Short Communication

Production of Haploid Barley Plants from Barley $\times$ Maize and Barley $\times$ Italian ryegrass Crosses

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Barley (*Hordeum vulgare* L.) haploids were obtained from two intergeneric crosses (barley $\times$ maize and barley $\times$ Italian ryegrass). In both crosses, 2,4-D treatment after pollination was necessary to obtain the barley haploid embryos. Especially in barley $\times$ maize crosses, it was proved that application of a 75ppm 2,4-D solution increased the efficiency of embryo differentiation (number of embryos obtained/number of florets pollinated) and haploid production (number of haploids obtained/number of florets pollinated). The efficiency of embryo differentiation and haploid production in barley $\times$ maize crosses ranged from 0.0% to 19.6% and from 0.0% to 6.9%, respectively, among the 14 barley cultivars examined. In the crosses of barley with Italian ryegrass, these values ranged from 0.0% to 16.7% and from 0.0% to 10.4%, respectively, among the 3 barley cultivars examined.

KEY WORDS: *Hordeum vulgare*, *Zea mays*, *Lolium multiflorum*, haploid production, intergeneric cross.

Introduction

Haploids of barley (*Hordeum vulgare* L.) can be produced by using interspecific crosses with *H. bulbosum* (KASHA and KAO 1970).

LAURIE and BENNETT (1986, 1987) reported that haploid wheat embryos were produced by crossing wheat with maize (*Zea mays* L.) through the elimination of maize chromosomes.

In the report on the wide hybridization of barley with different species of *Gramineae* by ZENKTELER and NITZSCHE (1984), globular embryos were obtained from crosses of barley $\times$ *Agropyron repens*, *Alopecurus agrestis*, *Pennisetum americanum* and *Lolium perenne*, but they degenerated 6 to 10 days after pollination, and no any embryos were obtained from crosses of barley $\times$ maize.

In this report we describe the production of barley haploids by the use of barley $\times$ maize crosses. Haploid production from crosses of barley $\times$ Italian ryegrass (*Lolium multiflorum* LAM.) is also described.

Materials and Methods

Maize F₁ hybrid, B 14 $\times$ CI 64 ($2n=2x=20$), was grown under a 23°C and 16-hour day-length regime. Vernalized plants of Italian ryegrass cv. Waseaoba ($2n=2x=14$) were kindly provided by Dr. K. NAKASHIMA, National Grassland Research Institute, and maintained under the same conditions as the maize material.

Emasculated spikes of barley were covered with polyethylene bags and pollinated with

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freshly collected pollen of maize and Italian ryegrass at the time of barely anthesis. A solution about 0.3ml to 0.5ml of a 75ppm 2,4-dichlorophenoxyacetic acid (2,4-D) was injected into the uppermost internodes of the barley stems immediately after pollination. The spikes were sprayed with 75ppm gibberellic acid (GA₃) 1 day after pollination. To evaluate the effect of 2,4-D treatment, 25, 50, 75, and 100ppm concentration of 2,4-D were applied in a cross of barley (cv. Chikukei 7565) × maize. Fourteen barley cultivars crossed with maize and three cultivars with Italian ryegrass with application of 75ppm 2,4-D solution.

Ten to eleven days after pollination, differentiated embryos were transferred to the modified B₅ medium as described by JENSEN (1975) and were incubated in the dark at 25°C for 1 week. Germinated embryos were maintained under a 25/20°C (day/night) and 12-hour day-length regime.

Four weeks after germination of the cultured embryos, the developed plants were randomly selected for chromosome counting.

Results and Discussion

1. Haploid production from barley × maize crosses

In the barley × maize crosses, regenerated plants were obtained (Fig. 1a). Table 1 shows the effect of 2,4-D concentration on efficiency of embryo differentiation and plant regeneration. When 2,4-D was not applied, the ovaries failed to grow and most of them were shrunken. The efficiency depended on the 2,4-D concentrations. Application of 2,4-D at 75ppm concentration was the most effective method to obtain the embryos and plants from a cross of barley × maize. Table 2 shows the varietal difference of embryo differentiation and plant regeneration of barley × maize. Embryos were obtained from 9 out of 14 cultivars examined, and plants from 7 out of 14 cultivars with application of 75ppm 2,4-D solution immediately after pollination. The efficiency of embryo differentiation and plant

Table 1. Effect of 2,4-D treatment on embryo differentiation and plant regeneration in a cross of barley cultivar Chikukei 7565 × maize

Concentration of 2,4-D ¹⁾ (ppm)	No. of florets pollinated	No. of embryos obtained	No. of regenerated plants obtained
0	70	0 (0.0) ²⁾	0 (0.0) ³⁾
25	80	3 (3.8)	1 (1.3)
50	60	3 (5.0)	0 (0.0)
75	204	32 (15.7)	14 (6.9)
100	129	4 (3.1)	2 (2.3)

¹⁾ 2,4-D was injected into the stem immediately after pollination.

²⁾ Figures in parentheses indicate the percentage of embryo differentiation (No. of embryos obtained/No. of florets pollinated).

³⁾ Figures in parentheses indicate the percentage of plant regeneration (No. of regenerated plants obtained/No. of florets pollinated).

regeneration ranged from 0.0% to 19.6% and 0.0% to 6.9%, respectively, among the barley cultivars. Chikukei 7565 showed a high rate of embryo differentiation (15.7%) and plant regeneration (6.9%). All the ten regenerated plants from crosses of barley with maize (8 from cv. Chikukei 7565 and 2 from cv. Haruna Nijo) which were randomly sampled had 7 chromosomes (Fig. 2a), and since the other regenerated plants showed the same morphological characteristics as the plants examined for the chromosome number, all plants were considered to be haploids.

2. Haploid production from barley × Italian ryegrass crosses

In the cross of barley × Italian ryegrass, regenerated plants were obtained (Fig. 1b). Embryos were obtained from 2 out of 3 cultivars examined and plants from 1 cultivar with application of 75ppm 2,4-D solution immediately after pollination (Table 3). When 2,4-D was not applied, the ovaries failed to grow and most of them were shrunken. Chikukei 7565 showed a high rate of embryo differentiation (16.7%) and plant regeneration (10.4%), with

Table 2. Varietal difference in embryo differentiation and plant regeneration through barley × maize crosses

Cultivar	No. of florets pollinated	No. of embryos obtained	No. of regenerated plants obtained
Chikukei 7565	204	32 (15.7) ¹⁾	14 (6.9) ²⁾
Yoshikei 15	64	6 (9.4)	1 (1.6)
Yoshikei 19	88	0 (0.0)	0 (0.0)
Yoshikei 21	104	3 (2.9)	3 (2.9)
Yoshikei 28	42	1 (2.4)	0 (0.0)
Kyushu Nijo 9	101	8 (7.9)	3 (3.0)
Kyushu Nijo 10	74	0 (0.0)	0 (0.0)
Nishino Gold	122	0 (0.0)	0 (0.0)
Kanto Nijo 25	104	1 (1.0)	1 (1.0)
Misato Golden	117	0 (0.0)	0 (0.0)
Amagi Nijo	93	1 (1.1)	0 (0.0)
Haruna Nijo	56	11 (19.6)	2 (3.6)
Tsuyushirazu	127	3 (2.4)	3 (2.4)
Nishinochikara	119	0 (0.0)	0 (0.0)

¹⁾, ²⁾ See notes of Table 1.

Table 3. Varietal difference in embryo differentiation and plant regeneration through barley × Italian ryegrass crosses

Cultivar	No. of florets pollinated	No. of embryos obtained	No. of regenerated plants obtained
Chikukei 7565	336	56 (16.7) ¹⁾	35 (10.4) ²⁾
Yoshikei 15	84	0 (0.0)	0 (0.0)
Kanto Nijo 25	126	5 (4.0)	0 (0.0)

¹⁾, ²⁾ See notes of Table 1.

Fig. 1. Regenerated plants obtained from barley $\times$ maize cross 5 weeks after embryo rescue (a) and from barley $\times$ Italian ryegrass cross 2 weeks after embryo rescue (b).

Fig. 2. Root tip chromosomes of regenerated plants having 7 chromosomes equivalent to the number of haploid barley chromosomes.

a: from barley $\times$ maize cross, b: from barley $\times$ Italian ryegrass cross.

values higher than those in the barley $\times$ maize crosses (15.7% and 6.9%, respectively). All the thirteen regenerated plants (from cv. Chikukei 7565) which were randomly sampled had 7 chromosomes (Fig. 2b). Since other regenerated plants had the same morphological characteristics as the plants which were examined for the chromosome number, plants obtained from the barley $\times$ Italian ryegrass crosses were also considered to be barley haploids.

These techniques of haploid production from crosses of barley with maize and with Italian ryegrass offer a new possibility for a barley haploid breeding program. A proper use of three techniques (barley $\times$ *H. bulbosum*, maize and Italian ryegrass crosses) may widen a range of application of haploid breeding method in barley.

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属間交雑（オオムギ×トウモロコシおよびオオムギ×イタリアンライグラス）

によるオオムギ半数体の作出

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トウモロコシおよびイタリアンライグラスの花粉を利用した属間交雑を行い，オオムギ半数体を作成した。2種類の属間交雑とも，授粉直後に2, 4-Dを穂首節間に注入することによって，半数体を作成することができた。また，トウモロコシとの交雑において75 ppmの2, 4-Dは幼胚着生率および半数体作出率を高めるために最も効果的であった。二条オオムギ14品種とトウモロコシとの交雑における幼胚着生率および半数体作出率はそれぞれ0.0~19.6%，0.0~6.9%であった。二条オオムギ3品種とイタリアンライグラスとの交雑では0.0~16.7%および0.0~10.4%であった。いずれの交雑においても筑系7565が最も高い半数体作出率を示した。

以上のように，2種類の属間交雑によってオオムギ半数体が得られることが明らかとなった。今後，属間交雑と半数体作出によく使用されている*H. bulbosum*による方法を適宜使い分けることによって，半数体育種の適用範囲を拡大し，効率をさらに高めることが可能と考えられる。