1. Genetic stability in microspore-derived doubled haploids

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1. Introduction

Doubled haploids (DHs) have the potential to greatly shorten the time needed to produce completely homozygous lines compared to conventional breeding. The use of DHs in crop improvement has been reviewed by a number of authors (Baenziger *et al.*, 1984; Luckett and Darvey, 1992; Wernsman, 1992).

Anther or microspore culture, whereby haploid plants are derived from

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microspores by androgenesis, is by far the most efficient method for producing DHs in a wide range of crop species (Maheshwari *et al.*, 1982). In recent years consistent progress has been made using androgenesis for several important crops, particularly among members of the Brassicaceae, Solanaceae and Gramineae, and anther culture is now routinely employed for the production of haploids and homozygous diploids in these crops. The production of cultivars via microspore culture has been reported for rice, wheat, tobacco, maize and pepper (Evans, 1989).

Microspore culture involves the regeneration of plants through a tissue culture phase. Tissue culture methods have long been associated with the phenomenon of somaclonal variation, in which the replacement of an organised tissue structure by unorganised proliferative callus cells leads to genetic changes (Larkin and Scowcroft, 1981; Evans *et al.*, 1984; Karp and Bright, 1985; Evans and Sharp, 1986). Evidence indicates that the genome of higher plants undergoes constant change, and the regeneration of plants from single cells may effectively capture this variation (Scowcroft, 1985). Similar observations have been made on progeny of plants originating from gametic cells cultured *in vitro* (Powell *et al.*, 1984; Morrison and Evans, 1987, 1988). The variation observed among plants regenerated from cultured gametic cells has been termed "gametoclonal variation" (Evans *et al.*, 1984).

Gametoclonal variation may be a disadvantage or an advantage in crop improvement. To produce useful homozygous lines from microspore culture it is usually important that the genetic stability of the lines be maintained. Genetic stability is also necessary for systems relying on the regeneration of uniform material for directed and controlled genetic manipulation such as transformation (Lorz and Brown, 1986), and for the conservation of germplasm through cryopreservation (Bajaj, 1990c). However, somaclonal or gametoclonal variation has also been advocated as a novel source of genetic variability for plant breeders (Evans and Sharp, 1986; Larkin, 1987). One advantage of gametoclonal variation is the opportunity to change one or a few characters without altering the remaining part of the genome, which is often not possible using conventional breeding methods (Orton, 1980b). In this context, gametoclonal variation has a particular potential advantage because it allows the immediate expression of recessive genes. This can be achieved directly without the need for selfing. Because of their origin from single haploid cells, microspore-derived DHs can also provide a unique opportunity to study the factors causing genetic changes in tissue culture. Variability may be expressed more directly in haploids, making it easier to ascertain possible mechanisms and causes (Ziauddin and Kasha, 1990). However, in practice, most of the variability observed in plant cell and tissue cultures has been neither novel nor useful, and on rare occasions when agronomically useful variation has been recovered, the variant plant often shows a number of undesirable genetic changes (Vasil, 1988). The cause of this variation is not fully understood, and because changes can be epigenetic and unstable, its value to agriculture has been questioned (Drew, 1993).