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# Progress in Polyhaploid Production Techniques of Hexaploid Wheat through Wide Crosses

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## ABSTRACT

Polyhaploid production techniques of hexaploid wheat (*Triticum aestivum* L.) through wide crosses were evaluated in terms of pollen sources, 2,4-D application, embryo rescue and chromosome retention. Pollen sources included *Hordeum bulbosum* L., *Zea mays* L., *Pennisetum glaucum* (L.) R. Br., *Sorghum bicolor* (L.) Moench, and *Tripsacum dactyloides* (L.) L. Maize-mediated polyhaploid production was more stable than the other methods because of a lesser genotypic influence on embryo formation. Application of 2,4-D onto wheat after pollination was critical to promote seed setting and embryo formation in all cross combinations. Embryo rescue was necessary at an appropriate embryo developmental stage to obtain plant regeneration. Paternal chromosomes were eliminated by the stage of active growth of the polyhaploid seedlings. Polyhaploid production frequencies ranged between 10 and 20% of pollinated wheat florets, suggesting germplasm genotypic effects.

## INTRODUCTION

The main objective of wide hybridization in the tribe Triticeae is alien genetic transfer, which is facilitated by tissue culture and cytogenetic manipulation (Sharma and Gill 1983, Mujeeb-Kazi and Asiedu 1990). Chromosomal stability of wide hybrids is dependent on cross combinations. Failure of alien genetic transfer after fertilization, as a consequence of the preferential elimination of chromosomes of one parent, was first reported in *Hordeum* hybrids (Symko 1969, Kasha and Kao 1970). Artificial rescue of haploid embryos was required since endosperm did not develop in most of seeds set. Monoploid production technique of barley (*Hordeum vulgare* L.,  $2n=2x=14$ ) through crosses with *H. bulbosum* L. was developed in the 1970's (Jensen 1976).

Intergeneric crosses of hexaploid wheat (*Triticum aestivum* L.,  $2n=6x=42$ ) with *H. bulbosum* also produced

immature embryos capable of regenerating polyhaploids ( $3x=21$ ) wheat plants (Barclay 1975). A polyhaploid production technique using *H. bulbosum* crosses was reviewed and its application restricted to cross-compatible wheat genotypes (Inagaki 1990). This paper reviews the development of various techniques for polyhaploid production of hexaploid wheat through wide crosses with the members of the Gramineae family.

## BREEDING

### H. bulbosum crosses

Cross-compatibility between hexaploid wheat and *H. bulbosum* depends on both parental genotypes (Snape *et al.* 1979, Falk and Kasha 1981, Inagaki and Snape 1982, Sitch and Snape 1986). The dominant genes *Kr1* and *Kr2*, located on wheat chromosomes 5B and 5A, respectively, control the cross-incompatibility (Falk and Kasha 1983, Sitch *et al.* 1986). The absence of fertilization in cross-incompatible wheat genotypes is attributed to the failure of pollen tube to penetrate the embryo sac (Snape *et al.* 1980, Sitch and Snape 1987a). Therefore, at present, wheat genotypes used for polyhaploid production are restricted to those that are cross-compatible with *H. bulbosum*. These wheat genotypes presumably have their origin in Asia (Falk and Kasha 1981, Inagaki and Snape 1982, Li and Hu 1986). According to the genealogical pedigrees of Japanese cultivars (Fukunaga and Inagaki 1985), wheat cultivars derived from the hybrid progenies of local varieties are highly cross-compatible (Inagaki 1986). On the other hand, *H. bulbosum* genotypes with heterogeneity show great variation in their ability to affect cross-compatibility of wheat. However, *H. bulbosum* genotypes that may be sufficiently cross-compatible with all wheat genotypes have not yet been found (Inagaki 1986, Sitch and Snape 1986).

When the cross-compatible wheat genotypes are used for crosses, the production efficiency is enough to obtain

200 polyhaploids from 1000 wheat florets (40 spikes) pollinated by *H. bulbosum* (Inagaki 1989). It also takes 26-30 weeks from crossing to harvest of doubled haploid grains by using environment-controlled facilities. This technique requires further development because of the restriction of wheat germplasm.

### Application of plant growth regulators

Reduction of the cross-incompatibility barrier of hexaploid wheat has been attempted by means of some synthetic plant hormones. Marshall *et al* (1983) reported that the application of a 2,4-dichlorophenoxyacetic acid (2,4-D) solution onto emasculated wheat florets prior to pollination induced parthenocarpic seed development and increased ovule size due to cell expansion. This however did not have a favorable effect on seed setting in crosses with *H. bulbosum*. To avoid physiological damage of 2,4-D on wheat florets, Inagaki (1986) adopted a method to inject repeatedly a 2,4-D solution (100 mg/l) in the wheat culms from emasculation to pollination. The development of wheat caryopses after *H. bulbosum* pollination was similar to that observed during self-pollination. The embryo formation frequency was twice that of the control when a cross-compatible wheat genotype was utilized. This crossing method also produced embryos with a cross-incompatible genotype at very low frequencies, suggesting that the 2,4-D application enhanced seed and embryo development after fertilization rather than increasing fertilization itself. Effectiveness of the 2,4-D treatment was further confirmed during production of wheat x barley hybrids (Koba and Shimada 1991, Riera-Lizarazu *et al.* 1992a). Other hormones such as indole-3-acetic acid and gibberellic acid did not reduce the cross-incompatibility mechanism (Falk and Kasha 1982).

### Pollen source

Alien genetic transfer to hexaploid wheat has been attempted in crosses with the members of the *Panicoides* subfamily (Zenkeler and Nitzsche 1984). Cytological evidence indicates that the fertilization of wheat with maize (*Zea mays* L.) pollen was successful, irrespective of the presence of *Kr* gene(s), and produced hybrid zygotes (Laurie and Bennett 1986, 1987a). In these zygotes, the maize chromosomes were rapidly lost within two days after pollination (Laurie and Bennett 1987b), necessitating artificial rescue of proembryos at early developmental stages (Laurie and Bennett 1988a). Suenaga and Nakajima (1989) found an enhancing effect of 2,4-D treatment on the embryo development in wheat caryopses. Inagaki and Tahir (1990) demonstrated that maize pollination of, and subsequent 2,4-D treatment onto wheat also resulted in production of wheat embryos capable of regenerating polyhaploid plants, even for wheat varieties that were cross-incompatible with *H. bulbosum*. Genotypic

differences in embryo formation frequency were significant only for the wheat parent. Polyhaploid production through maize crosses has been further confirmed using diverse wheat varieties (Inagaki and Tahir 1990, Laurie and Bennett 1991). Some species related to maize, like teosinte (*Z. mays* L. spp. *mexicana*) and eastern gamagrass (*Tripsacum dactyloides* (L.) L.) are alternative pollen sources for wheat polyhaploid production (Ushiyama *et al.* 1991, Riera-Lizarazu and Mujeeb-Kazi 1993). For increasing flexibility to handle wheat materials, detaching the wheat spikes pollinated with maize and culturing them in a nutrient solution containing 2,4-D was developed (Ushiyama *et al.* 1991, Riera-Lizarazu *et al.* 1992b).

In sorghum (*Sorghum bicolor* (L.) Moench) and pearl millet (*Pennisetum glaucum* (L.) R. Br.) crosses, successful fertilization and elimination of paternal chromosomes from hybrid zygotes were observed (Laurie and Bennett 1988b, Laurie 1989b). Efficient formation of polyhaploid embryos in these crosses indicated that the 2,4-D application was essential (Ohkawa *et al.* 1992, Inagaki and Mujeeb-Kazi 1994a). Significant embryo formation frequency differences existed among wheat varieties. Sorghum crosses however, expressed a strong genotypic barrier to embryo formation (Inagaki and Mujeeb-Kazi 1994a). Therefore, the maize-mediated polyhaploid production appears more stable than the other methods because of its lesser genotypic effect on embryo formation.

A technique of storing pollen for long periods is helpful for crosses onto wheat parents without having to synchronize flowering times of both parents. Long-term pollen storage at ultra-low temperatures is feasible in maize (Barnabas and Rajki 1981) and pearl millet (Hanna 1990). Dried maize pollen with 10 to 12% water content, stored for three months at -80°C produced embryos on wheat at half the frequency of fresh pollen (Inagaki and Mujeeb-Kazi 1994b). Stored maize pollen can thus be used for wheat polyhaploid production when and where fresh pollen is not available.

### Polyhaploid production efficiency

A technique of polyhaploid production in hexaploid wheat through wide crosses consists of two steps; hybridization and embryo rescue. A factor affecting polyhaploid production efficiency was the developmental stage of the wheat florets at crossing. This was critical in crosses with *H. bulbosum* (Sitch and Snape 1987b), maize (Laurie 1989a) and pearl millet (Inagaki and Bohorova 1994). Environmental conditions of humidity and temperature also affected embryo formation frequency in crosses with *H. bulbosum* (Inagaki and Snape 1982, Inagaki 1986, Sitch and Snape 1987c). Further, the developmental stage of wheat embryos formed was critical with respect to plant regeneration frequency in crosses with *H. bulbosum* (Inagaki 1985) and pearl millet (Inagaki and Bohorova 1994). In general, pollination at an early developmental

stage of wheat florets under a high level of humidity and temperature resulted in higher frequencies of embryo formation. Artificial rescue at a suitable embryo developmental stage was required to attain a higher frequency of plant regeneration. These procedures are routinely utilized in other Triticeae wide crosses (Mujeeb-Kazi *et al.*, 1987, 1989).

Crosses of diverse hexaploid wheat varieties with maize resulted in embryo formation frequencies of 32.7% (Laurie and Reymondie 1991), 21.7% (Inagaki and Tahir 1990) and 15.6%, 43.7% and 60.7%, respectively. These values are comparable to those obtained for the *H. bulbosum* crosses. Consequently, the polyhaploid production frequency with maize ranged from 20.1% to 9.5%, suggesting some genotypic effect of the wheat and maize germplasm used for crosses.

Cytological examination of the regenerated wheat plants indicated that most of them were euhaploids with 21 chromosomes (Inagaki and Tahir 1990, Riera-Lizarazu *et al.* 1992b, Inagaki and Mujeeb-Kazi 1994a). Retention of paternal chromosomes was detected at the proembryo stage in crosses with maize and sorghum (Laurie and Bennett 1986, 1988a), at the tillering stage of plants from crosses with pearl millet and maize (Ahmad and Comeau 1990, Comeau *et al.* 1992), and at the doubled haploid plant stage derived from the crosses with *H. bulbosum* (Inagaki 1987). These facts do not eliminate the possibility of translocating chromosome segments of pollen parents to the wheat genome through somatic associations. However, variations in agronomic traits of doubled haploids were negligible in *H. bulbosum* (Inagaki 1987, Snape *et al.* 1988) and maize (Laurie and Snape 1990,

Suenaga and Nakajima 1993a) crosses. Significant distortion of segregation ratios in the doubled haploids derived from hybrid progenies was not observed in *H. bulbosum* (Inagaki and Tahir 1991, Inagaki and Egawa 1994) and maize (Suenaga and Nakajima 1993b) crosses.

## CONCLUSION

Two decades were spent to establish a technique for producing polyhaploids from diverse genotypes of hexaploid wheat through wide crosses. Significant technical developments were attributed to pollen selection from different subfamilial species and the application of 2,4-D. At present, doubled haploids derived from hybrid progenies can be used as materials of recombinant inbred lines in genetic analyses and breeding purposes. However, it needs more development to extend this technique to the related crop species. Polyhaploid production in durum wheat by maize crosses was demonstrated (Riera-Lizarazu *et al.* 1992b), but dependent upon its genotypic variation (Inagaki and Tahir unpublished).

The initial objective of wide crosses is to augment the genetic diversity of the existing germplasm. Process of fertilization and embryo formation have been already confirmed in crosses of hexaploid wheat with maize, sorghum and pearl millet. The third step in producing symmetrical or asymmetrical hybrids is to develop a technique for retaining some paternal chromosomes in these distant hybrids. Cytogenetic manipulation and molecular approaches will be increasingly required to materialize this step.

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