High-oleic acid Australian Brassica napus and B. juncea varieties produced by co-suppression of endogenous Δ12-desaturases
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Abstract
Genetic engineering methods have been used successfully to modify the fatty acid profile of elite Australian germplasm of Brassica napus and B. juncea. Co-suppression plasmids carrying oleate desaturase genes from each species have been constructed and transferred into Australian elite breeding lines of B. napus and B. juncea using Agrobacterium tumifaciens plant-transformation techniques. Modifications to existing Brassica transformation protocols and the use of an intron-interrupted hygromycin-resistance gene as the selectable marker have resulted in improved transformation efficiencies. Silencing of the endogenous oleate desaturase genes has resulted in substantial increases in oleic acid levels, up to 89% in B. napus and 73% in B. juncea.

Introduction
The Australian canola industry has grown rapidly in recent years and has considerable scope for further expansion to meet a growing domestic utilization of canola oil as well as a large world trade in canola seed. Two factors will be important driving forces of expansion.

Firstly, there are increased domestic and export market opportunities for canola oil that can be realized through the development of high-oleic acid canola to replace saturated palm oil in food-service applications. High-oleic acid oils have been shown to have equivalent heat stability to saturated fats and are therefore suitable replacements for them in commercial food-service applications that require long-life stability. Additionally, high-oleic acid oils are more nutritionally beneficial because oleic acid has cholesterol-lowering properties, whereas saturated fatty acids tend to raise blood cholesterol levels.

Secondly, there are agricultural opportunities to increase canola production by expanding into the drier regions of the Australian cereal belt. This is being addressed through both the development of earlier-maturing varieties of Brassica napus canola and the introduction of B. juncea as an alternative source of canola-quality oil. Current double-low germplasm of B. juncea has around 45% oleic acid, significantly below the level present in B. napus and B. rapa. For B. juncea to become a viable alternative source of canola oil, oleic acid must be raised to at least 60%.

Increases in oleic acid content can potentially be achieved by reducing the activity of oleate desaturase (oleoyl-phosphatidylcholine Δ12-desaturase), the enzyme which converts oleate into linoleate in the developing seed. Ethylmethanesulfonate mutants which raise oleic acid up to 80% have been successfully obtained [1]; however, mutants having further increases above this level have been associated with undesirable agronomic effects [2]. Molecular mechanisms of inactivating genes, such as antisense and co-suppression, can be implemented in a tissue-specific manner enabling the inactivation of all copies of Δ12-desaturase in the developing seed without affecting gene expression in other tissues. Antisense and co-suppression methods have already been successfully employed to raise oleic acid up to 85% in both B. napus [2] and soybean [3].

In order to produce high-oleic acid quality in Australian Brassica germplasm we have implemented a Δ12-desaturase gene co-suppression strategy. This paper describes the progress made in achieving transformation in elite Australian breeding lines of double-low B. napus and B. juncea and in raising oleic acid content in both species.

Experimental
Plant material
The advanced breeding lines of B. napus R125 and BLN1239 were provided by Agriculture Victoria and NSW Agriculture respectively. A zero-erucic acid, low-glucosinolate line of B. juncea (815-1-6-2) was provided by CSIRO Plant Industry.

Binary plasmids
The oleate desaturase gene from B. juncea had previously been cloned [4] and the corresponding gene from B. napus was cloned by reverse transcriptase PCR. Co-suppression constructs were made by positioning either the B. napus or

Key words: canola, desaturation, Indian mustard.

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B. juncea Δ12-desaturase gene in the sense orientation downstream from a truncated form of the napin seed-storage promoter (FP1). These constructs were cloned blunt-ended into the Not1 site of the p35SH-iC binary vector described previously [5].

Transformation methods
The transformation protocol followed that of Bade and Damm [6] with minor modifications. A selection of putative transformed B. napus plants carrying the Δ12-desaturase co-suppression constructs were analysed by standard Southern analysis techniques. A total of 20 out of 21 plants analysed were positive for the presence of the selectable marker gene.

Results
Selfed was harvested from 44 B. napus (31 of BLN1239 and 13 of RI25) and eight B. juncea hygromycin-resistant Tₐ plants carrying the Δ12-desaturase co-suppression constructs. Bulk samples of 20 seeds per plant were analysed for fatty acid composition by GC separation of fatty acid methyl esters obtained from acid-methylated oil samples [7]. Three B. napus Tₐ plants (two of BLN1239 and one of RI25) had oleic acid levels above 80% (control, 60–65%), while three lines of B. juncea had oleic acid levels above 62% (control, 40–45%).

Single seed analysis of fatty acid composition was subsequently carried out on ten individual whole Tₐ seeds from each of the high-oleic acid Tₐ plants. The highest oleic acid found in single Tₐ seeds was 89% in B. napus and 73% in B. juncea (Figure 1). The levels of polyunsaturated fatty acids (linoleic and linolenic acid) were reduced markedly in these seeds, down to 4% in B. napus (compare with 26% in controls) and 16% in B. juncea (44% in controls). In both species the high oleic lines contained levels of saturated fatty acids (palmitic and stearic) similar to those of control lines.

Discussion
In B. napus, 43 primary (Tₐ) transgenic plants carrying the Δ12-desaturase co-suppression construct have so far been analysed. Of these, three lines have very high levels of oleic acid, up to 89% compared with the normal level of 63%. These levels are similar to those reported previously to occur in B. napus by suppression of Δ12-desaturase gene activity [2]. High-oleic acid derivatives of both B. napus lines (RI25 and BLN1239) were obtained. Southern analysis indicated that RI25*9-2 carries two copies of the transgene and BLN1239*12-2 carries six copies. The variation in oleic acid content observed between individual Tₐ seeds presumably reflects segregation for the transgene, and suggests that the highest oleic acid phenotype observed may be retained in homozygous segregants isolated from the progeny of selected Tₐ seeds.

In B. juncea, only seven transformants of CS815-1-6-2 have so far reached maturity. Of these, three lines had elevated oleic acid content, up to 73% compared with the normal level of 42%. This is the first time that these levels have been reported in B. juncea and it enables this species to be immediately converted to a source of

![Figure 1](distribution_of_oleic_acid_percentage.pdf)
Inhibition of polyunsaturated fatty acid accumulation in plants expressing a fatty acid epoxygenase

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Abstract

Earlier, we described the isolation of a Crepis palaestina cDNA (Cpal2) which encoded a Δ12-epoxygenase that could catalyse the synthesis of 12,13-epoxy-cis-9-octadecenoic acid (18:1E) from linoleic acid (18:2). When the Cpal2 gene was expressed under the control of a seed-specific promoter in Arabidopsis, plants were able to accumulate small amounts 18:1E and 12,13-epoxy-cis-9,15-octadec-2-enoic acid in their seed lipids. In this report we give results obtained from a detailed analysis of transgenic Arabidopsis plants containing the Cpal2 gene. The seeds from these plants accumulate varying levels of 18:1E, but show a marked increase in 18:1E and equivalent decrease in 18:2 and 18:3. We further observed that the co-expression of a C. palaestina Δ12-desaturase in Arabidopsis appears to return the relative proportions of the C18 seed fatty acids to normal levels and results in a 2-fold increase in total epoxy fatty acids.

Introduction

Epoxy fatty acids are valuable raw materials for the production of resins, glues, plastics, polymers and other surface coatings. Currently, epoxy fatty acids are derived by the chemical epoxygenation of the carbon double bonds present in highly unsaturated vegetable oils, such as soybean and linseed oils, or by synthesis from petrochemicals [1]. Earlier, we described the isolation of a Crepis palaestina cDNA (Cpal2) which encoded a fatty acid Δ12-epoxygenase that could catalyse the synthesis of vernolic acid (12,13-epoxy-cis-9-octadecenoic acid; 18:1E) from linoleic acid (18:2). When the Cpal2 gene was expressed in Arabidopsis under the control of a seed-specific promoter, plants were able to accumulate small amounts of 18:1E and 12,13-epoxy-cis-9,15-octadec-2-enoic acid (18:2E) in their seed lipids. This amount of epoxy fatty acid was significantly less than the amount found in the seeds of C. palaestina, where they comprise over 60% of total seed lipids [2].

Transgenic expression of the Cpal2 and other Δ12-acyl-modifying enzymes in Arabidopsis not only resulted in low levels of accumulation of the

References


Received 26 June 2000

Key words: Arabidopsis thaliana, Crepis palaestina, Δ12-desaturase, Δ12-epoxygenase, epoxynated fatty acid.
Abbreviations used: 18:1E, 12,13-epoxy-cis-9-octadecenoic acid; 18:2E, 12,13-epoxy-cis-9,15-octadec-2-enoic acid; ODP, oleic desaturation proportion.
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