EARLY SELECTION FOR FATTY ACID COMPOSITION IN MICROSPORE-DERIVED EMBRYOIDS OF RAPESEED (*BRASSICA NAPUS L.*)

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ABSTRACT

The availablity of new qualities of rapeseed oils with, e.g., a very high oleic acid content (HOAR), a very high erucic acid content (HEAR) or a low linolenic acid content is increasing rapidly due to the tools of mutation breeding, interspecific protoplast fusion and molecular gene transfer. However, the changes in quality are accompanied by mutation or introduction of genes and thus make existing segregating patterns even more complex. Application of the DH-technique in combination with a method of early selection for oil quality in the cotyledons of microspore-derived embryoids (MDEs) could facilitate further breeding progress, as outlined in the following investigations.

INTRODUCTION

Microspore-derived embryoids (MDE) of rapeseed have the advantage that their cotyledons contain storage lipids which are in their composition similar to the ones found in the seeds. At the late cotyledonary stage the microspore-derived embryoids are large enough to allow a dissection of one cotyledon under aseptic conditions and the determination of its fatty acid composition. The remaining part of the embryoid can be cultured further and regenerated to a plant. This offers the chance of an early selection for fatty acid composition in segregating populations of microspore-derived embryoids.

EXPERIMENTALS

Doubled haploid lines derived from the spring rapeseed cultivars 'Duplo' (low in erucic acid) and 'Janetzki' (~45% C22:1) as well as from different resynthesized spring rapeseed lines high in erucic acid (~58%) obtained from W. Lühs (Giessen) were used for crossing. In addition, lines were included carrying a translocation with the erucic acid gene from *Brassica nigra* and backcrossed to the spring rapeseed cultivar 'Andor'. F_1-seeds were harvested and grown with their parent lines in the greenhouse to produce donor plants for the microspore culture. Microspore culture, dissection of one cotyledon of the embryoids and plant regeneration from the remaining part, as well as fatty acid analyses were performed essentially as described by Albrecht et al. (1995).

From the segregating microspore population derived from F1-plants derived from the cross between doubled haploid lines of 'Duplo' and 'Janetzki' a total of 134 embryoids were

regenerated. The genotypes were divided into three classes based on the sum of eicosenoic acid (C20:1) + erucic acid (C22:1), i.e. **group 1**: ≤ 2% C22:1+C20:1 (n=37), **group 2**: ≤38% C22:1+C20:1 (n=86), and **group 3**: > 38% C22:1+C20:1 (n=11). Discrimination of genotypes with no or very little C20:1+C22:1 in the cotyledons from those with an intermediate content was easily possible as shown in Fig. 1. For the other genotypes there was a gradual increase in C20:1+C22:1 content, which made a clear separation into classes with an intermediate and high content difficult. This is also reflected by the results of a Chi^2 test for a 1:2:1 segregation (low : medium : high content; Chi^2=20,86) which was significant. However, the Chi^2-test confirmed a 3:1-segregation (medium + high : low; Chi^2=0,487). The contents of eicosenoic and erucic acid in the dissected cotyledons and in the seeds derived from plants regenerated from the remaining parts of the embryoids were highly correlated (r_S=0.85**). For the C20:1+C22:1 class a correlation of rS=0.65** was calculated (Fig.2). These results demonstrate that 60-70% of the regenerated MDEs may be discarded (89 of 134) at an early *in vitro* stage and only the remaining embryoids with the highest erucic acid content *in vitro* have to be transferred to the greenhouse in order to obtain the 10 genotypes with the highest erucic acid content in the seeds (or 18 out of the 20 highest).

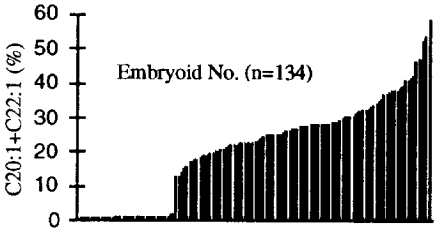

Fig.1: C20:1+C22:1 content of cotyledons dissected from MDEs of 'Duplo'x'Janetzki'

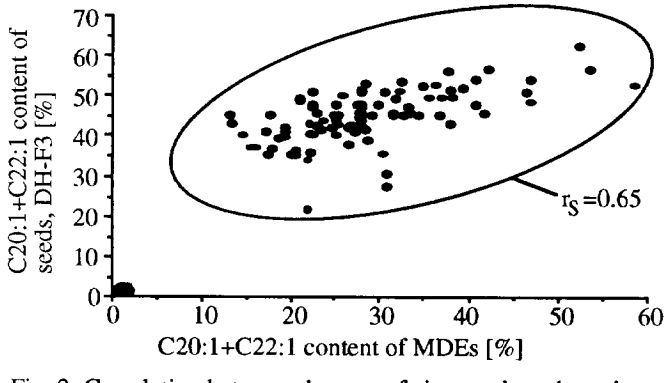

Fig. 2: Correlation between the sum of eicosenoic and erucic acid content of cotyledons of microspore-derived embryoids and seeds obtained from the plants regenerated thereof

In *Brassica* species, erucic acid content of the storage lipids is limited by the theoretical threshold of 66% because of the strict stereospecificity of the acyltransferase enzyme(s). However, while most of the present days' cultivars and breeding lines yield no more than about 50% erucic acid of their seed oil, a search for more efficient alleles among resynthesized rapeseed led to the generation of spring and winter types with an erucic acid content of ca. 60% (Lühs and Friedt 1994). Further increase might be obtained by introgression of additional erucic acid genes from other cruciferous species and by the introduction of a sn2-specific acyltransferase that effectively

incorporates erucic acid. To test the effect of an additional erucic acid gene on segregation of the erucic acid content in cotyledons of MDEs, F_1-plants of a cross between a high erucic acid *B. napus* 'Resyn' line with 56% C22:1 (EEEE) with a rapeseed line carrying the erucic acid gene from *B. nigra* as a translocation (eeeeE*E*) have been used for microspore culture. The results of fatty analyses of cotyledons is shown in Fig.3. Although a totally different segregation pattern was obtained, the highest contents reached for C20:1+C22:1 were not different from the results obtained for the cross 'Duplo' x 'Janetzki'. Whether the plants regenerated from the embryoids with the highest contents will indeed reach about 66% C20:1+C22:1 in their seedoil still needs to be shown. Those genotypes would represent prime candidates for transformation with the sn2-specific acyltransferase gene isolated from *Limnanthes* (Hanke et al. 1994).

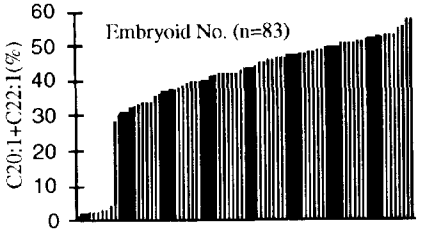

Fig.3: C20:1+C22:1 content of cotyledons dissected from MDEs of 'EEEE'x'eeeeE*E*'

Since the introgression of new genes will result in more complex segregation patterns in offsprings, the production of homozygous DH lines via microspore culture in combination with an early selection for the desired fatty acid composition represents an efficient tool for the selection of specific genotypes from a reduced population size. Whether the described microspore-derived embryoid system can also be used for *in vitro* selection of high stearic acid, high oleic, or low linolenic lines needs to be demonstrated with appropriate cross progenies.

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REFERENCES

Albrecht, S., Möllers, C. and Röbbelen, G. (1995). Selection *in vitro* for erucic acid content in segregating populations of microspore derived embryoids of *Brassica napus*. *Plant Breeding*, in press.

Lühs, W. and Friedt, W. (1994). Stand und Perspektiven der Züchtung von Raps (Brassica napus L.) mit hohem Erucasäure-Gehalt im Öl für industrielle Nutzungszwecke. Fat Science Technology **96**, 137-146.

Hanke, C., Peterek, G., Wolter, F.-P. and Frentzen, M. (1994). Cloning of cDNAs from Limnanthes douglasii showing similarity to the plsC gene of Escherichia coli encoding 1-Acylglycerol-3-phosphate acyltransferase. Proceedings of the 11th International Meeting on Plant Lipids, Paris, June 26 - July 1, 1994.