Development of rapeseed plants with optimised oilseed quality

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ABSTRACT

The seed oil of specific cultivars of *Brassica napus* L. is one of the primary sources of erucic acid. This long-chain fatty acid can be used as an important raw material for industry with a wide range of potential applications.

One way to develop plants with high erucic-acid content is the production of somatic hybrids between *B. napus* and *Sinapis alba* having a higher erucic acid content than *B. napus.* Mesophyll protoplasts from *B. napus* line '11502' and from cv. 'Lisandra' were fused with protoplasts from *S. alba* cv. 'Mustang' and cv. 'Litember' *via* PEG induction. The erucic-acid content was measured by half-seed analysis of seeds obtained from hybrids by self-pollination or backcrosses with *B. napus*. Seeds from the combination '11502' (+) 'Litember' displayed different contents of erucic acid compared to the *B. napus* control.

An alternative way is the development of transgenic plants with high contents of erucic acid in their seed oil due to an introduced LPAAT gene of *Limnanthes douglasii* and KCS gene of *B. napus* combined with ATP:citrate-lyase gene. The culture of C58 ATHV harbouring pPZP 111 was used to transform hypocotyl explants of the resynthesised line '306' with a modified method of De Block et al. (1989). Transgenic plants were self-pollinated. The harvested seeds were analysed for their fatty-acid composition. First results showed no differences in the erucic-acid content between the control plants and the transformed plants. Further gene constructs will be tested.

Key words: Erucic acid, somatic hybrids, gene transfer, *B. napus, S. alba*

INTRODUCTION

One of the most important aims of transgenic oilseed breeding is the genetic modification of seed oil by maximising specific fatty acids to obtain oil which can be used for oleochemistry. Vegetable oils have a broad range of technical applications from soaps to lubricants. The different fatty-acid specificity of acyltransferases permits varying fatty-acid composition of plant lipids. Lysophosphatidic acid acyl-transferase (LPAAT) of oilseed rape, *B. napus*, cannot incorporate 22:1 at the *sn*-2 position of the glycerol backbone (Frentzen 1993). In contrast, LPAATs from different *Limnanthes* species enables those organisms to do so (Brough et al. 1996). Because of this limitation *B. napus* is theoretically not able to achieve more than 66% erucic acid in the seed oil based on traditional breeding methods.

Oilseed rape is easy to handle in tissue-culture systems and this has allowed the establishment of a number of protocols for somatic hybridisation and genetic engineering.

The main procedure used for protoplast fusion is the polyethylene glycol (PEG) method by which high frequencies of heterofusion products can be obtained. Successful utilisation of electric stimulation has also been reported (Brewer et al. 1999; Müller and Sonntag 1999). Heath and Earle (1995) synthesised rape seed plants with an erucic-acid content of 57.4% and a low linolenic-acid content (3.5%) through symmetric hybridisation between *B. oleracea* and *B. rapa*. Schröder-Pontoppidan et al. (1999) found 61.5% erucic acid in F6 offspring of somatic hybrids between rapeseed cv. 'Hanna' and *Lesquerella fendleri.*

Another method which is most commonly used for the transfer of novel traits is *Agrobacterium tumefaciens*-mediated transformation. An efficient tissue culture regeneration system is essential for successful transformation of plant cells. The major strategy to change the fatty-acid

composition is by expressing a homologous gene in an antisense direction. Two aims were followed in these transformation experiments: 1. changes in the amounts of fatty acids and 2. production of novel fatty acids (Broun and Somerville 1997).

MATERIALS AND METHODS

Seeds of *B. napus* cv. 'Maplus' were kindly provided by Norddeutsche Pflanzenzucht "Hans-Georg-Lembke" KG Hohenlieth, 'RS306' originated from the reciprocal cross 'Yellow Sarson' x cauliflower cv. 'Super Regama' by Justus-Liebig University of Gießen and breeding line '11502' by the plant breeding company KWS Saatzucht AG Einbeck. These three cultivars contain about 50-60% 22:1 in their seed oil. The zero-erucic acid seeds from cv. 'Lisandra' were obtained by Deutsche Saatveredelung Lippstadt-Bremen GmbH. Seeds of *Sinapis alba* cv. 'Litember' and cv. 'Mustang' were obtained by the gene bank of the IPK Gatersleben.
Mesophyll protoplast isolation from leaves of 4-weeks-old *in vitro* grown plants and the identification of the somatic hybrids were carried out according to Müller and Sonntag (2000). PEG was used to induce protoplast fusion. Self-pollination and backcrossing were used for the production of seeds.
For transformation experiments hypocotyls from 6-days-old etiolated seedlings of 'RS306' were incubated with the *Agrobacterium* strain C58 ATHV. pPZP111 containing ATP:citrate-lyase gene (ACL) were used in co-transformation with pNKAT55 containing KCS (*ß*-ketoacyl-CoA synthase) and *L. douglasii* LPAAT genes. Further pRE1 with genes for ACL, KCS and LPAAT was used. Transformation was performed according to De Block et al. (1989) with minor modifications in the duration of the co-cultivation and in the intensity of the kanamycin selection. Regenerated transgenic plants were cultivated in the greenhouse and before self-pollination and harvest of seeds the plants experienced a vernalisation period of 8 weeks at 4° C.
Fatty-acid composition of the seed oil of somatic hybrids and transformants was analysed with gas chromatography using the half-seed technique described by Thies (1994).

RESULTS

Two combinations were made between *B. napus* and *S. alba*. The shoot regeneration efficiency ranged from 12.6-41%. From 207 regenerated plants analysed by flow-cytometry, 12 plants were identified as hybrids. The symmetric hybrids of *B. napus* cv. 'Lisandra' and *S. alba* cv. 'Mustang' were pollen-sterile while *B. napus* '11502' and *S. alba* cv. 'Litember' developed seeds after self-pollination. Fatty-acid composition of seeds was compared between somatic hybrids backcrossed with either the zero-erucic *B. napus* parent cv. 'Lisandra' or the high-erucic *B. napus* line '11502' (Fig.1).

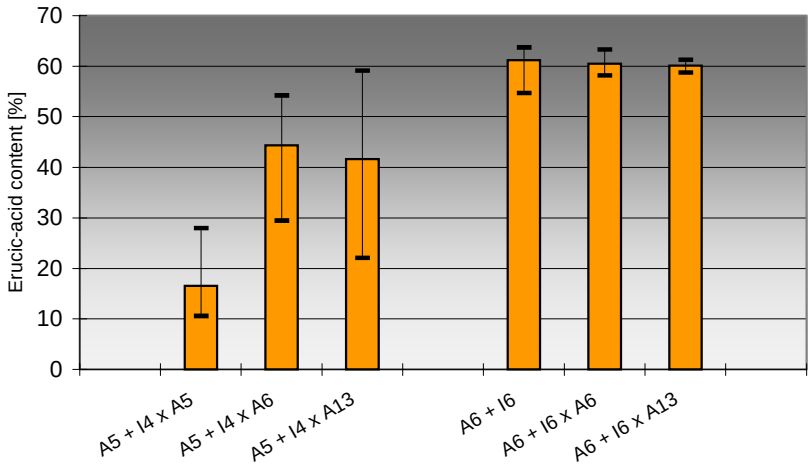

Figure 1: Means and ranges of erucic-acid contents [in %] in F1- and BC1-seeds of somatic hybrids from *B. napus* and *S. alba* according to half-seed analysis
(A5 = *B. napus* 'Lisandra', A6 = *B. napus*-line '11502', A13 = *B. napus* 'Maplus', I4 = *Sinapis alba* 'Mustang', I6 = *Sinapis alba* 'Litember')

The results demonstrated that backcross progeny involving two high-erucic parents had considerably higher erucic-acid contents. Four of five BC1 plants had erucic-acid contents of more than 62%. The erucic-acid content was very heterogeneous in the offspring of somatic hybrids between *B. napus* 'Lisandra' and *S. alba* 'Mustang'.

Up to now 135 transgenic plants were obtained from the transformation of line 'RS306'. Successful gene transfer was confirmed by NPTII ELISA assay. Transformation efficiency varied between the constructs used. It was lower in the co-transformation experiments: 9% in comparison to 12% with the triple construct. Transgenic and untransformed plants of 'RS306', which had been cultivated in the greenhouse, showed great morphological variation. Some of the plants displayed deformations, absence of pollen formation or abnormal seed set. Of the co-transformed 'RS306' lines 26 of 70 transformants yielded more than 0.4g seeds/plants. Seeds of these plants were analysed for their fatty-acid composition. The seeds of the plants being transformed with the triple construct remain yet to be harvested.

The erucic-acid contents in the 26 transformants showed no differences to the original cultivar. Therefore it is necessary to carry out further experiments and to add the results of the seed-oil composition of the transformants obtained with the triple construct containing ACL, KCS and LPAAT.

DISCUSSION

First data based on half-seed analyses of symmetric somatic hybrids of *B.* napus line '11502' and *Sinapis alba* 'Litember' indicated increased erucic-acid contents up to 65% in the offspring when backcrossed with high-erucic acid lines. This is consistent with results obtained by Heath and Earle (1995) from fusions between *B. oleracea* and *B. rapa*. Based on these results further back-crossings are planned for stabilising the high erucic-acid level in following generations.

ACKNOWLEDGEMENTS

Funding by the Fachagentur für Nachwachsende Rohstoffe, Gülzow, Germany, is acknowledged.

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