A Highly Efficient Haploid Producing Clone of *Hordeum bulbosum* Collected in Morocco

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True seeds of *Hordeum bulbosum* were collected in the northern part of Morocco in 1987, and 16 *H. bulbosum* clones were produced from the introduced seeds. All clones obtained were diploid ($2n=2x=14$), and all regenerated plants obtained from hybridization between barley and these clones were haploid ($2n=x=7$). Percentages of embryo formation (number of embryos obtained/number of florets pollinated) and haploid production (number of haploids obtained/number of florets pollinated) ranged from 3.7 to 67.8% and from 1.2 to 35.7%, respectively. One *H. bulbosum* clone was selected for its high haploid production efficiency. The present results show that this clone can be utilized to improve the doubled haploid barley breeding program.

KEY WORDS: *Hordeum bulbosum*, haploid, barley, genetic resources, Morocco.

Introduction

Haploids of barley can be produced from the interspecific cross between *Hordeum vulgare* L. ($2n=2x=14$) and *H. bulbosum* ($2n=2x=14$) (KASHA and KAO 1970). The haploid production efficiency is genetically controlled and influenced by genotypes of both species (JENSEN 1976, PICKERING 1983). To increase the haploid production efficiency, it is useful to detect and exploit genetical variations among *H. bulbosum* genotypes in producing barley haploids. PICKERING and RENNIE (1990) selected superior *H. bulbosum* clones obtained from hybridization of two clones which have been widely used in doubled haploid breeding programs.

In 1987, a joint field survey was conducted in Morocco and some *H. bulbosum* true seeds were collected by one of the authors (DAMANIA *et al.* 1987). Since *H. bulbosum* is self-sterile (LUNDQVIST 1962), it was considered that collected seeds were heterogeneous and might have various genetic variations. The objective of this study was to investigate the difference in haploid production efficiency and select the superior *H. bulbosum* clones for doubled haploid barley breeding programs.

Materials and Methods

Thirty true seeds of *H. bulbosum* collected at a river bank 38 km north of Tetouan, located in northern part of Morocco, were sown on 27 November 1987 in 1/5000a Wagner pots at the Fukuoka Agricultural Research Center. Sixteen *H. bulbosum* clones were obtained and repotted into the same size pots with a single plant per pot on 20 June 1988. These clones were vernalized at a temperature at 5°C with an 8-h photoperiod for 8 weeks. After vernalization, the clones were grown in a greenhouse maintained under a 24-h photoperiod at a minimum 15°C until flowering. A Cb2920 clone which was supplied by Dr. T.

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FUKUYAMA, Niigata University and which had been used in our breeding program was included as a check clone. All clones were tested for the percentages of embryo formation (number of embryos obtained/number of florets pollinated) and haploid production (number of haploids obtained/number of florets pollinated) using the two-rowed barley cultivar Kanto Nijo 25 as the female plant. Kanto Nijo 25 had a uniquely high percentage of haploid production when crossed with the check clone Cb2920 (FURUSHO *et al.* 1990), and it was considered that differences among *H. bulbosum* clones for haploid production could be clearly compared. The method for obtaining haploids followed the procedures reported by FURUSHO *et al.* (1990).

The analysis of variance was performed and comparisons for efficiencies of embryo formation and haploid production were made using Duncan's multiple range test. In each pollination, about 20 florets (i.e., one spike) from each of 17 clones per replicate were pollinated, and the percentage data were transformed by arcsine prior to statistical analysis. Each pollination was replicated 5 to 11 times (i.e., 5 to 11 spikes were pollinated). The chromosome numbers of *H. bulbosum* clones and 290 plants out of all 344 regenerated plants were counted in the squash preparations of root-tips stained with acetocarmine.

Table 1. Embryo formation and haploid production from *H. vulgare* crossed with *H. bulbosum* clones collected in Morocco

Clone no.	No. of florets pollinated	Embryos/florets pollinated (%)	No. of plants regenerated	Haploids/florets pollinated (%)	No. of plants examined chromosome numbers
Hb254-1	171	49.7 cde ¹⁾	45	26.3 bc ¹⁾	38 ²⁾
Hb254-2	61	67.2 ab	12	19.7 c	10
Hb254-5	160	48.8 cde	14	8.8 def	10
Hb254-6	178	37.6 ef	12	6.7 def	7
Hb254-7	81	3.7 g	1	1.2 g	1
Hb254-8	82	42.7 def	4	4.9 f	4
Hb254-9	111	48.6 cde	21	18.9 c	21
Hb254-10	88	60.2 abcd	29	33.0 ab	25
Hb254-12	102	52.0 abcde	6	5.9 ef	4
Hb254-13	89	28.1 f	10	11.2 de	3
Hb254-15	149	55.0 abcde	40	26.8 bc	33
Hb254-16	130	41.5 def	12	9.2 def	11
Hb254-17	143	67.8 a	51	35.7 a	43
Hb254-18	117	47.9 cde	14	12.0 d	13
Hb254-29	98	50.0 bcde	20	20.4 c	15
Hb254-30	93	59.1 abcd	9	9.7 def	9
Cb2920 (Check)	161	64.0 abc	44	27.3 bc	43

¹⁾ Means followed by the same letter (within a column) are not significantly different at $P \leq 0.05$, as determined by Duncan's multiple range test of arcsine transformed data.

²⁾ All the regenerated plants examined had 7 chromosomes which is equivalent to the number of haploid barley chromosomes.

Results and Discussion

Cytological examination showed that all *H. bulbosum* clones obtained from seeds collected in Morocco were diploid. Although the degree of winter habit of these clones was not identified, all clones flowered after vernalization treatment under 5°C, 8-h photoperiod for 8 weeks. Cytological examination also showed that all the 290 regenerated plants obtained from hybridization with *H. bulbosum* were haploid (Table 1), 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 haploid. It was reported that a hybrid which contained both parental sets of chromosomes was obtained from crossing barley with *H. bulbosum* (PICKERING 1980). However, no hybrids were obtained, and the *H. bulbosum* chromosomes were completely eliminated from the samples in this study.

The percentages of embryo formation and haploid production in crosses between barley and 16 *H. bulbosum* clones from Morocco and Cb2920 are shown in Table 1. These values ranged from 3.7 to 67.8% and from 1.2 to 35.7%, respectively. ANOVA revealed that differences among *H. bulbosum* clones were significant. There were no clones significantly higher than the check clone Cb2920 with respect to embryo formation. However, the haploid production percentage of Hb254-17 clone (35.7%) was significantly higher than that of the check clone Cb2920 (27.3%) employed in our haploid breeding program.

Identifying the superior haploid producing clones (SIMPSON *et al.* 1980, PICKERING and RENNIE 1990) or exploiting the better culture techniques (CHEN and HAYES 1989) have been utilized to increase the efficiency of haploid production. Although it is difficult to directly compare the efficiencies of embryo formation and haploid production with their results, the values for the selected clone in this study were higher than that of the check clone Cb2920 and moderately high for a haploid barley breeding program. The selected clone also produced haploid plants exclusively. Thus, a highly efficient haploid producing clone obtained in this study can be utilized to improve a doubled haploid barley breeding program.

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モロッコで収集した半数体作出能力の高い*Hordeum bulbosum*

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モロッコで収集した野生オオムギ (*Hordeum bulbosum* L.) について, 二条オオムギとの交雑によって得られる半数体の作出能力を調べた。収集した種子から養成した 16 クロンの *H. bulbosum* はすべて二倍体であった。

二条オオムギ系統関東二条 25 号を母に, 得られた *H. bulbosum* 16 クローンおよび比較クロン Cb 2920 を花粉親として種間交雑を行ったところ, 胚とそれを培養して 344 個の植物体が得られた。これらのうち 290 個体について染色体数を観察したところ, すべてオオムギ半数体であった。染色体を観察しなかった個体も形態的にすべて半数体であると判断され, 本研究に用いた *H. bulbosum* はすべて染色体が完全に消失するクロンであると考えられた。また, 胚着生率 (胚着生数/授粉小花数) および半数体作出率 (半数体数/授粉小花数) はそれぞれ 3.7~67.8%, 1.2~35.7% であり, *H. bulbosum* クローン間に有意な差が認められた。そのうちの 1 クロンは Cb 2920 よりも半数体作出率が有意に高かった。

したがって, このクロンを用いることによって二条オオムギにおける半数体育種法の効率をさらに高めることができる。