Increase of erucic acid content in oilseed rape (*Brassica napus*) through the combination with genes for high oleic acid

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ABSTRACT

Erucic acid (C22:1) is a valuable renewable resource which has several applications in the oleochemical industry. High C22:1 rapeseed (HEAR) contains ca. 50% C22:1. For the technical utilization it is desirable to increase the C22:1 and to decrease the eicosenoic acid (C20:1), polyunsaturated fatty acid (C18:2+C18:3) and saturated fatty acid (C16:0+C18:0) contents. In the present experiment, HEAR was crossed to high oleic acid rapeseed (HOAR, ca. 85% C18:1) with the hypothesis that a combination of the involved genes should lead to an enhanced content of C22:1 and of total monounsaturated fatty acids (MUFA, C22:1+C20:1 +C18:1). A near-infrared spectroscopical (NIRS) calibration for C22:1 was developed for single seeds and the calibration was used to select in a non-destructive manner F2-seeds high in C22:1. Selected F2-seeds were sown in the field and following selfing F3 seeds were harvested. The results of the fatty acid analysis showed recombinant genotypes with increased total MUFA content (up to 87%), decreased polyunsaturated (<8%) and saturated fatty acid content (<3.5%). There was no significant difference in C22:1-content, but an increased eicosenoic acid (C20:1) content was observed in comparison to the HEAR parental cv. Maplus. This new type of oil should have improved properties for use as lubricant and hydraulic oil.

Key words. Erucic acid - oil quality - NIRS – monounsaturated fatty acid – High oleic acid

INTRODUCTION

Erucic acid (C22:1) is a valuable and renewable resource which has several potential applications in the oleochemical industry for the production e.g. of plastics, lubricants, slip and coating agents, soaps, printing inks, surfactants, etc.. Current high erucic acid rapeseed (HEAR) cultivars contain only around 50% C22:1 in the seed oil. Because of the several applications, increase of the C22:1 content has attracted considerable interest. Combining the genes for high C22:1 with those for high C18:1 could be one way to further increase the C22:1 content. An increase in C22:1 could be expected, because C18:1 content is rather low in HEAR and this may limit the elongation to C22:1. The combination with genes for high C18:1, which is related to a reduced content of polyunsaturated fatty acids, may increase the pool of C18:1 available for elongation to C22:1. In the present study high C18:1 rapeseed lines (ca. 85% C18:1) were crossed to the high C22:1 cv. Maplus. Since C22:1-content is inherited by 2 genes and the HO-trait in the present material is inherited by 1 major and additional minor genes (Schierholt et al. 2001) a large number of F2 seeds need to be analysed to identify the desired recombinants. This was done in the present study by using a single seed NIRS calibration for C22:1. Selected high C22:1 F2-seeds were sown in the field and F3 seeds were harvested following selfing.

MATERIAL AND METHODS

Four high C18:1 and low linolenic (C18:3) sister DH-lines ('00'-quality, winter rapeseed) were crossed to the high C22:1 winter rapeseed cv. Maplus ('+0', 50% C22 :1). The 4 DH-lines had similar C18:1 (82-86%), C18:2 (2.7–4.7%) and C18:3 (2.6-4.3%) contents. F1-plants were grown in the greenhouse and selfed to obtain F2 seeds. A subsample of the F2 seeds were scanned by NIRS (FOSS 6500) using a single seed adapter and analysed for their fatty acid composition (FAC) by gaschromatography as described by Thies (1971). A calibration was developed using WinISI 1.04. This had an coefficient of determination in cross-validation 1-VR

of 0.58 and a SECV of 6.5%. The calibration was used to select out of 3200-3600 scanned seeds from each of the 4 F2-populations those 200 seeds with the highest C22:1 content. Selected seeds were grown together with the parental cv. Maplus in the field and after selfing, F3 seeds were harvested in 2002 and analysed for FAC by gaschromatography.

RESULTS

In the F2 seed population a segregation for C22:1 following a 1:4:6:4:1 pattern was found, as expected for a digenically inherited trait (data not shown). The F3-seeds derived from selected high C22:1 F2-seeds of the 4 crosses were not different for C22:1 or C18:1. Hence, the data were combined. Fig. 1 shows that genotypes with more than 20% C22:1 are enriched following single seed selection in F2 for high C22:1 content by NIRS. Thus, NIRS can be used to pre-select seeds with a high C22:1 content. However, there are a number of outliers that probably

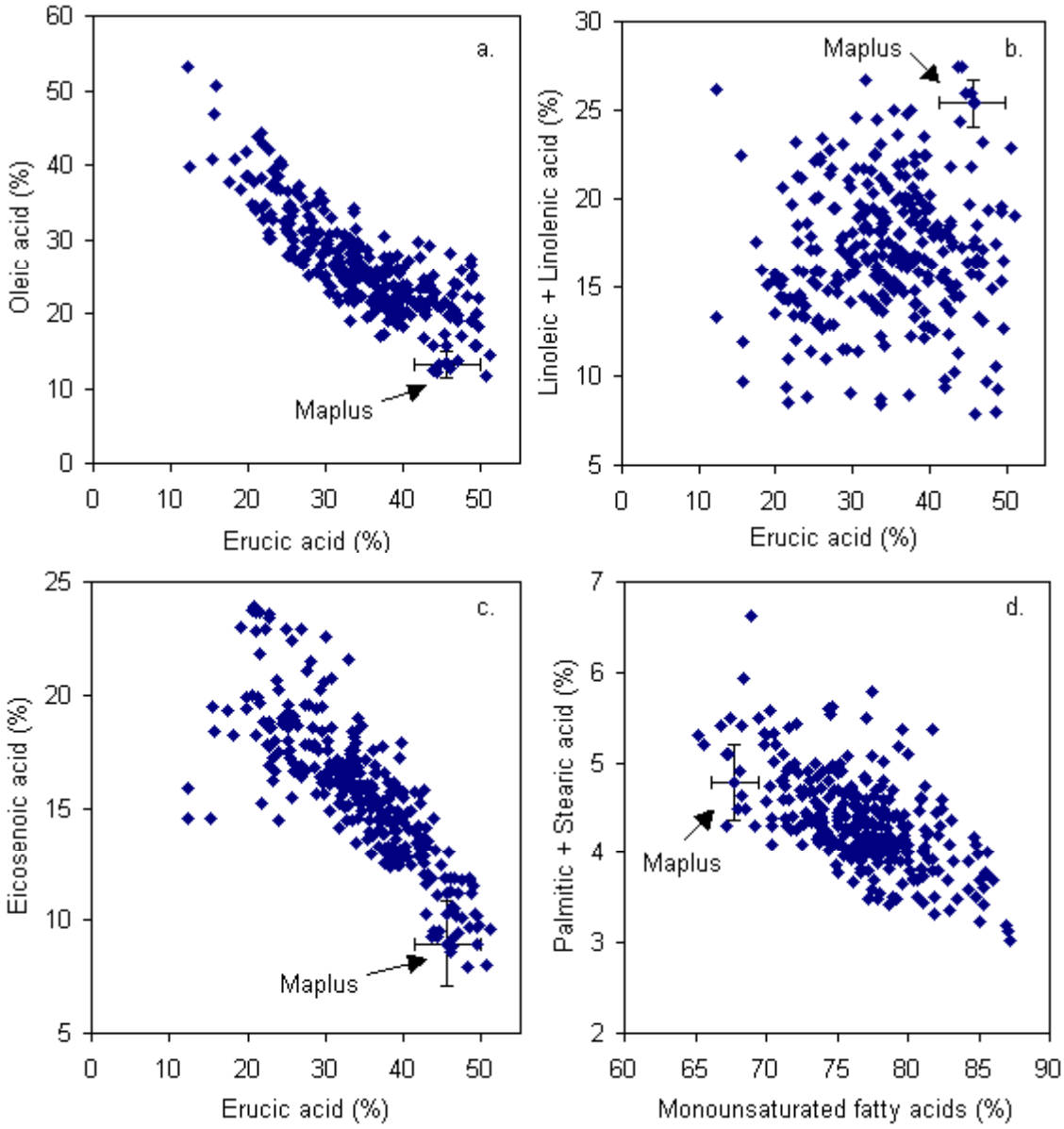

Fig. 1a-d: Fatty acid composition of F2 plants (F3 seeds) derived from crosses between high erucic acid and high oleic acid rapeseed, preselected for a high erucic content (n=351).

belong to the neighbouring EEEe and EEee classes. These can be due to wrongly estimated C22:1-content in selected F2-seeds by NIRS and due to environmental influences on the C22:1-content in single F2-seeds. An improved calibration and larger, more uniform seeds harvested from the field, instead of seeds from the greenhouse, should help to improve the selection for high C22:1-genotypes among F2-seeds.

Some of the high C22:1 F2-plants had a 15% increased C18:1 content compared to the high C22:1 parent cv. Maplus (Fig. 1a), which corresponded to a reduced C18:2+C18:3 content (Fig. 1b). However, this did not lead to an enhanced C22:1 content. The high C22:1 high C18:1 lines tended to have a higher C20:1 content (Fig. 1c). There was a wide variation for the MUFA content from 65-87% (Fig. 1d). The 3 lines with the highest MUFA content had about 47% C22:1, 12% C20:1, and 27% C18:1. A negative correlation between MUFA and saturated fatty acids was also found (Fig. 2d) with levels of saturated fatty acids as low as 3.0%. From 2 high C22:1 high C18:1 F2-plants, single F3-seeds were analysed for their FAC (Tab.1). Results confirmed homozygosity for the high C22:1 alleles and the increased C20:1 content.

Tab. 1: Mean fatty acid composition of single F3-seeds (n=20) of two selected high erucic high oleic acid F2 plants and of the parental cv. Maplus

Plant	C16:0+C18:0	C18:1	C18:2+C18:3	C20:1	C22:1	MUFA
6575-1	3.2±0.3 [b]	29.9±1.5 [b]	6.7±0.8 [a]	12.4±1.3 [b]	46.7±2.8 [a]	89.1±1.1 [b]
6586-3	3.6±0.6 [b]	28.1±2.3 [b]	8.0±2.3 [a]	11.1±1.6 [b]	47.9±4.4 [a]	87.0±3.3 [b]
Maplus	5.0 ±0.6 [a]	13.9±2.9 [a]	25.7±2.5 [b]	7.2 ±1.8 [a]	46.7±4.6 [a]	67.9±3.1 [a]

[a,b] – significantly different at p<0.05%, Tukey-Kramer

DISCUSSION AND CONCLUSION

The results show that an increased C18:1 content in the high C22:1 background does not lead to an enhanced C22:1 content. However, some recombinants showed a low content of polyunsaturated (7%) and saturated (3%) fatty acids, and a correspondingly high content of monounsaturated fatty acids (87%). Such an oil should have an improved stability and properties at low ambient temperatures. High oleic acid rapeseed (HOAR) oil has a similar MUFA content compared to the present material. However, erucic acid has a higher viscosity than oleic acid which should make this oil more useful for several applications.

The by around 5% increased C20:1 content in the present high erucic high oleic acid material indicates that there is only a limited influence of the high oleic acid genes on the fatty acid elongation, and that there are other biochemical limitations that restrict the enhanced erucic acid formation. The plant material generated in the present study may be useful in future experiments to identify these limitations.

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