THE INDUCTION OF MICROSPORE EMBRYOGENESIS IN *BRASSICA NAPUS VIA* ANTHER CULTURE AND ISOLATED MICROSPORE CULTURE

by

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ABSTRACT

THE INDUCTION OF MICROSPORE EMBRYOGENESIS IN *BRASSICA NAPUS VIA* ANTHER CULTURE AND ISOLATED MICROSPORE CULTURE

The aim of the work was to establish reliable and predictable methods for the induction of microspore embryogenesis *via* anther culture or isolated microspore culture of *B. napus*. Experimental investigations of some critical factors for the induction of microspore embryogenesis, e.g. genotype, growing conditions of donor plants, microspore developmental stage, thermal treatment, composition and concentration of carbohydrates in the medium, as well as carbohydrate metabolism of cultured anthers, were carried out in a strictly controlled system.

Experiments with isolated microspore culture of individual buds confirmed that embryos were mainly obtained from late uninucleate to early binucleate microspores. Drastic reductions in embryo yield were observed when slightly older or younger microspores were used. Bud length, anther length and the ratio of petal to anther length were evaluated as morphological markers of microspore developmental stage. In general, embryogenesis occurred in anthers from the buds of 3-4 mm long, or anther length between 2.0-2.5 mm, or the ratio of petal to anther length between 1/2-3/4. The strong correlations between these bud characteristics and the developmental stage of microspores indicated that they are good indicators of microspore developmental stage and can be used to select those buds containing potentially embryogenic microspores.

Genotype, donor plant growing conditions and age of inflorescence all substantial effects on embryo yield in *B. napus*. Embryos were only obtained from anther cultures when donor plants were grown in glasshouse or growth cabinet conditions with low temperature and supplementary illumination. Substantial genotypic variation in the

frequency of responsive anthers and the embryo yield per cultured anther was evident for each of the effective donor plant growing conditions. Generally, Nindoo, Topas and Tower gave higher embryo yields than the other three genotypes tested, (Regent, Shiralee and Altex). However, genotype ranking varied with inflorescence age as well as donor plant growing environment. Significantly lower yields of embryos were obtained from buds sampled at the third harvesting date, 14 days after the opening of the first flower. It was also observed that towards the end of flowering, anthers were generally smaller, thinner, and somewhat yellowish. Planting density of the donor plants was also found to have a substantial influence on donor plant physiological conditions and subsequently, on the embryo yields.

The pretreatment of buds by storage at low temperatures following their detachment from donor plants increased the yield of embryos, but did not trigger the initiation of microspore embryogenesis. Bud pretreatment at elevated temperatures proved to be deleterious to microspore embryogenesis. However, a heat shock treatment in the initial period of incubation after the onset of culture was required to initiate the process of microspore embryogenesis in *B. napus*, with incubation at 32.5°C for 3 days being the most effective for isolated microspore culture. Treatments at other temperatures, including short periods of 40°C were either comparatively ineffective or completely inhibitory.

A period of starvation at the beginning of isolated microspore culture (3 days incubation at 25°C on distilled water or a medium lacking sucrose or a medium containing mannitol prior to transfering to the standard induction medium) failed to induce microspore embryogenesis in *B. napus*. In contrast, induction of embryogenesis was obtained when a high concentration of metabolisable sugar was added in the induction medium, with 8% sucrose the most effective. The fact that sucrose favours embryo induction is obviously due to nutritional rather than osmotic differences between the various sugars. Liquid medium was not found to be better than solidified media. Of special interest is that the induction medium solidified with wheat starch also gave good results in terms of the

frequency of embryogenic anthers. It is not clear whether the starch has a dual role as both a gelling agent and a carbon source.

The analysis of soluble sugars and histochemical observations of insoluble carbohydrates were carried out in both *in vivo* developing and *in vitro* cultured anthers. It was found that there were dramatic increases in the contents of fructose and glucose as well as massive accumulation of starch grains in cultured anthers immediately following the initiation of anther culture. This suggests that sucrose is rapidly adsorbed from the induction medium and is of importance in the induction of microspore embryogenesis.

DECLARATION

I HEREBY DECLARE that the work presented in this thesis has been carried out by myself and does not incorporate any material previously submitted for another degree in any university. To the best of my knowledge and belief, it does not contain any material previously written or published by another person, except where due reference is made in the text. I am willing to make the thesis available for photocopy and loan if it is accepted for the award of the degree.

Qing Liu

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