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A comparison of *Hordeum bulbosum*-mediated haploid production efficiency in barley using in vitro floret and tiller culture *

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Summary. A high efficiency of *Hordeum bulbosum*-mediated haploid production in barley has been achieved using a floret culture technique in which florets pollinated with *Hordeum bulbosum* are cultured on modified N_6 media containing 0.5 mg/l kinetin and 1.2 mg/l 2,4-D. Cultures were maintained at 25 °C with a 16 h photoperiod for 9 days before embryo rescue. In a comparison of haploid production efficiency using five F_1 hybrids from winter × winter and winter × spring barley crosses, 41.6 haploid plants/100 florets pollinated were produced using floret culture. Using detached tiller culture, 13.5 haploid plants/100 florets pollinated were produced. Higher efficiencies achieved with floret culture are attributed to the formation of larger, differentiated embryos. Such embryos lead to higher frequencies of plant regeneration. The F_1 from a winter × winter cross was inferior in haploid production compared to F_1s from winter × spring crosses. No genotype × technique interaction was observed.

Key words: (*Hordeum vulgare* L.) – *Hordeum bulbosum* – Haploid production – Floret culture – Detached tiller culture

Introduction

Of the various haploid production techniques available in barley (*Hordeum vulgare* L.), the *Hordeum bulbosum* method, because of its comparatively high efficiency, is favored in breeding programs intent on capitalizing upon sexual variation.

Haploid production efficiency with the bulbosum method is determined by a number of factors and their complex interactions, including genotypic effects of *H. vulgare* (Pickering 1983a), the interaction of *H. vulgare* and *H. bulbosum* (Simpson et al. 1980), and culture environment (Jensen 1977). In general, haploid production efficiency (HPE) can be expressed as

HPE = (seeds/florets) (embryos/seeds) (plants/embryos).

Seed set with most barley genotypes is high when compatible *H. bulbosum* genotypes are used as pollen donors (Simpson et al. 1980; Pickering 1983a). In practice, many programs, including our own, use bulk pollen from an array of *H. bulbosum* clones (Devaux 1986; P. Guerrero, personal communication). Simpson and Snape (1981) reported that more than 80% of florets pollinated gave seeds in winter barleys, while Pickering (1983a) reported that 81.7% of florets pollinated developed seeds in five spring habit cultivars. Exceptions are two-rowed cultivars of *H. vulgare* with *H. distichum* var. 'laevigatum' in their pedigree, which gave low seed set (< 30%) (Pickering and Hayes 1976). Pickering (1983b) showed that low seed set, or partial incompatibility in cultivar 'Vada' is under the control of a single dominant gene located on chromosome 7 in *H. vulgare*.

Not all seeds formed in the interspecific cross of *H. vulgare* and *H. bulbosum* have embryos. Bjornstad (1986) reported that 22.9% – 83.0% of seeds actually had embryos. Furthermore, not all embryos are capable of regenerating plants. Huang et al. (1984) reported that 21.7% – 30.1% of embryos cultured gave green plants. Undifferentiated embryos germinate and regenerate far fewer plants than differentiated ones (Pickering 1983a).

In studying the genetics of incompatible responses in *Hordeum vulgare* × *H. bulbosum* crosses, we have developed a floret culture technique that significantly in-

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creases the efficiency of *Hordeum bulbosum*-mediated haploid production. The objective of this investigation was to compare the floret and detached tiller culture techniques in terms of haploid production efficiency and its determinants. Both floret and detached tiller culture offer the breeder flexibility in sampling field-grown gamete donors. The culture techniques were tested using five F_1 populations of both winter × winter and winter × spring parentage.

Materials and methods

Five six-rowed F_1 hybrids from winter × winter and winter × spring barley crosses (pedigrees given in Table 1) were grown at the Hyslop Agronomy Farm, Corvallis, Oregon, USA in 1987/1988. Each of the five F_1 hybrids was tested with floret and detached tiller culture using a 2 × 5 factorial in a randomized block design with three replications. Six tillers from each F_1 hybrid were sampled from the field daily from May 17–19, 1988. Tillers, harvested 2 days prior to anthesis, were taken at random from solid-seeded 2-row, 3-m plots of each F_1 population. Of the six tillers taken daily from each F_1, three tillers were randomly assigned to each of the two culture techniques.

Inflorescences were emasculated within 2 h of tiller harvest. Tiller culms were then trimmed to 2–3 nodes and surface-sterilized in 20% Clorox[1] (5.25% sodium hypochlorite) for 10 min followed by five rinses with sterile distilled water.

Tillers were cultured following a modification of the protocol described by Kasha et al. (1978). Individual detached tillers were placed in 25 × 200 mm test tubes filled with 60 ml of sterile nutrient solution (Table 2). Cultures were sealed with Parafilm and placed in a greenhouse mist bench at 18/16 °C (day/night). Mist bench relative humidity was maintained at approximately 90% using a Mee 1000 microclimate control system with remote humidistat control. A 16-h photoperiod was achieved with supplemental lighting providing by General Electric Lucalox LV400 high pressure sodium lights suspended 1.5 m above the bench surface. Twenty-four hours after emasculation, spikes were pollinated as described by Jensen (1977) with bulk pollen from an array of four *H. bulbosum* clones.

Detached tiller culture

Detached tillers were maintained under high humidity in sterile nutrient solution, as described above. Twenty-four hours after pollination and once daily for 4 consecutive days, florets were treated, by the drop method, with 75 mg/l GA_3 as described by Kasha et al. (1978). Spikes were covered with glassine bags, except for a 4-h-period after each GA_3 application. Fourteen days after pollination, embryos were rescued and cultured on modified B_5 media as described by Jensen (1977).

Floret culture

Twenty-four hours after pollination with *H. bulbosum*, inflorescences were severed and surface-sterilized in 20% Clorox (5.25% sodium hypochlorite) for 10 min, followed by five rinses in sterile distilled water. Individual florets were cut at the rachis and approximately 20 florets were placed upright per disposable petri dish (60 × 15 mm) containing 10 ml of modified N6 (Chu et al. 1975) media (Table 2). Cultures were maintained in an incubator at 25 °C with a 16 h, 4 W/m² photoperiod supplied by General Electric F450CW fluorescent lights. Nine days after inoculation, embryos were rescued and transferred to modified B_5 media, as described by Jensen (1977) for plant regeneration.

With both detached tiller and floret culture, rescued embryos were maintained in an incubator at 25 °C in the dark for 10 days. Embryo cultures were then exposed to a 16 h, 4 W/m² photoperiod at 25 °C for 5 days. Counts of regenerated plants were made when plants had developed two-three leaves. Only vigorous green plants were considered in regeneration counts.

[1] Mention of commercial products does not represent an endorsement of the product by the Oregon Agricultural Experimental Station to the exclusion of other comparable products.

Table 1. Pedigrees of barley F_1 hybrids used as gamete donors for a comparison of floret and detached tiller culture as aids to *H. bulbosum*-mediated haploid production

Cross no.	Pedigree	Cross type
1	B-1285/Astrix × Wintermalt/Scio// NY6005-19/J-126	winter × winter
2	Wintermalt//Perga/Boyer × ID13597	winter × spring
3	Wintermalt//Perga/Boyer × Azure	winter × spring
4	Wintermalt/Scio//NY6005-19 J-126 × ID13597	winter × spring
5	Wintermalt/Scio/NY6005-19 J-126 × Azure	winter × spring

Table 2. Medium composition used for in vitro tiller and floret culture as aids to *H. bulbosum*-mediated haploid production in barley

Composition	Medium	
	Tiller	Floret
	(mg l^{-1})	
NO_3	1,000	2,830
NH_4NO_3	500	–
$(NH_4)_2SO_4$	–	463
KH_2PO_4	150	400
K_2HPO_4	25	–
$CaCl_2 \cdot 2H_2O$	150	166
$MgSO_4 \cdot 7H_2O$	150	185
$MnSO_4 \cdot 4H_2O$	10	4.4
$ZnSO_4 \cdot 7H_2O$	5	1.5
H_3BO_3	5	1.6
$Na_2MoO_4 \cdot 2H_2O$	0.5	–
KI	0.5	0.8
$CuSO_4 \cdot 5H_2O$	0.05	–
$CoCl_2 \cdot 6H_2O$	0.05	–
$FeSO_4 \cdot 7H_2O$	11.6	11.6
Na_2EDTA	15.5	15.5
Glycine	–	2.0
Thiamine · HCl	–	1.0
Pyridoxine · HCl	–	0.5
Nicotinic acid	–	0.5
Myo-inositol	–	2,000
2,4-D	–	1.2
Kinetin	–	0.5
Glutamic acid	–	160
Sucrose	–	90,000
Agar	–	8,000
pH	5.7	5.8

The total number of florets pollinated, seed set, number of embryos cultured, and number of plants regenerated were recorded for each sample. Percentage data were transformed by arcsin for statistical analysis.

Results

Because no genotype × technique interaction was detected in the analyses of variance of seed set, embryos/seeds, and plants/embryos (Table 3), only main effects are used for treatment and genotype comparisons.

Seed set

Seed set was comparable with the two culture techniques. Observed levels of seed set – 60.0% and 59.6% for floret and tiller culture, respectively – are low when compared to values reported for compatible spring barley. Caryopses from floret culture were green and turgid at the time of embryo rescue, while dry or yellow caryopses were observed in detached tillers.

The winter × winter F_1 hybrid (cross 1) had a significantly lower seed set than the winter × spring crosses, while seed set within the winter × spring crosses was comparable (Table 4).

Embryo development

The number of embryos per 100 seeds differed significantly ($P<0.01$) between floret culture and detached tiller culture. From every 100 seeds rescued, the floret culture technique produced up to 82.9 differentiated embryos, as compared to the maximum of 51.8 achieved with detached tiller culture. The floret culture technique produce larger (0.5 – ≥ 1.5 mm) and more differentiated embryos than the floret culture technique (data not shown). F_1s did not significantly differ in the number of embryos produced per 100 seeds (Table 4).

Plant regeneration

The number of plants regenerated per 100 embryos cultured differed significantly ($P<0.01$) between floret and detached tiller culture. Across genotypes, nearly twice as many plants/100 cultured embryos were produced using floret culture as opposed to detached tiller culture (81.9 versus 45.3). The larger and more differentiated embryos produced with floret culture germinated faster than did embryos from detached tiller culture (data not shown). Genotypes did not differ significantly in regeneration frequency (Table 4).

Overall efficiency

The number of haploid plants produced per 100 florets pollinated, haploid production efficiency (HPE), is a function of three parameters: seed set, number of embryos per 100 seeds, and number of plants per 100 em-

Table 3. Mean squares from the analysis of variance of seed set, number of embryos cultured per 100 seeds, and number of plants regenerated per 100 embryos for five barley F_1 hybrids and two culture techniques for *H. bulbosum*-mediated haploid production

Source of variation	df	Seed set (% of florets)	Embryos cultured (% of seeds)	Haploid plants (% of embryos)
		Mean squares		
Replications (R)	2	86.94	484.93*	32.02
Culture Tech (C)	1	0.97	3,347.52**	4,291.25**
Genotype (G)	4	227.49**	108.42	62.31
G × C	4	26.73	87.73	150.56
Error	18	44.85	94.74	122.37

*, ** Significance at the 0.05 and 0.01 probability levels, respectively

Table 4. Comparison of the main effects of genotypes on seed set, embryos cultured and plants regenerated for two culture techniques used as aids to *H. bulbosum*-mediated chromosome elimination

Genotype	Seed set (% of florets)	Embryos cultured (% of seeds)	Plants regenerated (% of embryos)
Cross 1	42.77 b	55.79 a	65.83 a
Cross 2	66.27 a	70.98 a	65.96 a
Cross 3	62.69 a	67.74 a	58.85 a
Cross 4	58.11 a	70.32 a	67.30 a
Cross 5	69.38 a	72.07 a	60.27 a

Means followed by the same letter are not significantly different at the 0.05 probability level, as determined by Duncan's multiple range test of arcsin transformed data

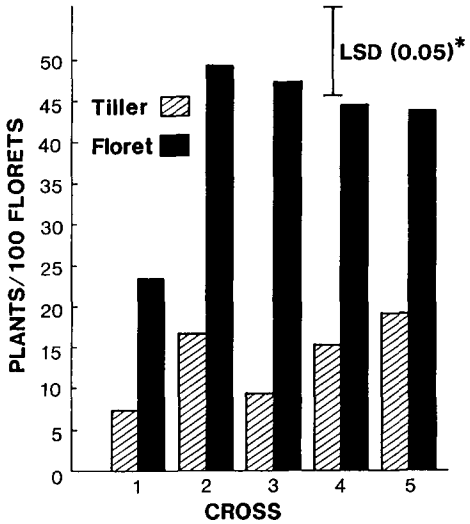

Fig. 1. A comparison of haploid production efficiency (HPE) using floret and detached tiller culture as aids to *Hordeum bulbosum*-mediated haploid production. * *Vertical bar* designates LSD (0.05) for testing differences between arcsin-transformed culture treatment means. Data presented are actual percentage values

bryos. The HPE of floret culture was 41.6 plants per 100 florets pollinated. In contrast, the HPE of detached tiller was 13.5 plants per 100 florets pollinated. The difference was highly significant ($P < 0.01$). Floret culture gave consistently higher HPE values over all crosses (Fig. 1).

The higher efficiencies of haploid production achieved with floret culture are due to more embryos/100 seeds and more plants/100 embryos. With the floret culture technique, larger differentiated embryos are produced at higher frequencies, and such embryos, in turn, lead to higher rates of plant regeneration. The genotypic variation for HPE was significant ($P = 0.03$). Cross 1, the winter × winter cross, because of significantly lower seed set, had a lower HPE than the four winter × spring crosses (Table 4).

Discussion

Seed set in the five hybrids from winter × winter and winter × spring barley crosses, ranging from 42.77% – 69.38%, was lower than reported values for compatible spring barley genotypes (Pickering 1983a; Huang et al. 1984). The winter × winter cross had a significantly lower seed set than the winter × spring crosses. Nonetheless, there is no evidence to indicate that growth habit per se is involved in crossability with *Hordeum bulbosum*.

Despite relatively low seed set, high haploid production efficiencies (23.4% – 49.36%) were achieved using floret culture of winter × winter and winter × spring F_1s. From the components of haploid production efficiency (HPE), it is apparent that the high HPE of floret culture technique resulted from the high percentage of seeds with differentiated embryos (82.91%) and the high percentage of cultured embryos capable of regenerating plants (81.97%).

The haploid production efficiency achieved with detached tiller culture, although much lower than with floret culture, is comparable to that reported by Huang et al. (1984) for compatible spring genotypes in which the seed set was more than 80%, and is higher than that reported for winter barley (Simpson et al. 1980; Devaux 1987).

Numerous attempts have been made to increase the efficiency of the bulbosum technique through hormone treatments (Kasha et al. 1978), optimization of culture media and culture conditions for plant regeneration (Jensen 1977), screening for compatibility of *H. bulbosum* clones (Pickering 1980), and the use of special pollination bags (Pickering 1982). Compared to these approaches, floret culture provides a simple defined system allowing for optimum embryo development. Floret culture may be useful in other wide cross haploid production systems and perhaps for sexual gene transfer via wide crosses (Laurie and Bennet 1988), as well as in wide crosses where seed survival, not crossability, is a factor (Stitch and Snape 1987).

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