3 | 168.5354 | 56.1785** |
| Error | 9 | 111.7210 | 12.4134 |
| Total | 15 | 420.9269 | |

$^{NS}$ and ** Not significant and significant at the 1% level, respectively

**Table 3.** Classification of crossabilities between wheat and barley, after *t*-tests with transformed data to angles[a]

| Cross combination | Cross-ability (%) | Class |
|---|---|---|
| Norin 12 × Betzes | 8.25 | a |
| Norin 61 × Betzes | 6.04 | a |
| Norin 61 × Nyugoruden | 3.78 | a |
| Shinchunaga × Betzes | 5.00 | a b |
| Shinchunaga × Kinai 5 | 5.00 | a b c |
| Shinchunaga × Nyugoruden | 2.20 | a b c d |
| Shinchunaga × Harunanijou | 1.52 | b c d |
| CS × Nyugoruden | 0.89 | b c d e |
| CS × Betzes | 0.64 | b c d e |
| Norin 61 × Kinai 5 | 0.56 | b c d e |
| Norin 12 × Harunanijou | 0.49 | b c d e |
| Norin 12 × Nyugoruden | 0.47 | b c d e |
| Norin 12 × Kinai 5 | 0.00 | b c d e |
| Norin 61 × Harunanijou | 0.79 | c d e |
| CS × Harunanijou | 0.00 | d e |
| CS × Kinai 5 | 0.00 | e |

[a] Cross combinations with same letters are not significantly different at the 5% level

leucine aminopeptidase (LAP). Among these enzymes, five were used as markers for barley chromosomes in aneuploid hybrids, namely, EP (chromosome 1), G6PDH (chromosome 2), PGM (chromosome 4), 6PGDH (chromosome 5), and GOT (chromosome 6).

## Results

### *Crossability*

Crossabilities were calculated as percentages of the number of embryos formed divided by the number of florets pollinated in individual cross combinations, as shown in Table 1.

Among wheat cultivars, Norin 12, Norin 61, and Shinchunaga showed higher crossabilities then Chinese Spring, not only with Betzes barley but also with the other three barley cultivars. However, they were not significantly different at the 5% level by two-way analysis of variance, after transforming percentages ot angles (Table 2). A clear difference, significant at the 1% level, was found among barley cultivars as the male side. Betzes barley showed the highest crossability with wheat. Therefore, it appeared that genetic factors that control crossability between wheat and barley were present in both wheat and barley parents.

In order to compare the crossabilities in individual cross combinations, *t*-tests were carried out between the means of each cross combination, after transforming the data for individual ears to angles and using variation between ears within cross combinations as error (Table 3). Crossabilities of three cross combinations, Norin 12 × Betzes, Norin 61 × Betzes, and Norin 61 × Nyugoruden, were significantly higher than those of the others. Comparing the crossabilities among four wheat cultivars × Betzes barley, Norin 12, Norin 61, and Shinchunaga showed significant differences from Chinese Spring. This result clearly shows the genetic difference in crossability among wheat cultivars, although a wide range in crossability was observed among the crosses involving each wheat cultivar.

In total, 82 embryos were obtained from the 3,845 florets pollinated. All of the embryos, except four, germinated following embryo culture and grew well to seedlings. One embryo was used for another purpose not related to the present investigation. A total of 74 adult plants was obtained.

### *Somatic chromosome variation*

Among the 74 plants, 60 were used for examination of somatic chromosome number. Two or three root tips of

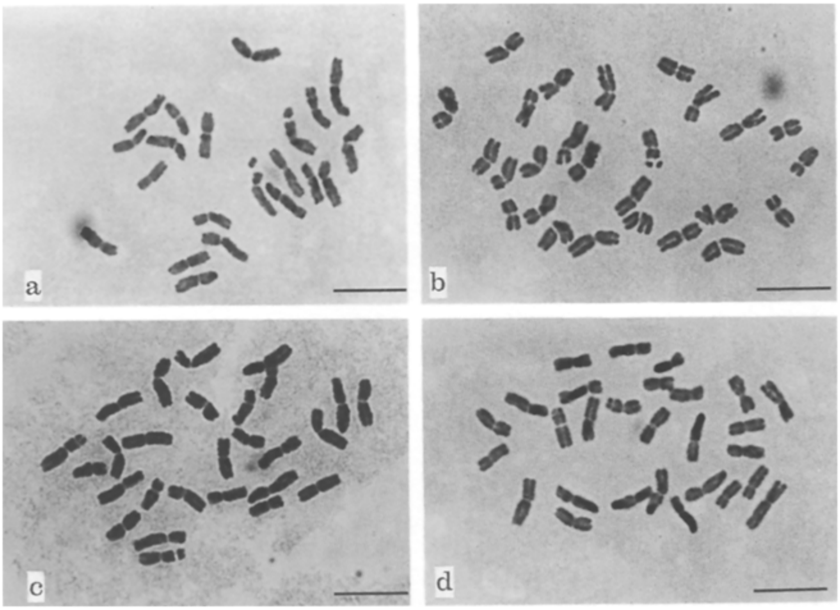

**Fig. 1a–d.** Somatic chromosomes of the hybrids between common wheat, *T. aestivum* and cultivated barley, *H. vulgare*. **a** Norin 61 × Betzes, 2n = 21; **b** Norin 61 × Betzes, 2n = 26; **c** Shinchunaga × Betzes, 2n = 27; **d** Norin 61 × Betzes, 2n = 28. Bar = 10 μm

**Table 4.** Chromosome number of the hybrid plants from the crosses between wheat and barley

| Cross combination | No. plants observed | Chromosome no. (no. plants) |
|---|---|---|
| CS × Betzes | 1 | 27 (1) |
| CS × Nyugoruden | 1 | 21 (1) |
| Norin 12 × Betzes | 12 | 21 (3), 21–22 mosaic (2) |
| | | 24 (2), 24–25 mosaic (1) |
| | | 28 (4) |
| Norin 12 × Harunanijou | 1 | 28 (1) |
| Norin 61 × Betzes | 14 | 21 (3), 21–22 mosaic (1) |
| | | 23 (2), 21–23 mosaic (1) |
| | | 26 (1), 22–23 mosaic (1) |
| | | 27 (1), 24–26 mosaic (1) |
| | | 28 (1), 27–28 mosaic (1) |
| | | 29 (1) |
| Norin 61 × Nyugoruden | 5 | 27 (1), 28 (4) |
| Norin 61 × Harunanijou | 2 | 28 (2) |
| Norin 61 × Kinai 5 | 1 | 27–28 mosaic (1) |
| Shinchunaga × Betzes | 8 | 21 (1), 24–26 mosaic (1) |
| | | 26 (1), 25–27 mosaic (1) |
| | | 27 (1), 28 (3) |
| Shinchunaga × Nyugoruden | 3 | 28 (1), 26–27 mosaic (1) |
| | | 27–28 mosaic (1) |
| Shinchunaga × Harunanijou | 8 | 28 (8) |
| Shinchunaga × Kinai 5 | 4 | 28 (4) |
| Total | 60 | 21 (8), 21–22 mosaic (3) |
| | | 23 (2), 21–23 mosaic (1) |
| | | 24 (2), 22–23 mosaic (1) |
| | | 26 (2), 24–25 mosaic (1) |
| | | 27 (4), 24–26 mosaic (2) |
| | | 28 (28), 25–27 mosaic (1) |
| | | 29 (1), 26–27 mosaic (1) |
| | | 27–28 mosaic (3) |

individual seedlings were observed. As shown in Table 4 and Fig. 1, chromosome number of the plants varied from 21 to 29. Twenty-eight seedlings had 28 chromosomes as excepted for the true hybrid. Eight seedlings had 21 chromosomes and were assumed to be wheat haploids. The other 23 seedlings were aneuploid hybrids and 1 had 29 chromosomes. About half of the aneuploid hybrids showed mosaicism for chromosome number, i.e., chromosome number differed by one or two among root tips in a plant.

This indicates that aneuploid hybrids occurred by elimination of barley chromosomes during embryogenesis from hybrid zygotes that originally had 28 chromosomes. Plant with a haploid chromosome number (21) probably originated by complete elimination of barley chromosomes. Chromosome elimination was not observed in any of the 11 hybrids in which Harunanijou was the male parent (Norin 12 × Harunanijou, Norin 61 × Harunanijou, and Shinchunaga × Harunanijou).

### *Chromosome constitution of the hybrids*

In order to examine the chromosome constitution of the hybrid plants, isozyme analyses were carried out using five isozyme markers of barley chromosomes (Fig. 2, Table 5). Among 19 plants examined, 2 with 28 chromosomes showed all five isozyme markers of barley. Four having 21 chromosomes showed none of them. Thus, the former were probably hybrids having 21 wheat and seven barley chromosomes and the latter were wheat haploids.

Elimination of barley chromosomes was characterized in the 13 aneuploid hybrids. The number of plants missing individual barley chromosomes, estimated from

**Table 5.** Patterns of presence of the isozyme bands that mark barley chromosomes in the hybrids between wheat and barley (+: present, −: absent)

| Hybrid | Cross combination | No. of chromosomes | Barley chromosome (isoenzyme marker) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 (EP) | 2 (G6P) | 4 (PGM) | 5 (6PG) | 6 (GOT) |
| WB-36 | Scn × Haruna | 28 | + | + | + | + | + |
| WB-63 | Scn × Betzes | 28 | + | + | + | + | + |
| WB-25 | CS × Betzes | 27 | − | + | + | + | + |
| WB-61 | Scn × Betzes | 27 | + | + | + | + | + |
| WB-67 | N61 × Betzes | 27 | + | + | + | + | − |
| WB-45 | Scn × NG | 26, 27 | + | + | + | − | + |
| WB-78 | Scn × Betzes | 25, 27 | − | + | + | − | + |
| WB-64 | Scn × Betzes | 26 | − | − | + | + | + |
| WB-59 | Scn × Betzes | 24, 26 | − | + | + | − | + |
| WB-74 | N61 × Betzes | 24, 26 | − | + | + | − | + |
| WB-54 | N12 × Betzes | 24, 25 | + | + | + | − | − |
| WB-77 | N12 × Betzes | 24 | + | − | + | − | + |
| WB-66 | N61 × Betzes | 23 | − | − | + | − | − |
| WB-72 | N61 × Betzes | 23 | − | + | − | + | − |
| WB-50 | N61 × Betzes | 21, 22 | − | − | − | − | − |
| WB-26 | N12 × Betzes | 21 | − | − | − | − | − |
| WB-60 | Scn × Betzes | 21 | − | − | − | − | − |
| WB-68 | N61 × Betzes | 21 | − | − | − | − | − |
| WB-69 | N61 × Betzes | 21 | − | − | − | − | − |

Abbreviations – Scn: Shinchinuga; CS: Chinese Spring; N61: Norin 61; N12: Norin 12; Haruna: Harunanijou; NG: Nyugoruden; EP: Endopeptidase; G6P: Glucose-6-phosphate dehydrogenase; PGM: Phosphoglucomutase; 6PG: 6-phosphogluconate dehydrogenase; GOT: Glutamate oxaloacetate transaminase

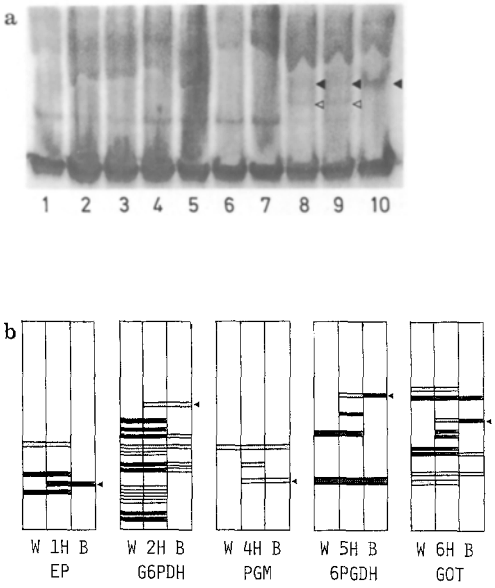

**Fig. 2 a and b.** Electrophoretic patterns of isozymes of the hybrid plants between wheat and barley. **a** Patterns of eight hybrid plants, wheat and barley for the enzyme 6PGDH. *Lanes 1–10;* common wheat cv Norin 61, hybrids WB-26, WB-66, WB-50, WB-71, WB-77, WB-64, WB-61, WB-47, and Betzes barley (for code number of the hybrids, see Table 5). *Solid* and *open arrowheads* indicate the band of chromosome 4 of barley and the hybrid band, respectively. **b** Diagram of the isozyme patterns of wheat (W), barley (B), and the hybrid plants each having barley chromosomes

**Table 6.** Number of eliminated barley chromosomes estimated from the absence of the marker isozyme bands in the 13 aneuploid hybrids

| No. of aneuploid hybrids | Barley chromosome eliminated | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 4 | 5 | 6 | 3 or 7 | 3 and 7 |
| 13 | 8 | 4 | 2 | 8 | 5 | 4 | 2 |

the absence of the isozyme markers, is summarized in Table 6. Both barley chromosomes 1 and 5 were eliminated in eight plants, while chromosome 4 was eliminated only in two. Although chromosomes 3 and 7 could not be examined, absence of both chromosomes 3 and 7, or either 3 or 7, was deduced from the chromosome number and from the presence or absence of isozyme markers of the other five chromosomes (Table 6).

### *Morphology of the hybrids*

Ears of the hybrid plants are shown in Fig. 3 together with those of the parent cultivars. Ear morphology of all the hybrid plants resembled that of wheat rather than barley. The plants had alternating florets in one spikelet at each ear node. Aneuploid hybrids with a few barley chromosomes showed similar ear morphology to that of

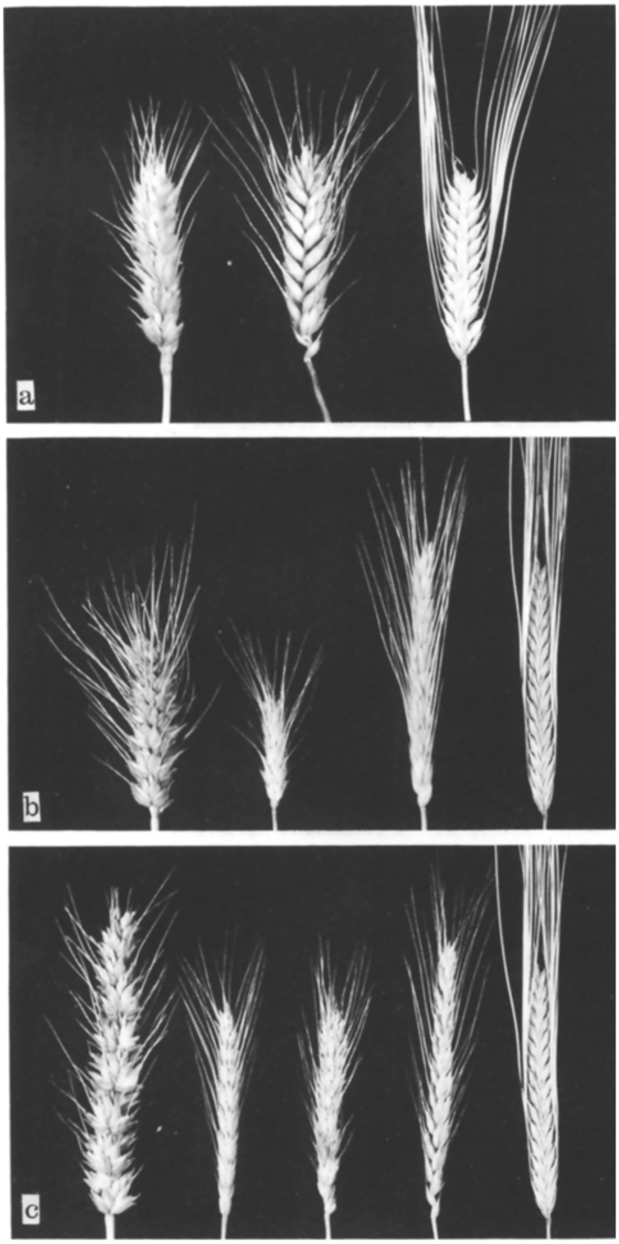

**Fig. 3a–c.** Ears of hybrid plants between common wheat, *T. eastivum*, and cultivated barley, *H. vulgare*, and their parents. From *left* to *right*: **a** Norin 12, hybrid (2n = 28), Harunanijou; **b** Norin 12, haploid (2n = 21), hybrid (2n = 28), Betzes; **c** Norin 61, haploid (2n = 21), hybrid (2n = 22, 23 mosaic), hybrid (2n = 29), Betzes

haploid wheat plants. Hybrids with 27–29 chromosomes showed longer and slender ears with more spikelets than the wheat parents.

## Discussion

High crossabilities between wheat and barley were found in the present experiment. There are three main factors that affect crossability between wheat and barley, namely: (1) the genotypes of the parental cultivars, (2) the environmental conditions in which parental plants grow and crosses are carried out, and (3) the embryo rescue technique.

Snape et al. (1979) reported that none of the European wheat used was crossable with *H. bulbosum* and that it also had poor crossability with rye. Riley and Chapman (1967) showed that almost all wheats of European and western Asia origin have poor crossability with rye. Since the wheat cultivars used in the present experiment were of Chinese and Japanese origin, they were expected to show high crossability with barley. In addition, wheat cultivars Chinese Spring and Shinchunaga were both classified as having the *kr1* and *kr2* alleles, which confer high crossability with rye to wheat (Sasaki 1987). Because Norin 61 was derived from Shinchunaga, it was also assumed to have *kr* alleles. Inagaki (1986) reported that Shinchunaga and Norin 61 showed high crossability with *H. bulbosum*. Because of the high correlation between the crossabilities of wheat with rye and *H. bulbosum* (Snape et al. 1979; Falk and Kasha 1981), these two cultivars were expected to show a similar degree of crossability to barley as Chinese Spring does. However, a large difference was observed among these three cultivars, i.e., Shinchunaga and Norin 61 showed higher crossabilities than Chinese Spring. Norin 12 also showed extremely high crossability with Betzes barley. This fact suggests the presence of crossability gene(s) other than *kr1* and *kr2* in Shinchunaga and other Japanese wheat cultivars.

Environmental conditions, especially temperature, might be important to crossability. Inagaki (1986) reported that seed set at 15 °C was significantly lower than that at 20 °C and 25 °C in wheat-*H. bulbosum* crosses. The present experiment was carried out in a greenhouse whose temperature was not higher than 25 °C during the day and was higher than 15 °C at night. Thus, high crossabilities could be obtained under the present temperature conditions.

In intergeneric crosses among cereals, gibberellic acid (GA) treatment has often been used to increase crossability. Larter and Chaubey (1965) showed that GA application promoted pollen tube growth and ovary development in barley-rye crosses. However, since the effect of GA seemd to be ambigous in our previous work (Shimada et al. 1987), we applied 100 mg/l 2,4-D to wheat as described in Inagaki (1986). Following the 2,4-D treatment of the wheat stems, tissues inside the stems developed well and ovaries became as large as normally fertilized ovaries, although they were filled with liquids without endosperm. The hybrid embryos floating in the liquids developed well until at least 20 days after pollination. Thus, a high frequency of embryo germination was obtained after transfer to embryo culture, i.e., only 4 out of 81 embryos inoculated did not germinate. It is not known whether or not 2,4-D affects pollen tube growth, but its effect on ovary and embryo developments, which are necessary prerequisites of the embryo rescue, have been clearly shown by Inagaki (1986). Other chemicals such as indole-3-acetic acid (IAA) (Falk and Kasha 1982), ε-amino-η-caproic acid (EACA), and L-lysine (Taira and Larter 1977) also have been used in intergeneric hybridization. Combinations of these chemicals may further improve the crossability of intergeneric crosses.

Chromosome elimination in intergeneric hybrids is a well-known means of haploid production in barley and wheat crosses with *H. bulbosum* (Barclay 1975; Kasha and Kao 1970; Kasha and Subrahmanyam 1973). In these cases, chromosomes of *H. bulbosum* were all eliminated. The occurrence of stable aneuploid hybrids, however, was reported in crosses between wild tetra- or hexaploid *Hordeum* species and diploid cultivated barley, *H. vulgare* (Barclay 1976; von Bothmer et al. 1986; Linde-Laursen and von Bothmer 1986; Pickering and Morgan 1985). Recently, Linde-Laursen and von Bothmer (1988), using C-banding, showed that barley chromosomes 1, 5, 6, and 7 were preferentially eliminated in aneuploid hybrids between wild hexaploid barley, *Hordeum lechleri*, and cultivated barley. In wheat-barley crosses, Islam et al. (1981) obtained wheat haploids and aneuploid hybrids in which barley chromosomes were partially eliminated. However, because of the small number of hybrids produced, preferential chromosome elimination could not be determined in their wheat-barley hybrids. Among 13 aneuploid hybrids obtained in the present experiment, barley chromosomes 1 and 5 were eliminated with the high frequencies, followed by chromosome 6. Although the presence or absence of chromosome 7 was not tested directly, it was assumed from the data of Linde-Laursen and von Bothmer (1988) that almost all of the four plants that were considered to have lost chromosome 3 or 7 in Table 6 might have lost chromosome 7. Thus, chromosome 7 might have been eliminated in six plants. If so, the tendency of chromosome elimination in the hybrids between wheat and barley showed close similarity with that of the hybrids between wild hexaploid barley and diploid cultivated barley.

Fukuyama (1987) reported in *Hordeum vulgare* (4x) × *H. bulbosum* (4x) that the genotype of the *H. bulbosum* parent exerted a greater effect on the frequency if chromosome elimination in the hybrids than that of the *H. vulgare* parent. In the present experiment, the frequency of chromosome elimination was clearly different among barley cultivars, since no chromosomes of Harunanijou were eliminated in 11 hybrid plants. This result clearly shows that the frequency of elimination of barley chromosome is influenced by the genotype of barley itself. Chromosome elimination might occur by inhibition of spindle function caused by the disturbance of protein synthesis (Bennett et al. 1976; Noda 1984). Pickering and Morgan (1985) proposed a hierarchy for eliminating chromosomes in hybrids among *Hordeum* species, *H. lechleri* (6x) and *H. proceum* (6x) > *H. depressum* (4x) > *H. vulgare* (2x) > *H. bulbosum* (2x). Common wheat, *Triticum aestivum*, may take the same or higher position than *H. lechleri* (6x) in this hierarchy.

In conclusion, hybrids between wheat and barley were obtained at high frequencies by selecting the cultivars used, controlling the environmental conditions, and treating with 2,4-D. By using these hybrid plants, incuding aneuploids, new barley chromosome addition lines can be produced.

*Acknowledgements.* The authers are grateful to Drs. A. J. Worland and D. A. Laurie, IPSR Cambridge Laboratory, UK, for their critical reading of the manuscript. Thanks are due to Dr. H. Niizeki for his guidance. This work was supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries, Japan.

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