DIFFERENTIAL REGENERATION CAPABILITY OF A WILD TYPE AND A GENIC MALE STERILE LINE OF RAPESEED (BRASSICA NAPUS) CULTURED IN VITRO.

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INTRODUCTION

In breeding programs, male sterile plants of various crop species have often been used for the production of hybrids (Kaul 1988). Usually, cytoplasmic male sterile (CMS) lines are used in hybrid programs as there are problems in maintaining genic male sterile (GMS) lines (Frankel and Galun 1977). One possible way to maintain GMS lines is through in vitro clonal propagation. Regeneration of GMS plants through micropropagation was recently achieved in Brassica juncea (Agrawal et al. 1990). However, different species of Brassica show variation in the in vitro regeneration of plantlets. Also, the regenerative capacity depends on the source, size and age of the explant (Jain et al. 1988; Pua et al. 1989).

Rapeseed (Brassica napus) is an important oilseed crop throughout the world. The objective of the present study was to develop a suitable method for the in vitro regeneration of a large number of GMS plants of B. napus for investigations into the physiological and biochemical mechanisms in male sterility. And to determine the potential of this approach for generating GMS plants for hybrid production.

MATERIALS AND METHODS

The wild type (B. napus cv. Westar) and GMS (Line 3-8) plants of B. napus were grown in growth chambers with a 16/8 hr photoperiod at a $23^0C/18^0C$ (day/night) temperature regime. Light of 180 $\mu Es^{-1}m^{-2}$ was provided by high intensity fluorescent tubes and incandescent bulbs.

For culturing, Murashige and Skoog (1962) medium (MS medium) was used with 3% sucrose, inositol (100 mg/l) and White's vitamins and glycine (White 1943). Benzylamino purine (BAP) and naphthaleneacetic acid (NAA) were added separately or in various combinations, and the pH of the medium was adjusted to 5.8. Difco-Bacto-Agar (0.6%) was used for solidification of the medium and 20 ml aliquots were poured in 100 ml jars and sterilized.

Internodal stem segments were excised from 8-week old plants of wild type and GMS line and sterilized with 20% Javex (commercial bleach containing sodium hypochlorite). These segments were cut into 0.5 cm pieces and transferred to the culture medium. The jars were incubated in growth chamber with 16/8 hr photoperiod at 25^0C for 4 weeks. Each treatment had a minimum of 10 replicates. Observations on number of roots and shoots per explant were recorded at 3- and 7-day intervals, for 4 weeks.

RESULTS

1) Regeneration of GMS plants: Different concentrations of

NAA and BAP (in various combinations) were tested for shoot and root regeneration on GMS explants. The GMS line showed an absolute requirement of NAA for root formation, and the maximum number of roots were produced with $5x10^{-6}$ M NAA. Benzylamino purine was necessary for shoot production. A total of 2-3 plantlets per explant developed with $5x10^{-6}$ M NAA and $5x10^{-5}$ M BAP. After 4 weeks in culture, the plantlets were transferred to peat pellets and finally to pots in the green house. At anthesis, these plants produced male sterile flowers.

2) Differential response by wild type and GMS explants: Explants of the wild type, similar to the GMS line, showed an absolute requirement of NAA for root formation. No roots were produced in the absence of NAA. Both the lines showed best rooting response with $5x10^{-6}$ M NAA, in combination with BAP. The major differences between the two lines was that in almost all cases, the number of roots produced per explant in the GMS line was greater (more than 30) than in the wild type (15-20). The rate of production of roots was also greater in the GMS line in comparison to the wild type.

Shoot formation ability by the wild type explants was opposite to that of rooting ability. Wild type explants regenerated 6-7 shoots per explant in various concentrations of BAP (along with 10^{-5} M NAA) tested. In contrast, GMS explants regenerated shoots only in the presence of $5x10^{-5}$ M BAP, and the maximum number of shoots was 2-3. Benzylamino purine was essential for shoot formation in both lines, as no shoots were produced in its absence.

DISCUSSION

This study has shown that GMS plants of B. napus can be regenerated from internodal segments through in vitro propagation. Although the number of plants produced was not high, it was adequate for the maintenance of the GMS line. The observations also show that the two genotypes viz. wild type and GMS line, differ in their regenerative ability in response to NAA and BAP. The GMS explants produced more roots, whereas the wild type explants produced more shoots.

In separate studies, different workers have reported that various species of B. napus differ in their regenerative capacity (Dunwell 1981; Chuong et al. 1985; Barsby et al. 1986). Some reports also suggest that these differences may be due to differences in the endogenous levels of plant growth substances (Elenshenser et al. 1978). As higher cytokinin/auxin ratio favours shoot formation (Murashige and Skoog 1962), it may be argued that the GMS line is deficient in cytokinins. Alternatively, it is possible that this line may have a higher level of auxins than wild type, as is the case in a tomato GMS mutant (Singh and Sawhney, personnel communication).

In conclusion, the two genotypes have a different response in vitro, in terms of root and shoot formation. Studies are in progress to analyze the endogenous levels of cytokinins and auxins in the wild type and the GMS line.

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