I.1 Overview of Haploidy

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1 Introduction

The term haploid sporophyte is generally used to designate sporophytes having the gametic chromosome number, and although the first haploids in flowering plants were identified over 80 years ago (Belling and Blakeslee 1922), it was not until Guha and Maheshwari (1964) reported the first in vitro culture anther-derived haploids from *Datura* that their potential for crop improvement was seriously contemplated as the value of quickly achieving homozygous lines was recognized. Since then, haploids and doubled haploids have been reported in a vast number of species and several cultivars have been developed using doubled haploids (Maluszynski et al. 2003a; Thomas et al. 2003). Consequently, the use of germplasm collection, classical plant breeding methods and genetic engineering for crop improvement can be supplemented with doubled haploid technology (Baenzinger 1996; Khush and Virmani 1996; Gepts 2002). Here we present a brief overview of the occurrence and experimental induction of haploidy.

2 Natural Occurrence of Haploids

Naturally occurring haploids have been reported in a number of species including tobacco, rice and maize (see review by Harlow et al. 1996). In *Brassica* homozygous diploid lines from naturally occurring haploids were reported by Thompson (1974). In barley, *Hordeum vulgare*, the *hap* initiator gene controls haploidy and spontaneous haploids were recovered at high frequency from barley (Hagberg and Hagberg 1980). In maize, the indeterminate gametophyte gene (*ig*) results in a monoploid embryo either from the sperm cell or from the egg cell (Kermicle 1969). Although doubled haploids can be recovered from such spontaneous haploids, the frequencies are too low for breeding purposes.

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Haploid-inducing lines of *Zea mays* have been used to produce haploids by development of the unfertilized egg cell (Eder and Chalyk 2002) at frequencies of up to 8.0% and one line of maize showed a high frequency of haploidy (Coe 1959). The phenomenon of semigamy where disturbances during fertilization result in embryo formation from the egg cell without participation of the sperm cell occurs naturally in cotton (Turcotte and Feaster 1974). In this process both egg and sperm nuclei divide independently and may produce a chimera from which haploids can be isolated. In some cases, the haploid embryo is produced from either the egg cell or the sperm cell.

3 Induction of Haploidy

With the recognition of the importance of doubled haploids in plant breeding, extensive efforts were made to induce haploid embryogenesis and increase the frequency at which doubled haploids could be recovered. There are now four methods generally applicable to the production of haploids in plants at frequencies useful for a breeding program, and a recent monograph detailed the protocols applicable to haploid and doubled haploid production in a number of species (Maluszynski et al. 2003b). These methods are:

1. Androgenesis, where cultured anthers or isolated microspores undergo embryogenesis/organogenesis directly or through intermediate callus.
2. Gynogenesis, where cultured unfertilized isolated ovules, ovaries of flower buds, develop embryos from cells of the embryo sac.
3. Wide hybridization crosses followed by chromosome elimination from one parent of a cross, usually the pollinating parent.
4. Parthenogenesis, where there is development of an embryo by pseudogamy, semigamy or apogamy.

3.1 Androgenesis

Under the appropriate culture conditions, responsive microspores undergo cell division and organize embryos (sporophytes) rather than gametophytes. A number of factors influence embryogenic response of cultured anthers and microspores and these have been extensively reviewed (Ferrie et al. 1995; Sopory and Munshi 1996; Wang et al. 2000; Touraev et al. 2001). For some species and genotypes these requirements may be more stringent than for others.

Culture response is influenced by genotype and donor plant growth conditions. The nuclear stage of the microspore is a key factor for embryogenic response and the mid-uninucleate stage to the early binucleate stage is the responsive stage in most cases. There is a requirement for elevated temperature and/or nutritional or osmotic stress during the initial stages of culture

(Touraev et al. 2001). The type and levels of carbohydrates or polymers also influence embryogenic outcome (Ilic-Grubor et al. 1998; Touraev et al. 2001). In some cases, there may be a requirement for temperature or osmotic pretreatment of anthers, isolated microspores or flower buds to ensure success. Even when all these conditions are met, some species and genotypes may not respond to culture. During microspore embryogenesis all stages characteristic of zygotic embryo development are evident. After 21–28 days in culture, fully developed embryos are recognizable. Because of the potential to produce large numbers of embryos, the use of isolated microspore culture is increasing in importance in both monocots and dicots. However, of 193 species reported in 1996, anther culture was used in 105 species (Maluszynski et al. 1996).

3.2 Gynogenesis

This is an alternative route to haploid embryogenesis where under the appropriate culture conditions the unfertilized egg cell of the embryo sac develops into an embryo by yet unknown mechanisms. Other cells of the embryo sac, antipodals or synergids may produce the embryo (Mukhambetzhanov 1997). The culture response is still genotype dependent (Alan et al. 2003; Bohanec et al. 2003) and culture media composition and stage of embryo sac development are important considerations for successful culture (Keller and Korzun 1996; Sita 1996). Depending on the species, unfertilized ovule, ovary or flower bud can be cultured. In some members of the Chenopodiaceae, Lilliaceae and Cucurbitaceae, gynogenesis is the main route to doubled haploid production. Even where anther or microspore culture is successful, gynogenetic haploids have been produced, e.g. in barley, maize, rice and wheat (Sita 1996). Embryogenic frequency is low in many cases, but relatively high frequencies have been reported in other cases (Martinez et al. 2000; Alan et al. 2003). Genetic stability of the doubled haploids and the absence of albinism are attributes of this method (Touraev et al. 2001).

3.3 Wide Hybridization Crosses

This method of haploid production, through the elimination of all the chromosomes of the pollinating parent of a wide cross, is sometimes referred to as the bulbosum method as it came into prominence with the recovery of haploid *Hordeum vulgare* in a cross with *Hordeum bulbosum* as the pollinating parent (Kasha and Kao 1970). After fertilization, there is usually endosperm failure and the embryo must be rescued and cultured in vitro. Doubled haploids are recovered by treating either the embryo or the plantlet with colchicine. This approach is now widely used in cereals, especially wheat, where maize pollination yielded wheat haploids with high efficiency (Laurie and Bennett 1988; Kisana et al. 1993; Mujeeb-Kazi and Riera-Lizarazu 1996).

In a number of cereal species these crosses result in haploid recovery (see Khush and Virmani 1996). Other species of Poaceae can be used as pollinators (Falk and Kasha 1983), and with maize there are genotypic differences affecting the efficiency of pollination (Verma et al. 1999). The advantages of this method are genotype independence, drastic reduction in albinism and absence of gametoclonal variation. Doubled haploid lines produced by this method compared favorably with those produced by anther culture and by single seed descent (Guzy-Wrobelska and Szarejko 2003). However, instances of reduced fertility and pollinating parent chromosome retention have been reported (Riera-Lizarazu et al. 1996).

3.4 Parthenogenesis

With this method of haploid induction, the egg cell of the embryo sac usually develops into an embryo without the active involvement of the sperm nucleus. This process is referred to as pseudogamy; where the embryo develops from any haploid cell of the embryo sac other than the egg cell the process is called apogamy.

A distinction can be made between this form of parthenogenesis and embryo development by chromosome elimination and gynogenesis. In the latter two processes, there is usually endosperm failure and the embryo must be rescued for continued development in vitro. In the former, there is endosperm development and embryo maturation occurs in vivo.

Parthenogenesis can be induced by pollination with inactivated pollen or a variety of chemical treatments (Khush and Virmani 1996; Sestili and Ficcadeni 1996). The genes controlling haploidy such as indeterminate gametophyte (*ig*) in maize (Kermicle 1969) and the haploid initiator gene of barley (Hagberg and Hagberg 1980) induce embryo development by parthenogenesis. The frequency of parthenogenetic haploids is usually too low for plant breeding purposes (Khush and Virmani 1996). However, in potato (*Solanum tuberosum* L.) the use of special pollinator species induced haploidy at high enough frequency to be of value in breeding programs (Hutten et al. 1994; Peloquin et al. 1996; Straadt and Rasmussen 2003). Even though anther culture is reasonably successful as in potato (Rokka 2003), parthenogenesis through superior pollinators is still preferred to anther culture (Peloquin et al. 1996).

4 Conclusion

From the foregoing discussion, it can be concluded that the choice of method for haploid and doubled haploid production will depend on the species, genotype, efficiency of the generation, genetic stability of the doubled hap-

loids and the ease of application of the method. Doubled haploids can occur spontaneously, but in most cases chromosome doubling of haploids is required to restore fertility. This is achieved by the use of antimicrotubule agents. In many cases androgenesis can be efficient especially in responsive genotypes where isolated microspore culture is used. Apart from doubled haploid production, this method is convenient for mutagenesis, transformation, basic research studies and other uses relevant to crop improvement. Gynogenetic haploid production is efficient in a few species and has been employed in cases where androgenesis and other methods prove intractable. Chromosome elimination technique is widely applicable in monocots even though in many cases androgenesis is efficient. The advantages are greater genetic stability and absence of albinism.

Parthenogenetic haploids have been detected in nature at low frequency and are inducible by pollination with inactivated pollen. The use of special pollinator species has allowed the recovery of haploid embryos, arising from pseudogamy, at high efficiencies in members of the Solanaceae.

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