Paper 10
USE OF DOUBLED HAPLOIDS IN BARLEY BREEDING

D.E. Falk

Canterbury (NZ) Malting Co. Ltd, C/- Crop Research Division,
DSIR, Private Bag, Christchurch

INTRODUCTION

The technique of producing haploids, which can subsequently be doubled with colchicine, is an important development in barley breeding because it allows the recovery of completely homozygous, homogeneous inbred lines in a single generation. This bypasses all segregating generations in the production of true-breeding lines from a cross of selected parents. The technique can be used with F1 plants or later generation selections, and prevents the possible loss of desirable genotypes under competition during segregation. The derived lines are genetically suitable for immediate evaluation for both quantitative and qualitative characters.

There are several requirements of a system of haploid production if haploidy is to be useful as a breeding technique. The most important consideration is the ability to produce large numbers of haploids from any genotype at a reasonable cost. These should represent a random sample of gametes from the parental line. An efficient method of chromosome doubling is essential to recover the genotype in the diploid form. The doubled haploid lines must then be tested for agronomic productivity and environmental stability to establish their commercial value.

Under a cooperative breeding agreement with the Department of Scientific and Industrial Research, the author and a technician, Mr P.E. Guerrero, are employed by the Canterbury (NZ) Malting Company to maintain a doubled haploid breeding and testing programme, using mainly Crop Research Division facilities. The programme owes a great deal to Mr R.A. Pickering, who was seconded to the Malting Company from the Welsh Plant Breeding Station in 1981 to assist in developing materials and methods; 207 progenies produced under his care were screened in Denmark by G.M. Wright in 1982.

PRODUCTION OF HAPLOIDS

The *Hordeum bulbosum* method developed by Kasha and Kao (1970) is the most widely used and efficient way of producing haploids in barley for breeding purposes. In this method, barley haploids are produced as a result of selective chromosome elimination from hybrid embryos after crossing cultivated barley (*Hordeum vulgare*) with a related wild barley, *Hordeum bulbosum*. The chromosomes of *H. bulbosum* are eliminated during the early mitotic divisions in the developing embryos. After several days, only cultivated barley chromosomes are left in the dividing cells.

Because the development of the endosperm is abnormal (also due to chromosome elimination) normal seeds will not develop. The embryos must be "rescued" and placed on an artificial growth medium about 10-14 days after pollination. The embryos "germinate" on the medium to produce small "seedlings" which are transplanted to soil, where they continue to grow and develop as normal seedlings.

Haploid plants appear to be normal and vigorous, although they are somewhat smaller than diploid barley. They have abnormal meiotic cell division due to the lack of homologous chromosome pairing, and the resulting gametes generally abort. Therefore haploid plants must be treated to double the chromosome number before they can produce seed. Colchicine has been found to be effective in chromosome doubling in haploid barley plants (Thiebaut *et al.*, 1979), and 90-95% success is not uncommon.

Following colchicine treatment plants are grown to maturity, and those sectors derived from cells doubled by colchicine will self-pollinate and produce seed. The embryos in the seed contain the normal diploid number of chromosomes, the second set being derived as a duplicate of the orginal haploid set. The seeds will all be genetically identical and completely homozygous, and therefore absolutely true breeding.

Doubled sectors on treated haploid plants may include part of an ear or several ears, and can produce from one to several hundred seeds, although the average is around 20 per plant. Many of these seeds are small, and plants produced from small seeds may be weak. Thus at least one generation needs to be grown, to produce normal vigorous seed, before initiating definitive tests on the derived doubled-haploid lines.

Overall efficiences of 5 to 10 doubled haploid plants per 100 florets pollinated are normal with the *H. bulbosum* method (Kasha and Reinbergs, 1981). In some material,

frequencies as high as 23 doubled haploids per 100 florets have been reported (Jensen, 1975).

HAPLOIDY AS A BREEDING TOOL

Kasha and Reinbergs (1975) and Pickering (1980) have shown that simply inherited genes have a random segregation among doubled haploid lines derived by the *H. bulbosum* method. Choo *et al.* (1982) and Park *et al.* (1976) have demonstrated random genetic assortment for quantitative characters also.

In comparisons of the doubled haploid technique with conventional breeding systems, Park *et al.* (1976) and Reinbergs *et al.* (1975) concluded that the distributions of performance, including means and ranges and the frequencies of superior genotypes, were similar for pedigree, single seed descent, and double haploid methods. There were no differences in performance between the best lines derived from each of the methods. Turcotte *et al.* (1980) concluded that more superior lines were derived by use of the haploid technique, and that the improved selection efficiency from using homogeneous lines and the time savings in developing such lines were valuable. Although the results obtained using the haploid method as a breeding technique are limited, they show that it is an effective means of deriving a similar assortment of homozygous lines, in less time than conventional methods (Kasha and Reinbergs, 1979).

Reinbergs *et al.* (1976) determined that as few as 20 doubled haploid lines were sufficient to evaluate the potential of a cross, based on the mean, range, genetic variability and frequency distribution of derived lines. Kasha *et al.* (1977) concluded that there was sufficient recombination in the F1 to produce haploids that combined the desirable characteristics of both parents. They found some doubled haploid lines equivalent in performance to the F1 hybrid parents.

Doubled haploids can be used in conjunction with other breeding methods, for instance by selecting parents with specific characters in a segregating population to use directly or in F1 combinations. There may be some advantage in selecting plants from F2 and F3 generations as sources of doubled haploids (Snape and Simpson, 1981). Kasha and Reinbergs (1975) and Choo *et al.* (1979) have outlined a doubled-haploid recurrent-selection procedure that takes only two years per cycle, including replicated field evaluation. Kasha (1976) also points out that haploidy could be used to exploit a recurrent selection population developed through the use of genetic male sterility.

ACHIEVEMENTS

The first doubled haploid barley cultivar, Mingo, was released by CIBA GEIGY Seeds Ltd. in Canada in 1979, after three years in official trials (Ho and Jones, 1980). This line was one of seven doubled haploids produced in 1973, during the first season of the newly established programme. Their programme has expanded, and now produces several thousand doubled haploids annually. A seed increase is grown in New Zealand during the Canadian winter and replicated yield trials commence the following summer in Canada. A number of promising lines have come from this programme, although Mingo is the only licensed cultivar to date.

The Welsh Plant Breeding Station has been producing double haploids and increasing them in New Zealand since 1974. One doubled haploid line, identified as having potential in New Zealand in 1976, was released jointly by CRD and WPBS as the cultivar Gwylan in 1980. It is the second doubled haploid line to achieve cultivar status. Gwylan has continued to perform well in Canterbury, winning the 1982-83 South Island Barley Society Competition at 10.54 t/ha, becoming the first official 10-tonne barley crop in New Zealand (Anon, 1983).

DISCUSSION

Gwylan has not performed well in Wales, where it was bred. This underscores the point that there is no selection for adaptation in a haploid programme, because lines are not exposed to environmental stress until after they are fixed. Of course even with pedigree breeding and selection for the commercial environment in all generations, many crosses produce no lines worth extensive testing. The absence of early-generation selection in a haploid programme can be used to advantage where a centrally located haploid-producing facility provides homozygous material for screening in several diverse enviroments.

The use of a doubled haploid technique as a breeding tool requires consideration of several factors beyond whether a particular parental combination is likely to give desirable progeny. One factor to consider is the proportion of the progeny from a particular cross which will be automatically rejected due to a simply inherited, undesirable trait. For example, if an adapted, mildew-susceptible cultivar is crossed with an unadapted, mildew-resistant cultivar, approximately one half of the doubled haploid progeny will be susceptible to mildew, and therefore discarded. Furthermore, since one parent is not adapted to the local environment, only a small proportion of the progeny would be expected to carry the right combination of characters for good performance. Only a small proportion of the derived lines from such a cross could be expected to be desirable, and it may be better to backcross to the adapted parent first, and select resistant progeny to put through the haploid programme. A higher proportion of the derived lines would contain more of the genes required for adaptation. Another possibility would be to self-pollinate the F1 plant and select disease resistant, agronomically desirable plants in the F2 population. The F3 seedlings from selected plants could be screened for disease resistance and those from resistant families used for haploid production. This should result in a higher proportion of desirable derived doubled haploid lines.

Since the selected lines will also be approaching homozygosity through self-pollination, fewer gametes need

to be sampled per plant to recover a sample of the genetic potential of that genotype. The proportion of inferior derived lines should be lower when using a selected proportion of the population as compared to the random sample obtained from the original crossed plants.

The effective use of the doubled haploid technique as part of a breeding programme is expensive, and it is imperative to develop potential breeding materials that will maximise the probability of producing desirable doubled haploid lines. Crosses that have the potential to produce a rare, superior gene combination along with mostly inferior progeny should be handled by pedigree breeding, growing several thousand F2 plants. Some selection prior to the inclusion of a line as a parent in a haploid programme may ensure that lines with agronomic potential can be recovered, even from wide crosses. Intercrossing selected lines will generate further variability among progeny while maintaining a generally desirable background.

An efficient evaluation procedure must be established to identify superior derived lines as quickly as possible. Improved selections can then be cycled back into the breeding programme as parents, as suggested by Kasha and Reinbergs (1975).

The *H. bulbosum* method is the fastest available for deriving homozygous lines from barley breeding populations, and the doubling provides an assurance that all genes are fixed. Data from derived populations indicate that there are no significant differences in the yield potential of lines developed by the different breeding models. At least two cultivars of doubled haploid barley have been released and many more are being evaluated. The haploid method is also used to augment more traditional approaches by purifying lines in the final stages of development.

The most serious limitations on the use of the *H. bulbosum* method are the stringent requirements for optimum growing conditions of parental materials and derived haploids and the high level of technical expertise necessary in culturing haploid embryos and doubling the chromosome numbers of the haploid plants. The substantial savings in time and the increased efficiency of evaluating derived lines must be weighed against the investment in facilities and personel and the limited number of lines which can be produced in a year.

Before adopting the technique the individual breeding programme must be analysed to determine whether its use is desirable and economical. The development of more efficient methods of producing haploids could alter the economics of the use of haploidy.

The inclusion of a doubled haploid phase in a breeding programme puts extra pressure on a breeder to select the parents with the greatest probability of producing progeny with a high potential value and to evaluate the derived lines efficiently.

REFERENCES

Anon, 1983. Gwylan wins barley comp. *Arable Farming (Farming Press, Wanganui) June 1983:* 12.

Choo, T.M., Christie, B.R., Reinbergs, E. 1979. Doubled haploids for estimating genetic variances and a scheme for population improvement in self-pollinated crops. *Theoretical and Applied Genetics 54:* 267-271.

Choo, T.M., Reinbergs, E., Park, S.G. 1982. Comparison of frequency distributions of doubled haploid and single seed descent lines in barley. *Theoretical and Applied Genetics 61:* 215-218.

Ho, K.M., Jones, G.E. 1980. Mingo barley. *Canadian Journal of Plant Science 60:* 279-280.

Jensen, C.J. 1975. Barley monoploids and double monoploids: techniques and experience. *In:* "Proceedings of the third international barley genetics symposium". Ed. H. Gaul. Verlag Karl Thiemig, Munich: 316-345.

Kasha, K.J. 1976. Utilization of haploidy in plant breeding and mutation. *Acta Biologica Iugoslavia Serija F Genetika 8:* 101-110.

Kasha, K.J., Kao, K.N. 1970. High frequency haploid production in barley (*Hordeum vulgare* L.). *Nature 225:* 874-876.

Kasha, K.J., Reinbergs, E. 1975. Utilization of haploidy in barley. *In*: "Proceedings of the third international barley genetics symposium". Ed. H. Gaul. Verlag Karl Thiemig, Munich: 307-315.

Kasha, K.J., Reinbergs, E. 1979. Achievements with haploids in barley research and breeding. *In:* "The Plant Genome. Proceedings of the second international haploid conference". Eds. D.R. Davies and D.A. Hopwood. The John Innes Society, Norwich, U.K.: 215-230.

Kasha, K.J., Reinbergs, E. 1981. Recent developments in the production and utilization of haploids in barley. *In:* "Proceedings of the fourth international barley genetics symposium". Ed. R.W.H. Whitehouse. Edinburgh University Press: 655-665.

Kasha, K.J., Song, L.S.P., Park, S.J., Reinbergs, E. 1977. Fixation of heterosis: comparison of F1 hybrids with their respective homozygous lines developed using doubled haploid procedures. *Cereal Research Communications 5:* 205-214.

Park, S.J., Walsh, E.J., Reinbergs, E., Song, L.S.P., Kasha, K.J. 1976. Field performance of doubled haploid barley lines in comparison with lines developed by the pedigree and single seed descent methods. *Canadian Journal of Plant Science 56:* 467-474.

Pickering, R.A. 1980. Use of the doubled haploid technique in barley breeding at the Welsh Plant Breeding Station. *Annual Report, Welsh Plant Breeding Station, Aberystwyth 1979:* 208-226.

Reinbergs, E., Park, S.J., Kasha, K.J. 1975. Haploid technique in comparison with conventional methods in barley breeding. *In:* "Proceedings of the third international barley genetics symposium". Ed. H. Gaul. Verlag Karl Thiemig, Munich: 346-350.

Reinbergs, E., Park, S.J., Song, L.S.P., 1976. Early identification of superior barley crosses by the doubled haploid technique. *Zeitschrift fur Pflanzenzuchtung 76:* 215-224.

Snape, J.W., Simpson, E. 1981. The genetical expectations of doubled haploid lines derived from different filial generations. *Theoretical and Applied Genetics 60:* 123-128.

Thiebaut, J., Kasha, K.J., Tsai, A. 1979. Influence of plant development stage, temperature, and plant hormones on chromosome doubling of barley haploids using colchicine. *Canadian Journal of Botany 57:* 480-483.

Turcotte, P., St. Pierre, C.A., Ho, K.M. 1980. Comparison entre des lignees pedigrees et des lignees haploides doublees chez l'orge (*Hordeum vulgare* L.). *Canadian Journal of Plant Science 60:* 79-85.