17. Conditional lethal markers: spontaneous haploid selection in plants

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1. Introduction

Haploidy refers to any organism, tissue or cell, having the chromosomal constitution similar to the normal gametes of a given species (Chase, 1951). Haploid plants are useful for developing inbred lines (Gallais, 1986; Foroughi-Wehr and Wenzel, 1990) and for the selection of recessive mutations at the cellular level (Grafe et al., 1986; Marion-Poll et al., 1988). Haploids can be produced by two methods: a) in vitro, and b) in situ. In the last thirty years, a considerable effort has been made to produce haploids by in vitro culture of male and female gametophytes. The frequency of androgenesis has been successfully increased in a number of agricultural crops and new cultivars of wheat (De Buyser et al., 1981; Schaeffer and Baenziger, 1982), Brassica (Hoffmann et al., 1982; Keller et al., 1983), barley (Foroughi-Wehr and Friedt, 1984), tobacco (Collins et al., 1974; Nakamura et al., 1984), Asparagus (Doré, 1974; 1976), potato (Wenzel et al., 1980; Wenzel and Uhrig, 1981), maize (Wu et al., 1983; Kuo et al., 1986) have been released.

The ability to produce in situ haploids by different methods has been largely exploited. Physical and chemical treatments were given to male gametophytes in order to prevent the formation of two functional generative nuclei, while keeping the pollen capacity to germinate and to stimulate the parthenogenetic development of the embryo (Illies, 1964). Poplar haploids were produced by treating pollen grains with toluidine blue (Illies, 1974).

Irradiation has been used as a physical agent for treating pollen for a long time, and today X-rays or gamma rays are most often employed. Parthenogenetic haploids have been obtained by pollination with irradiated pollen of normal plants of *Brassica* (Doré, 1989), melon (Sauton and Dumas de Vaulx, 1987), petunia (Raquin, 1985), apple (Zhang et al., 1988), carrot (Rode and Dumas de Vaulx, 1987) and banana (Leblanc and Escalant, 1992). However, except in melon and petunia, irradiation of the pollen is not an efficient method for haploid production. Irradiation of the embryo sac followed by pollination with non-irradiated pollen produced *in situ* androgenic haploids in *Crepis tectorum* (Gerassimova, 1936), *Antirrhinum majus* (Ehrenberg, 1948) and *in vitro* ovary culture of *Petunia hybrida* and *P. parodii* (Raquin et al., 1989).

The possibility of chromosome elimination of one of the partners by interspecific and intergeneric hybridizations has been exploited. For example, a large number of barley haploids have been obtained from crosses between *Hordeum vulgare* (cultivated species) and *Hordeum bulbosum* (wild species) (Symko, 1969; Kasha and Kao, 1970). Regardless of the direction of the cross, the haploids recovered were of *Hordeum vulgare* genome. Cytological investigations have demonstrated that fertilization is normal but the chromosomes of *Hordeum bulbosum* are eliminated during the first cell divisions during embryo formation (Subrahmanyam and Kasha, 1973; Bennett et al., 1976). The process of chromosome elimination degenerates the endosperm. Since these embryos lack a functional endosperm, and they could not complete their growth on the mother plant, thereby, “embryo rescue” *in vitro* culture becomes necessary. Other interspecific hybridizations in potato (Hougas et al., 1964), melon (Dumas de Vaulx, 1979) and *Brassica* (Chen and Heneen, 1989) have facilitated isolation of haploid plants. By intergeneric crosses between wheat plants, used as a female parent, and maize plants used as pollinators, haploid wheat plants have been obtained after “embryo rescue” (Laurie and Bennett, 1988). The cost and efficiency of the method of producing haploids are significant factors for consideration. For example, haploid barley production is possible by two methods, anther culture and intergeneric crosses (*H. bulbosum* method). Out of the total number of haploid plants produced, 64% were recovered with the *H. bulbosum* method and 36% by anther culture (Devaux, 1992). The anther culture method needs to be improved for many genotypes.

Numerous reviews on haploid production exist (see Hu, 1985; Snape and Simpson, 1986; Snape, 1989; Bajaj, 1990); the aim of this one is to focus on the spontaneous haploids and to a certain degree on the modern methods of genetic engineering that offer tools to select them systematically. In fact, spontaneous haploids occur, in general, at very low frequencies (less than $10^{-3}$) which is a big restraint to their use.