The inheritance of genetic markers in microspore-derived plants of barley *Hordeum vulgare* L.


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Summary. Biochemical, molecular and morphological markers have been used to monitor the segregation of alleles at major gene loci in microspore-derived lines of four spring barley crosses and their parents. Significant deviations from the expected Mendelian ratios were observed for four of the ten markers studied in the cross. Distorted ratios were associated with loci located on chromosomes 4H and 6H. The differential transmission of alleles was in favour of the responsive parent (Blenheim) used in the anther culture studies. For the x-Amy-1 locus on chromosome 6H, the preferential transmission of Blenheim alleles was most pronounced in the haploid regenerants that were colchicine treated. These results are discussed in relation to the genetic control of androgenetic response in barley and with respect to the exploitation of another culture in barley improvement.

Key words: Doubled haploids – Microspore – Isozymes – RFLPs – Barley

Introduction

Haploidisation and subsequent chromosome doubling provides an opportunity to produce very quickly desired gene combinations from segregating material in the homozygous state. The increase in selection efficiency associated with doubled haploidy (DH) indicates that DH procedures offer considerable promise in plant breeding. Snape et al. (1986) have identified three criteria for the successful and cost-effective incorporation of DH into breeding programmes. These criteria are: the production of large numbers of DH of all genotypes should be easy and consistent, DH should be genetically normal and stable and the DH population should contain a random sample of the parental gametes.

Regeneration of plants from microspores is one of the methods available for haploid production in barley, *Hordeum vulgare* (Choo et al. 1985). Refinements in another culture procedures, which include the substitution of sucrose by maltose in the culture medium (C. P. Hunter, personal communication; Finnie et al. 1989; Powell 1990), have resulted in a dramatic improvement in the efficiency of green plantlet regeneration from microspores. In addition, on maltose-based culture media, the mode of regeneration of plantlets tends to be via an embryogenic route rather than via a callus phase. The absence of an intermediate callus phase will contribute to the genetic stability of microspore-derived plantlets of barley. Two of the three criteria identified by Snape et al. (1986) for the inclusion of DH procedures in barley breeding therefore appear to have been satisfied. The third criterion relating to the utilisation of barley anther culture involves determining whether DH derived by anther culture are an unbiased sample of the parental gametes. A random sample of gametes is desirable if the potential genetic variation within a cross is to be fully exploited.

The segregation of alleles at specific loci may be used to test for the random assortment of genetic information in DH extracted from *F*₁ hybrids. Genetic analysis of morphological and disease response traits has been carried out in early studies of anther-derived plants of *Nicotiana tabacum* (Nakata 1971; Nakata and Kurhara 1972) and *Hyoscyamus niger* L. (Corduan 1975). In *N. tabacum*, the phenotypic ratios were found to be in
agreement with the expected (1:1) segregation ratios, whereas in studies with \textit{H. niger}, significant deviations from the expected ratios were observed.

Plants recovered from microspore culture may be skewed in the direction of one of the parents by selective regeneration of certain genotypes. Deviations from the expected gametic ratio among microspore-derived plants of broccolis were reported by Orton and Bowers (1985). Powell et al. (1986) compared barley DH populations produced by anther culture and the \textit{H. bulbosum} technique, and observed deviations from the expected gametic frequency for three of five loci in the anther-culture-derived DHs and for one locus among the \textit{H. bulbosum} DHs. \textit{Japonica} rice cultivars are more responsive in another culture than \textit{Indica} cultivars, and a skewed distribution for 4 out of 12 isozyme markers was observed by Guiderdoni et al. (1989) in microspore-derived plants from a \textit{Japonica} \times \textit{Indica} rice hybrid. These results emphasise the need to monitor the segregation of alleles in DH populations derived from \textit{F\textsubscript{1}} hybrids.

In this study the inheritance of ten marker genes located on five of the seven pairs of barley chromosomes in populations of microspore-derived lines derived from two pairs of reciprocal \textit{F\textsubscript{1}} hybrids have been analysed. Both colchicine doubled haploid and spontaneous diploid lines have been included in the analysis.

\textbf{Materials and methods}

\textit{Plant material}

Microspore-derived plants were regenerated from reciprocal crosses between the spring barley cultivar, Blenheim, and two breeding lines, TS264 and E224. The parents were chosen to complement the good malting quality characteristics of Blenheim with improved disease resistance genes from TS264 and E224. Methods for plantlet regeneration from microspores have been described in detail previously (Finnie et al. 1989).

\textit{Detection of polymorphism in parental material}

\textit{Restriction fragment length polymorphism (RFLP)}. Leaf material was harvested from field-grown plots of anther culture lines derived from crosses between Blenheim \times E224 and E224 \times Blenheim. Total plant DNA was extracted from powdered, freeze-dried leaves by a modification of the CTAB extraction procedure (Murray and Thompson 1980). Following restriction endonuclease digestion (10 \mu g DNA), the resulting DNA fragments were fractionated on 1% agarose/TAE gels and transferred to HYBOND N + charged nylon membrane (Amersham) by alkaline blotting (Reed and Mann 1985). Probe labelling and hybridisation conditions were as described by Sharp et al. (1988). The sources of clones used are given in Table 1.

\textit{Protein polymorphism}. The isoelectric focusing (IEF) methods used have been described by Thompson et al. (1990). Polymorphic systems are given in Table 2 together with the chromosomal location of their structural genes.

\textit{Morphological markers}. The microspore-derived lines, together with the three parents, were grown in replicated field experi-

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Genetic marker & Chromosomal location (reference) & Method of detection & Parents exhibiting polymorphism \\
\hline
Hordein-1 & 1HS(1) & RFLP: pBl1(6) & Blenheim/E224 \\
Hordein-2 & 1HS(1) & RFLP: pCp387(6) & Blenheim/E224 \\
rDNA & 5HS(2) & RFLP: pBG35(7) & Blenheim/E224 \\
Leaf esterase-1 & 3HL(3) & IEF & Blenheim/E224/T5264 \\
Leaf esterase-2 & 3HL(3) & IEF & Blenheim/E224 \\
\beta-Amlyase-1 & 4HL(4) & IEF & Blenheim/TS264 \\
Water-soluble protein-3 & 4H(5) & IEF & Blenheim/TS264 \\
\alpha-Amlyase-1 & 6HL(3) & IEF & Blenheim/E224/T5264 \\
Water-soluble protein-2 & Unknown & IEF & Blenheim/TS264 \\
Juvenile growth habit & Unknown & Morphological & Blenheim/E224 \\
\hline
\end{tabular}
\caption{Genetic markers used in the segregation analysis}
\end{table}

\textbf{Results}

The protein and RFLP profiles for the three parents are given in Fig. 1. The segregation ratios of the parental phenotypes for each marker are presented in Table 2. Segregation of alleles at the hordein loci on chromosome 1H does not deviate significantly from the expected 1:1 ratio. Similarly, alleles at the rDNA locus do not deviate significantly from the expected ratio. The polymorphism detected in the Blenheim \times E224 cross and its reciprocal corresponds to the \textit{Nor-H3} (\textit{Rmr2}) locus, which segregates for the 6.2-kb fragment located to chromosome 5H (Saghai-Marof et al. 1984). The leaf esterase loci, \textit{Est-1} and \textit{Est-2}, which are located on chromosome 3H, are polymorphic for the three parental genotypes. Segregation of alleles at these two loci does not differ significantly from the expected ratio (Table 2). Two isozyme loci located on chromosome 4H were used to characterise microspore-derived lines from the Blenheim \times TS264 cross and its reciprocal. Alleles at both the \beta-\textit{Amy}-1 and \textit{Wsp}-3 locus do not deviate from the expected ratio in the Blenheim \times TS264 cross, although there is an apparent