MODIFICATION OF THE OIL PROFILE OF OILSEED RAPE BY SOMATIC HYBRIDISATION

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ABSTRACT

Progress on developing isolation, culture and regeneration protocols for three high erucic acid lines of oilseed rape (HEAR) and for the isolation of protoplasts of the cruciferous species *Lunaria annua* is described. All HEAR lines investigated regenerated plants from mesophyll protoplasts, with the cultivar 'Arthur' proving particularly responsive. The protoplast technology was adapted for use in a programme of somatic hybridisation with one target being the enhancement of the nervonic acid content of *B. napus*. Electrofusion protocols were developed, and putative somatic hybrid material of *B. napus* x *L. annua* regenerated as far as a late callus stage. A number of molecular markers were also screened for their application in characterising such novel material at early stages of development.

INTRODUCTION

The use of protoplast fusion to introgress genes encoding modifications of the fatty acid biosynthetic pathway offers an alternative method to gene transfer facilitated by such means as *Agrobacterium*-mediated transformation. Somatic hybrids have been successfully created among members of the Cruciferae family, enabling the combination of distantly related species, often as asymmetric hybrids, which can be used as a bridge for the transfer of traits between species.

Protoplast regeneration has been reported in a range of *Brassica* germplasm (Glimelius *et al.*, 1991). However, there have been no reports to date of the regeneration of plants from protoplasts of the industrially valuable long chain (C22:1) high erucic acid cultivars. These have assumed greater economic importance in recent years as an industrial crop suitable for use on set-aside land.

There are a number of desirable targets for the modification of the oil profile of *B. napus*, among which an important area is the enhancement of the long chain fatty acid component. The introgression of desirable traits such as elevated levels of nervonic acid (C24:1) from the species *Thlaspi* has been previously reported (Glimelius *et al.*, 1991), but following gas chromatographic analysis of the seed oil profiles of a range of cruciferous species (Millam, Craig and Christie, 1994), we selected the species *Lunaria annua* for further study, as a source of this potentially valuable fatty acid. There are a number of agronomic problems precluding the adoption of *L. annua* as a domestic crop species; consequently, it is our aim to incorporate the long chain fatty acid component of this species into the agronomically acceptable crop plant, oilseed rape.

EXPERIMENTAL

Protoplast isolation, purification and regeneration from high erucic acid *B. napus*

Protoplasts were isolated from 12 day old, dark grown, *in vitro* hypocotyl tissue using the methods of Glimelius (1984) and *in vitro* mesophyll tissue by the methods of Loudon, Nelson and Ingram (1989) modified by replacing the percoll purification step with the use of a 21% sucrose solution. The protoplasts were cultured in medium B (Pelletier *et al.*, 1986) at a density of 5000/ml in 50 mm dishes at 25°C in the dark for three days before exposing to light. After 30 days the cells were overlayed onto media TB1, and at the stage of calli development of diameter 1–2 mm onto TB2 for shoot regeneration (Barsby, Yarrow and Shepard, 1986).

TABLE 1. Summary of protoplast response of HEAR lines

Cultivar	Source material	Mean no. protoplasts per gram fresh weight	Number of plants regenerated to date
Martina	leaf	$1.78 \pm 0.55 \times 10^6$	11
	hypocotyl	$3.30 \pm 0.89 \times 10^5$	5
Askari	leaf	$1.70 \pm 0.40 \times 10^7$	25
	hypocotyl	$5.80 \pm 2.10 \times 10^5$	12
Arthur	leaf	$4.10 \pm 0.60 \times 10^6$	>120
	hypocotyl	$4.20 \pm 1.10 \times 10^5$	n.a.

Protoplast isolation and purification from *Lunaria annua*

Mesophyll protoplasts of *L. annua* were isolated and purified according to the methods of Craig and Millam (1994). From over twenty separate isolations, a mean yield of $2.86 \pm 1.51 \times 10^6$ per gram fresh weight was calculated. Those protoplasts not used in fusion studies were cultured in media C of Pelletier *et al.* (1983) where low levels of cell division were observed after ten days culture. Mesophyll protoplasts of *L. annua* (mean diameter 42.2 μm ± 5.2) were found to be significantly larger than those from *B. napus* (mean diameter 28.5 μm ± 6.2) and were morphologically distinguishable from *B. napus* protoplasts due to colour and chloroplast configurations.

Protoplast fusion and regeneration of fusion products

Fusions were set up using a 1:1 ratio of either hypocotyl or mesophyll protoplasts of HEAR lines, and mesophyll protoplasts of *Lunaria*. Freshly isolated protoplasts were re-suspended in a mannitol:calcium chloride solution and fused using a Braun Biojet 50 apparatus, with a fusion voltage of 90V and one 30 microsecond pulse. The fusion material was taken through the protocol as far as the TB2 stage but, to date, no shoot regeneration has been observed. High rates of fusion were also obtained by using PEG

(Polyethylene glycol 6000) – calcium chloride mediated fusion, but no cell divisions were observed following these procedures.

Development of randomly amplified polymorphic DNA-based markers (RAPDs) for identification of somatic hybrids

DNA was isolated from all three HEAR cultivars and from *L. annua* using standard mini-prep procedures, with an additional phenol:chloroform extraction stage. A range of primers created at SCRI were investigated for their suitability as a means of distinguishing between all three HEAR lines and also *Lunaria*. A portfolio of such markers was assembled to be used for the early verification of putative somatic hybrid material.

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