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BREEDING MALTING BARLEY USING HAPLOID TECHNIQUES

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ABSTRACT

A joint project to produce doubled haploid malting barley cultivars was established in 1980 between the Canterbury (NZ) Malting Co. Ltd and the Crop Research Division of the New Zealand Department of Scientific and Industrial Research at the Lincoln Research Centre. This programme uses the Bulbosum Method to produce haploids. Haploids are produced from crosses and selections deemed to have a high probability of producing a new cultivar possessing disease resistance, agronomic merit, high yield and malting quality. A steamlined evaluation system has been developed where doubled haploids are assessed for simply inherited characters simultaneously with seed increase in the early stages of the programme. Micromalting begins at the stage of the first unreplicated yield trials. When lines with obvious agronomic defects have been eliminated, standard cultivars of known yield potential and quality are used in replicated trials to identify superior doubled haploid lines. Superior doubled haploid lines are recycled as parents as soon as they are identified.

KEYWORDS

Hordeum bulbosum, Bulbosum Method, malting quality.

INTRODUCTION

The use of haploids in breeding self pollinated crops has several advantages over conventional methods of developing pure line cultivars. Haploidy, combined with efficient chromosome doubling, provides a single step procedure for obtaining complete homozygosity. Such a procedure is faster than accelerating generations to obtain a high level of homozygosity. The assurance of homozygosity allows for immediate evaluation of quantitative as well as qualitative traits. Recessive genes are not masked and there is no necessity for reselection after identification of a desirable line. With no genetic variation between generations and all genes fixed, heritabilities and genetic variances are at a maximum; environment is the only variable factor. Haploid techniques that give a random sample of gametes produce populations with a random assortment of the potential genotypes derived from the materials used as parents. The derived homozygous lines can be used directly as a source of cultivars, as a sampling technique to determine the most promising crosses or populations, as a method of determining the genetic structure of the source population, as a method of recovering genetic recombinations directly, and/or to assess and exploit the effects of various selection procedures applied to the source population before haploid production. If haploids are taken directly from F_1 plants, the method of propagating, during the process of reaching homozygosity, does not influence population structure.

The Bulbosum Method (Kasha and Kao, 1970) is the most commonly used method to produce barley haploids (Choo *et al.*, 1985). Studies have shown that this method gives high frequencies of haploids from most barley genotypes, random samples of the expected gametes, and populations comparable to those developed by pedigree bulk and single seed descent methods from the same crosses (for review see Kasha and Reinbergs, 1979; 1981; Choo *et al.*, 1985). The Bulbosum Method has been used to generate populations for genetic studies (Choo *et al.*, 1979; 1982; Choo and Reinbergs, 1979) and in conjunction with recurrent selection for population improvement (Patel *et al.*, 1985). High yielding lines have been produced in frequencies greater than expected in some crosses (Kasha *et al.*, 1977; Turcotte *et al.*, 1979). A number of programmes using the Bulbosum Method for plant breeding and genetical research have been established around the world. Doubled haploid cultivars have been released in Canada (Mingo, Rodeo), New Zealand (Gwylan) and the U.K. (Doublet).

This paper outlines the procedures which have been used at the Lincoln Research Centre in New Zealand to develop parental materials, produce and double barley haploids, and evaluate the resulting homozygous lines in a joint government-private industry programme.

HISTORY OF THE JOINT PROJECT

In June of 1980, the Canterbury (NZ) Malting Co. Ltd (CMC) and the Crop Research Division (CRD) of the New Zealand Department of Scientific and Industrial Research

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(DSIR) jointly established a project to produce doubled haploid barley. The objective of the project was to produce high yielding, disease resistant, two-rowed cultivars with malting and brewing quality adapted to New Zealand cultural practices. The joint project was housed at the CRD Lincoln Research Centre. The Bulbosumm Method was to be used to produce haploids. Mr Pablo Guerrero was employed as a Science Technician to produce and double the haploids.

In 1981, Mr Richard Pickering, of the Welsh Plant Breeding Station, spent six months at Lincoln upgrading facilities and techniques for haploid production. Several hundred doubled haploids were produced at that time and increased in an off-season nursery. In September of 1982 Dr Duane Falk was appointed visiting scientist to the CRD-DSIR to intensify the development of malting barley breeding materials for haploid production and to supervise the evaluation of the resulting doubled haploids. In May of 1983, Dr Falk was signed to a contract with the CMC as a Research Scientist with the Joint Doubled Haploid Barley Breeding Programme. The programme currently consists of a scientist responsible for breeding and evaluation, a science technician for haploid production and doubling and a part-time student, during the summer season, to assist with glasshouse and field work. Cooperative projects are carried out with the Plant Diseases Division and the Plant Physiology Division of DSIR, and other sections of the Crop Research Division as part of the overall breeding effort.

BREEDING MATERIALS AND METHODS

To produce high yielding, disease resistant, two-rowed malting barley cultivars adapted to New Zealand, cultivars of known high yield, disease resistance and malting quality have been collected from around the world. Old land races and cultivars from the early part of this century, as well as recently developed cultivars and breeding lines, have been brought to New Zealand for evaluation. Lines with reasonable adaptation, or resistance to New Zealand pathogens have been selected and crossed with locally adapted materials. This has broadened the germplasm base and simultaneously introduced many desirable characters. Cultivars from central Europe and the western parts of Canada and the United States have been the primary sources of malting quality as these regions produce mainly two-rowed malting barleys. These materials form the germplasm pool which is used as a source of parents for haploid production.

There are two major criteria for determining whether a cross will be used for haploid production or not. The first consideration is the *possibility* of producing a good cultivar; i.e. one or both parents having good disease resistance, yielding ability, malting quality and adaptation. The second consideration is the *probability* of producing a good cultivar from a particular cross. If it is a wide cross or if neither parent has the desired level of disease resistance or malting quality, there is very little chance of actually achieving a good combination of traits even though the potential may exist (Fig. 1). The expected mean, range and variance of a cross are used as guidelines for selecting or producing crosses for haploid production.

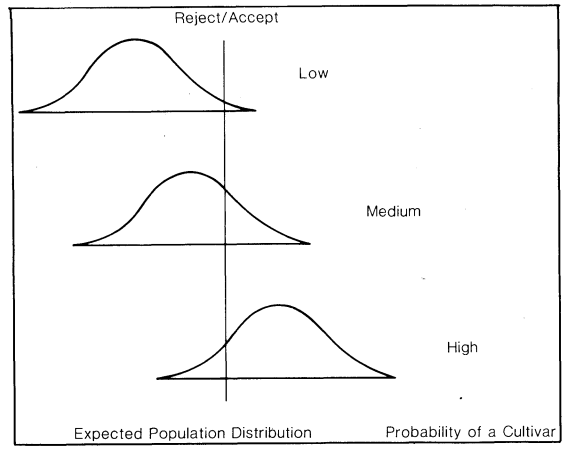

Figure 1. The probability of producing a new cultivar is a direct function of the potential of the material used as a source of haploids.

Many crosses are made between lines which possess one or more simply inherited defects along with their desired traits. It would not be efficient to run these materials directly through the haploid system because a large proportion of the resulting lines would be discarded solely on the basis of not having the more desired alleles at a few loci. Therefore, F1 plants from such crosses are grown and selfed to produce F2 seed. The F2 generation is grown to screen for the desired plant type. Selected plants are harvested individually and the progeny may be screened for disease resistance in laboratory or field nurseries. Selected plants are then crossed to other selected plants in the same family or related families or selected plants in unrelated crosses. The F1 plants from this second series of crosses may be run through the haploid programme if there is a sufficiently high probability of producing progeny with most of the desired characters. To accumulate more desired traits most F1 plants are used to produce selfed seed for further cycles of selection and crossing. Some outstanding selections from F2 or later generations are used as source materials for haploid production (Fig. 2).

Few haploids are being produced from cultivar x cultivar crosses; most are produced from crosses of first or second cycle selections and related cultivars or from crosses of selected doubled haploids with cultivars or other doubled haploids. The greater the probability that a cross will produce a desirable line, the more efficient it is to run it through the haploid system as the probability of producing discard lines is reduced. The effort applied to generating parents and crosses more suitable for exploitation via a haploid system is not great when compared to the time and effort used to produce and double the haploids and to

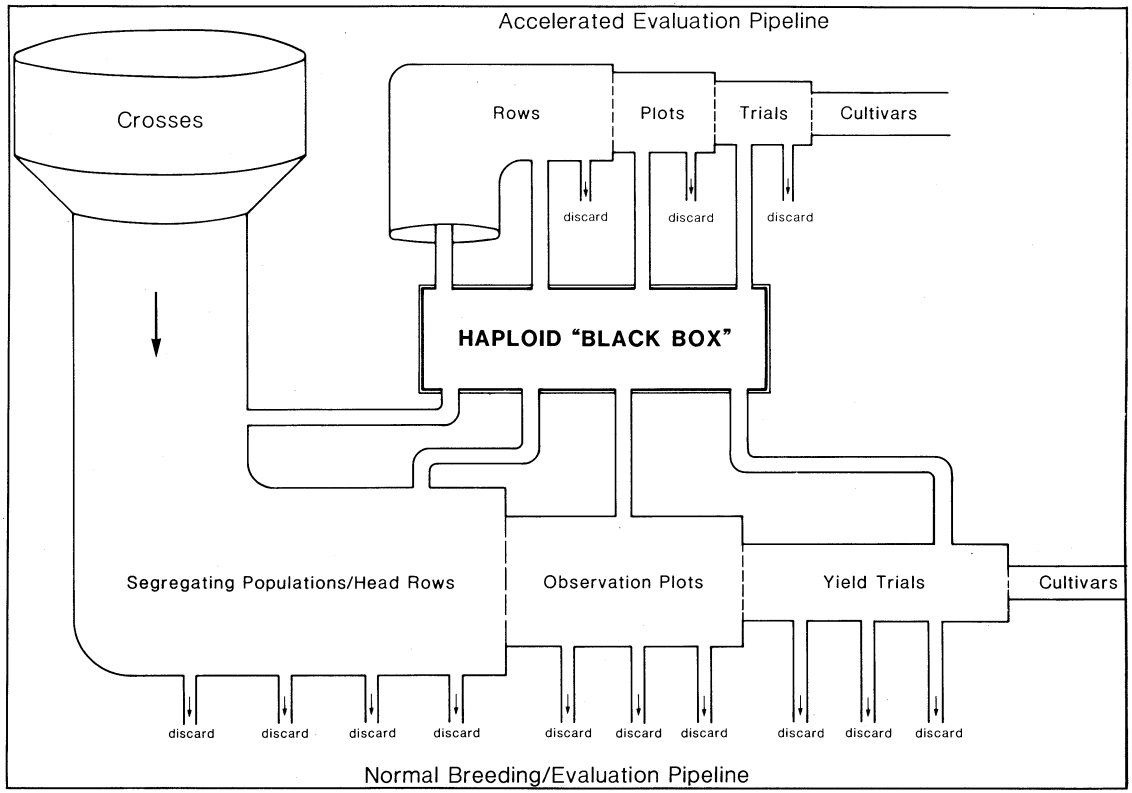

Figure 2. A haploid procedure can be used at any step in a normal breeding/evaluation programme to give pure lines which can be evaluated through an accelerated system due to their complete homozygosity and homogeniety.

evaluate the resulting lines. Not only should the proportion of good lines be higher but the potential of the best lines should also be greater.

HAPLOID PRODUCTION AND DOUBLING

Production of barley haploids follows the basic Bulbosum Method as outlines by Kasha (1974), Jensen (1976) and modified by Pickering (1980). Some further developments and adjustments have been made to suit the conditions and facilities at the Lincoln Research Centre.

Haploid production is on a year-round basis, although the spring and early summer generally give the highest efficiencies. Each week 10-20 seeds of the barley source materials are planted in pots. These are grown in a glasshouse at 22/17 °C with at 16+ hour daylength. Humidity is maintained at 80-90%. Mercury vapour and high presure sodium lights are used to extend the daylength and as supplemental lighting during periods of cloudy weather. Barley plants are limited to 3-4 tillers by weekly trimming.

At the time of emasculation, poorly developed flowers at the base and apex of each spike are removed. The lemma and palea are clipped back and the anthers removed with forceps. When the flowers have opened and the stigmas are feathery (2-3 days), they are pollinated with freshly collected pollen of 2x *Hordeum bulbosum* (clone Cb 2920/4) and then covered with a brown paper bag. Spikes are sprayed with GA_3 for three successive days after pollination. Spikes are maintained intact on the plants.

Embryos are cultured at 10-14 days after pollination. Poorly developed or dried seeds are not dissected and poorly differentiated embryos are not cultured. Cultured embryos are germinated in a dark cabinet at 15 °C until the coleoptile elongates. They are then transferred to a growth room at 18/14 °C with a 16 hour daylength. Illumination of 240 $\mu Em^{-2}sec^{-1}$ is provided by cool white flourescent tubes and tungsten lamps. Presumed haploid plantlets are transplanted to a free draining soil after several leaves and roots have formed. They are maintained in the same environment.

Colchicine treatment takes place when 4-6 healthy tillers have formed. The slit procedure of Pickering (1980)

is used to prepare the plants for treatment. An overnight ice water pretreatment (Falk, unpublished) is used to synchronize cell divisions prior to and during colchicine treatment. From ice water, plants are transferred to a 0.05% colchicine and 2% DMSO solution at 0 °C and held for one hour. The beaker containing the plants and colchicine is then placed in a 30 °C water bath for 5 hours. The solution is aerated (Guerrero, unpublished) continuously during the treatment. After the doubling treatment, plants are rinsed in running water for 30-60 minutes then placed in a beaker of aerated tap water overnight at room temperature.

Colchicine-treated haploids are planted in free-draining soil in shallow boxes and maintained in the glasshouse. At maturity seeds are harvested from doubled sectors of each plant. Up to four seeds of each doubled haploid line are grown in a 2 litre pot in the glasshouse for an increase generation. This eliminates any residual colchicine effects and provides an adequate quantity of good quality seed for evaluation under field conditions.

The efficiencies of various steps in the haploid production and doubling system are given in Table 1. The variation in seed set (13-94%) is caused by the use of some incompatible barley genotypes (Pickering and Hayes, 1976), and by high temperatures during the summer and cold, cloudy periods in the winter. During the winter, even with supplemental lighting, growing conditions are not as good as in the summer. Plant development is poorer, seed sets are lower, embryo differentiation is slower and recovery after colchicine treatment is less vigorous, all of which lead to an overall reduction in efficiency of the programme during the winter months.

Table 1. Haploid production efficiency.

Operation	Percentage Mean	Range
Seeds set per flower pollinated	62	13-94
Seeds dissected per seed set	77	27-92
Embryos cultured per seed dissected	48	24-100
Haploid plants per embryo cultured	56	32-76
Chromosome doubling per haploid plant	83	21-95
Doubled haploids per flower pollinated	11	1-35

EVALUATION OF DOUBLED HAPLOIDS

Each year the haploid programme generates about 1000 homozygous lines which are completely uncharacterized. Virtually the only information available on them is their pedigree. These lines must be assessed and evaluated in as short a time as possible or the advantage of saving time through the haploid procedure would be lost in the evaluation of the derived lines.

The evaluation system is organised in a hierarchical fashion with a series of steps from a single row, to a small plot, to replicated small plots (Table 2). The evaluation is integrated with the seed multiplication process. Initially small amounts of seed are available so it is most practical to grow lines under conditions that maximize seed returned and use negative selection to remove all lines with serious defects or undesired characters. At later stages when seed quantity is not limiting, assessment is based on yield at commercial planting rates in comparison to the best available cultivars.

The first field trial/seed increase is sown as a single, precision seeded observation row 2.5 m long with 10 cm between plants and 40 cm between rows. It takes 25 seeds per plot and returns from 200-400 grams of seed. Assessment is based on plant height, flowering date, straw strength, disease susceptability and general appearance. All lines with serious agronomic defects are discarded. Only selected rows are harvested. Selection is practised among crosses as well as among lines within a cross. Visual evaluation is quite effective as the extreme uniformity within lines maximizes the differences between lines. In addition a separate, inoculated disease trial may be run for lines from crosses known to have resistance to certain diseases. Such simultaneous evaluation is possible at this early stage because of the complete homozygosity and resulting homogeneity of the doubled haploid lines. From 30% to 50% of the lines are usually selected.

The second level of evaluation is an unreplicated observation plot of 6 rows at 17 cm spacings and 2.5 m long (2.5 m^2). Approximately 25 gms of seed are sown with 1.0 to 2.5 kg of seed being harvested under normal conditions. Height, flowering date, maturity date, lodging resistance,

Table 2. Number of doubled haploids in the evaluation system.

Year	Trial: Observation[1] rows	Observation[2] plots	Yield[3] trial	Cooperative[4] yield trial
1981/82	419	-	-	-
1982/83	884	206	-	-
1983/84	1068	457	12	-
1984/85	799	370	42	3
1985/86	790	554	40	7

[1]Single rows 2.5 m long.
[2]Single plots 2.5 m^2
[3]Replicated plots 2.5 m^2
[4]Cooperative trial series containing material from all CRD breeding programmes, grown at 4 sites.

disease susceptability and general appearance are again noted and used as selection criteria. All plots are machine harvested with relative yield being calculated on a moving mean basis. Lines with unacceptable yields are discarded. Retained lines are run through a set of sieves to identify those producing a large, uniformly plump grain. Selected lines are then micromalted at the Plant Physiology Division of DSIR, Palmerston North, using a 30 gm sample (see also D. Smith, 1986). From 10% to 20% of the lines are usually selected for continuing in replicated trials.

It is essential to identify those lines which may have outstanding malting quality at this stage so they can be accelerated to take further advantage of the time already saved by using the doubled haploid procedure. It is also desirable to identify the lines with serious quality defects so they can be eliminated from further consideration. This then leaves a higher proportion of material with acceptable quality and agronomic performance, across a range of environments, can be determined more critically.

The following season, all selected lines are grown with yield and quality standards in sets of replicated 2.5 m^2 plots at one or more sites. From this trial, lines are selected primarily on mean yield, seed characters and micromalting performance relative to the standards. Selected doubled haploid lines are advanced to a cooperative trial among CRD barley breeders grown at four sites, including both North and South Islands.

Simultaneously, doubled haploid lines enter the pure seed multiplication system at CRD. A standard disease assessment is conducted with all advancing doubled haploids and materials from other CRD breeding programmes. Lines with outstanding performance in these cooperative trials are advanced to large plot, multilocation trials as a preliminary to entering official trials. Doubled haploid lines are submitted for official trials as soon as adequate data demonstrating sufficient merit are available.

An off-season nursery may be used to grow plots of outstanding lines from the first observation rows sown in the field. This returns additional data as well as sufficient seed for replicated trials at several locations in the second season of field evaluation.

DISCUSSION

Many doubled haploids are used as parents as soon as they have been identified as outstanding for some characteristics in the early phases of the evaluation system. They are usually used in crosses with complimentary siblings or selected lines from related crosses to go directly into the haploid system. Crosses to unrelated materials are usually run through a selection cycle before going to the haploid system or for use in further crossing.

The objective of selecting and recycling lines, as soon as they can be identified as possessing outstanding merit, is to accumulate repaidly many desirable alleles in families and individuals. This is essentially the recurrent selection systems which was proposed for barley by Choo *et al.*, (1979) and practiced by Patel *et al.*, (1985). The use of recurrent selection procedures, coupled with haploid production, allows for the accumulation and recombination of desired alleles in a population and their subsequent rapid fixation and efficient identification in the derived doubled haploids as outlined by Fouilloux (1980). When a number of desirable alleles are present in an individual, the fastest way to produce homozygous lines for evaluation is through a haploid system. Such methods can be used to generate populations which have a high probability of containing a large portion of the desired alleles. Breeding progress, by going through several cycles of selection and recombination, is generally much greater than is possible by selection in a large population of genetically isolated (self-pollinated) individuals.

CONCLUSION

The Joint Doubled Haploid Project has evolved from the stage of collecting desirable germplasm to the point where it is accumulating desirable traits in elite, adapted populations which can be used to produce superior cultivars through the doubled haploid system. The material used as a source of doubled haploids has the most direct effects on the probability of producing superior cultivars and the evaluation system employed determines how soon such genotypes can be identified and released as cultivars or recycled as parents.

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