BREEDING STRATEGIES FOR THE DEVELOPMENT OF HIGH PERFORMING
WINTER RAPESEED WITH AN IMPROVED POLYENOIC
FATTY ACID COMPOSITION

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INTRODUCTION

In order to increase the amount of rapeseed oil consumed by the food industry, a more appropriate fatty acid (f.a.) composition of the oil is desired. From the nutritional point of view, an edible oil for human consumption should contain a high level of polyenoic fatty acids, in particular linoleic acid. On the other hand, the oxidative stability of an oil is related to the number of double bonds in its fatty acid chains. Due to the three double bonds in the linolenic acid, shelf live of seedoils containing high levels of this f.a. is reduced.

Canola oil exhibits a fatty acid composition of about 60 % - 65 % oleic (C18:1), 20 % - 25 % linoleic (C18:2), and around 10 % linolenic (C18:3) acid. Therefore, the aims of breeding are to increase the C18:2 fraction to 30 % and simultaneously to reduce the C18:3 content to less than 3 % of the total fatty acids in the seedoil.

Due to the limited variation of the two polyunsaturated fatty acids in the available germplasm, a mutation experiment was initiated with spring rapeseed. Mutants were selected exhibiting deviating f.a. compositions (Rakow 1973, Röbbelen and Nitsch 1975). For an effective use of such alleles for the sake of conditioning an improved oil quality in winter rapeseed, three different breeding strategies are applied to improve the initially low seed yield of the mutant material.

MATERIALS AND METHODS

The original mutants of spring rapeseed, <u>B. napus</u> L., contained seedoils with about 30 % C18:2 and 3 % C18:3. These desired alleles were transferred into winter breeding material by recurrent crossing using different pollinators . For each cross, from a broad F_2 population plants exhibiting high linoleic and low linolenic acid contents (0_1 +* quality) were reselected by gaschromatography (GC). Up to now three back-crosses have been executed. For the third backcross, the parents exhibited Canola quality; therefore, in the segragating population selection was also directed to low glucosinolate (gsl) content. In this BC_3F_2 population, open pollinated plants were first screened by the Palladium test (Thies 1982) for low gsl contents. The selected plants then were analysed for their f.a. composition resulting in 0_1 0 quality. In 1989/90, 5 0_1 + BC_2F_5 , 1 0_1 0 BC_3F_4 and 28 0_1 0 BC_3F_5

^{*} The designation refers in its first position to the given oil quality, i.e. "zero erucic" (= 0) and "low linolenic" (= 0) in this case, and in its second position to the meal quality, i.e. "high glucosinolate" (= +) in this case.

lines were field tested for seed yield (kg/ha) in a 6 x 6 lattice with three replications at Göttingen. Oil (%), protein (%) and gsl content (μ mol/g seed) were determined by NIR and f.a. composition (% of total f.a. in the seedoil) by GC.

In addition to backcrossing, seed yield is selected for by another two different recurrent programmes. In each of them, the base population has been derived from BC_2 lines with. an improved f.a. composition. For phenotypic selection, in which one cycle of selection is completed every year, the partially autogamous rapeseed species is forced into full allogamy by using a dominant gene (MsJ) for male sterility isolated from the variety "Janpol" (Mathias 1985). Offsprings of pollinated MsJ/msJ plants segregate into 50 % fertile (msJ/msJ) and 50 % sterile (MsJ/msJ) plants. By two backcrosses with a pollen mixture from 100 BC2 lines, the MsJ gene was transferred to a population (MsJ population) with an improved polyenoic fatty acid content containing still 12.5% of the genome of the MsJ source. Each year the population is sown in an isolated block in the field. Weak and diseased plants are eliminated before flowering. The population is examined several times at flowering to mark vigorous sterile plants. These are harvested and drilled for the next cycle of selection.

The second recurrent system is based on the performance of progenies of selfed plants (S_1) , in order to get more precise genotypic information for the selection. In May 1987, $10~BC_2$ lines each represented by two plants were selected and crossed in a diallel manner. Hybrid plants were self-pollinated the next spring and 98 S_1 populations were tested together with two Canola varieties for seed yield. The experimental design was a 10 x 10 lattice with two replications in Göttingen and Thüle. Seed yield, lodging and disease resistance were determined.

RESULTS

<u>Backcrossing</u>: To determine, in the backcrossing programme, the effect of simultaneous selection for f.a. composition and gsl content compared to selection for cil quality only, 0:0 lines were tested together with 01+ lines and two high yielding (00) Canola varieties. The analysis of variance indicated significant differences between genotypes for all traits. In the average, 0:0 lines yielded 32.6 % and 0:1+ lines 20 % less than the standard varieties (Tab. 1).

Table 1. Relative means of 010 and 01+ lines compared to the mean of two Canola varieties ("Ceres", "Lirabon"=100%) as control in seed yield and quality traits; Göttingen 1990

Quality	Yield	Oil	Protein	gsl	C18:2	C18:3
0:0 0:+ 0 0 standard	67.4 80.0 100	97.3 95.5 100	109.2 106.9 100	148.2 533.2 100	126.5 131.1 100	41.5 37.3 100
0 0	5400 kg/ha	47.1 %	20.3	20.5 μmol/g	25.7 %	11.8

However, the best 0_1+ genotype exhibiting 2.3 % C18:3 only, reached yield levels of 92.5 % of the actual breeding material. On the other hand, the best 0_1 0 line (2.6 % C18:3 and 20.2 µmol gsl/g seed) yielded 36.4 % less than the Canola varieties. In general, the material with the improved polyenoic f.a. composition had a lower oil content, whereas the protein content was increased. The gsl content of the 0_1 0 lines exceeded the Canola control by nearly 10 µmol/g seed, which indicates the urgency of further selection.

Phenotypic recurrent selection

The base population for the two recurrent selection systems consisted of 0_1+BC_2 lines derived from backcrosses with a broad spectrum of low erucic acid, high yielding varieties. The first cycle of phenotypic selection was started in 1988/89. The f.a. composition of the MsJ population after cycle 1 and 2 is shown in Table 2.

Table 2. Fatty acid composition (%) and linoleic desaturation rate (LDR= C18:3/C18:2) in the MsJ-population in two consecutive selection cycles

Cycle	Year	C16:0	C18:1	C18:2	C18:3	LDR
C ₁	1989	4.4	63.2	24.7	3.0	0.12
	1990	4.5	58.1	29.6	3.6	0.12

The ratio of C18:3 to C18:2 demonstrates that the population did not change with respect to the polyenoic fatty acid composition. From each cycle, seeds from about 30 fertile plants were stored for later evaluation of the selection gain as well as for comparison of genetic progress by phenotypic versus S_1 selection.

S₁ recurrent selection

The first selection cycle based on the performance of S₁ lines at two locations was completed in 1989. The O₁+ material yielded in the average 14.7 % less than the mean of the standard varieties "Ceres" and "Lirabon" (4080 kg/ha). The seed yield ranged between 2500 kg/ha and 4500 kg/ha indicating a broad variability in alleles controlling yield. Unexpectedly several S₁ lines reached and even exceeded the yield level of the standards already after the first recombination cycle. 10 S₁ lines were selected based on their seed yield, origin and fatty acid composition. Line 16 outyielded varieties by 11 % and exhibited no more than 2.8 % linolenic acid (Tab. 3).

The seed yield of the selected lines ranged from 3900 kg/ha to 4330 kg/ha. However, all lines exhibited only a moderate lodging and disease resistance. The polyenoic f.a. composition of the S₁'s variied between 25 % to 29.4 % for C18:2 and for C18:3 between 2.8 % and 5.1 %. Stored seed of these 10 S₁-lines was drilled in August 1989 and the recombination for the second cycle was provided for by crossing in a diallel manner making plant to plant crosses with 10 plants per S₁.

Table 3. Relative seed yield (00=100%), scores of lodging resistance (lodg) and disease resistance (res; 1=low, 9=high)) and f.a. composition (%) of the selected Silines compared to the mean of the standard varieties "Ceres" and "Lirabon"

S ₁ line	Yield	Lođg	Res	C18:1	C18:2	C18:3
16	111.0	4.0	4.3	57.6	27.9	2.8
45	103.2	3.2	5.3	61.1	25.6	3.6
59	101.5	4.3	5.3	61.6	28.2	3.7
62	100.5	5.7	6.2	62.3	29.2	3.0
34	100.0	4.5	5.3	59.2	25.0	5.1
90	100.0	3.7	4.8	61.1	26.9	2.9
84	99.0	5.8	6.0	58.4	27.5	2.8
17	98.0	5.2	5.5	57.8	26.7	4.1
53	97.8	6.7	5.7	56.8	29.4	4.1
58	95.6	5.3	5.8	63.5	27.5	3.7
00(standard)	100					
00(standard)	4080	7.3	7.7	54.3	24.5	11.3
	kg/ha	score	score	%	%	%

DISCUSSION

Lines with an improved fatty acid composition and fairly high seed yields appeared after two backcrosses already (see.also Kräling 1989). Moreover, by recombining BC_2 lines, one particulary high yielding S_1 line with the desired oil quality was identified. Therefore, a reduced linolenic acid content in the seedoil does not necessarily cause physiological disturbances as assumed by Thies (1971). Similar to mutants in flax (Tonnet and Green 1987) and sunflower (Sperling et al. 1990) we assume our mutants to result from a change in the ER desaturation system which only is active during embryo development.

Despite of an overall higher share of the winter rapeseed genome in the BC3, no higher yielding lines were identified in comparisiom to BC2. Therefore, an additional selection for low glucosinolate content reduces yield again. In comparing winter rapeseed lines selected exclusively for seed yield and lines selected simultaneously for low gsl and erucic acid content, Sauermann et al. (1987) demonstrated the lower yielding capacity at the quality selection path. The limited number of selected 0:0 plants in the segragating generation reduced selection gains for seed yield. However, using developed 0:0 lines in further crosses with Canola varieties, the genetic progress for seed yield will increase, since now selection is only necessary for fatty acid composition which allows for a higher number of selected 0:0 plants.

The evaluation of the effectiveness of the two recurrent selection systems will be finally determined only after the completion of several cycles. Simplicity and lower costs support mass selection, while for quantitative traits genotypes are identified with higher presicion by progeny tests. In the BSK maize population, S₁ selection was 5 times more effective for yield than mass selection (Hallauer 1985). In our S₁ test, a broad genetic variability was found in the material and, therefore, high genetic progress is to be expected.

CONCLUSION

- -Alleles for an improved polyenoic fatty acid composition do not restrict γ ield performance.
- -Simultaneous selection for low gsl and desired oil quality reduces selection gain for seed yield.
- $-S_1$ recurrent selection seems to be an effective method to accumulate favourable alleles for seed yield in a O_1 + base population.

REFERENCES

HALLAUER, A.R. 1985. Compendium of recurrent selection methods and their application. Critical Reviews in Plant Sciences 3: 1-33.

KRÄLING, K. 1989. Performance of winter rapeseed lines with an improved fatty acid composition. Vortr. Pflanzenzüchtg. 15(I): 15-9.

MATHIAS, R. 1985: A new dominant gene for male sterility in rapeseed, <u>Brassica</u> <u>napus</u> L.. Z. Pflanzenzüchtg. 94: 170-173.

RAKOW, G. 1973. Selektion auf Linol- und Linolensäuregehalte in Rapssamen nach mutagener Behandlung. Z. Pflanzenzüchtg. 69: 62-82.

RÖBBELEN, G. and NITSCH, A. 1975. Genetical and physiological investigations on mutants for polyenoic fatty acids in rape-seed, <u>Brassica napus L. Z. Pflanzenzüchtg. 75: 93-105.</u>

SAUERMANN, W., RÖBBELEN, G. and FRAUEN, M. 1987. Der Einfluss der Selektion auf 00-Qualität auf den Ertrag bei Vinterraps. Proc. Int. Rapeseed Conf., Poznan. Vol. 1: 34-39.

SPERLING, P., HAMMER, U. FRIEDT, W. and HEINZ, E. 1990. High oleic sunflower: Studies on the composition and desaturation of acyl groups in different lipids and organs. Z. Naturforsch. 45c: 166-172.

THIES, W. 1971. Der Einfluß der Chloroplasten auf die Bildung von ungesättigten Fettsäuren in reifenden Rapssamen. Fette Seifen Anstrichmittel 73: 710-715.

THIES, W. 1982. Complex-formation between glucosinolates and tetrachloropalladate (II) and its utilization in plant breeding. Fette Seifen Anstrichmittel 84: 338-342.

TONNET, M.L. and GREEN, A.G. 1987. Characterization of the seed and leaf lipids of high and low linolenic acid flax genotypes. Arch. Biochem. Biophys. 252: 646-654.