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Identification of Individual *Brassica oleracea* Plants with Low Erucic Acid Content

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Abstract: In the course of studies dealing with the inheritance of erucic acid content in the seed oil of *Brassica* species individual plants belonging to two accessions of *B. oleracea* conv. *capitata*, Kashirka 202 and Ladozhskaya DS 8395, were identified being very low in erucic acid content. Applying half seed selection their progeny confirm a monogenous inheritance. Low-erucic mutants of *B. oleracea* have been found and described first time.

Key words: *Brassica oleracea*, cabbage, low-erucic acid mutants

INTRODUCTION

Due to substantial progress in breeding and cultivation practice rapeseed and mustards-derived from several locally distributed members of the genus *Brassica*-have become one of the worldwide most important source of vegetable oil. Especially in several European countries with cool-temperate climates oilseed rape (*B. napus*) with ‘double-low’ seed quality (canola) dominates field crop production.

Regarding this quality characters, low-erucic acid mutants were found in *B. rapa* (AA)^[1], *B. napus* (AACC)^[2,3] and *B. juncea* (AABB)^[4].

In the amphidiploid *B. carinata* (BBCC) low-erucic acid mutants were not known up to the 90’s. Different strategies, such as the selection of transgressive segregants in the crossing progeny of diverse *B. carinata* accessions^[5], induced mutations and interspecific crosses with low-erucic *B. napus* and *B. juncea*^[6,7] were used to reduce the erucic acid content. With the first and last mentioned method low erucic acid forms could be selected successfully^[5,8].

For the monogenomic species *B. oleracea* and *B. nigra* low-erucic acid forms were not cited in the literature up to now^[1-3]. In the course of studies dealing with the inheritance of erucic acid content in the seed oil of *Brassica* species individual plants belonging to two accessions of *B. oleracea* conv. *capitata*, Kashirka 202 and Ladozhskaya DS 8395, were identified being very low in erucic acid content. In the presented data their morphological characters and their fatty acid composition were described.

MATERIALS AND METHODS

Plant material: Individual plants belonging to two accessions of *B. oleracea* conv. *capitata*, Kashirka 202 and Ladozhskaya DS 8395, were identified being very low in erucic acid content in 1999 in the Plant Breeding Institute of the Justus-Liebig-University of Giessen. Individual half seed plants were transferred in pots and plant characters like leaf characteristics, flower characteristics were obtained. They were cultivated in the greenhouse, artificially vernalised and self-pollinated by bagging. The seed progeny was analysed for fatty acid composition in order to check for true-breeding individuals.

The fatty acid composition of the *B. oleracea* material was determined on the basis of half-seed analysis as described earlier using GC^[9,10]. The preparation of half seeds was done according to the method described by Thies^[11].

RESULTS

Description of investigated *B. oleracea* forms: Kashirka and Ladozhskaya both have vernalization requirement. After vernalization experiments it can be said that Kashirka has a vernalization requirement of 14 weeks at 5°C and Ladozhskaya up to 18 weeks at 5°C.

Both genotypes are self-compatible and seed set could be obtained after pollination per hand (Fig. 1a and b). Their flowers are typical *Brassica* flowers (Fig. 1c). One stigma, 4 long and two



Fig. 1(a-d): Seed pod of Ladozhskaya (a) and Kashirka (b), typical Brassica flower (c) and leaf shape (d)

Table 1: Mean fatty acid composition (%) of individual half seed plants belonging to the two genotypes Kashirka and Ladozhskaya (mean of totally 21 and 23 plants, respectively)

	Genot./Fatty acid comp.	
	Kashirka	Ladozhskaya
Palmitic	3.58	3.95
Stearic	0.90	1.09
Oleic	57.40	56.21
Linoleic	22.10	20.64
Linolenic	12.93	14.59
Eicosenic	1.90	2.64
Erucic	0.17	0.57
Nervonic	0.30	0.33
Rest	0.71	0.70

short anthers, 4 sepals and 4 petals. The leaves of both accessions are small and transverse broad elliptic (Fig. 1d).

Quality characters

Fatty acid composition: After half seed analysis 23 individuals of Kashirka and 21 individuals of Ladozhskaya were identified with low erucic acid content.

The mean values of the fatty acid composition of these half seed plants can be seen in Table 1. The fatty acid composition of both cabbages is very similar. The biggest component for Kashirka and Ladozhskaya is oleic acid, (respectively 57.40 and 56.21%), followed by linoleic acid (22.10 and 20.64%, respectively) and linolenic acid (12.93 and 14.59%, respectively). Low amounts of palmitic, stearic and erucic acid were also detected. Specially erucic acid is under the 2% limit given for oils regarding canola oil quality.

Unfortunately only three half seed plants of Kashirka and one half seed plant of Ladozhskaya survived and could be selfed and they also display low erucic acid content. The obtained seed material was measured with NIRS (Near Infrared Restriction Spectroscopy)

for glycosinolate content. These values are 63.4 and 129.5 $\mu\text{mol g/DM}$, respectively. Regarding Canola quality they have only O-quality.

Additionally to the presented results the genomic Southern analysis and PCR-Amplifications of different *Brassica* species inclusively Ladozhskaya revealed that in the progeny of the *B. oleracea* accession Ladozhskaya DS 8395 a complete different pattern was observed as no signal in the selection zero of this cabbage meaning partial or complete deletion of the KCS gene. The latter result was verified through PCR analysis with a KCS-specific primer pair, where the 1.25 kb fragment characteristics for all other genotypes was not amplified in the case of zero erucic acid mutant^[2].

DISCUSSION

The gene pool of elite rapeseed breeding material has been further eroded by an emphasis on specific quality traits. As a consequence, genetic variability in this important oilseed crop is restricted with regard to many characters of value for breeding purposes. Resynthesis of novel genotypes with novel quality characters through artificial crosses between the diploid parents, assisted by embryo rescue techniques, will broaden the genetic basis of present breeding material.

Developing synthetic *Brassica napus* forms has provided important basic germplasm for further improvements of seed yield, disease and pest resistance as well as relevant seed quality traits^[3]. Regarding the later some examples of introgression breeding are documented, such as the resynthesis approach to create new genetic variation for low glucosinolate content^[4,15] or the approaches developing of yellow-seeded rapeseed via interspecific crosses^[16,17]. Regarding fatty

acid composition different cauliflower cultivars (*B. oleracea* ssp. *oleracea* convar. *botrytis* var. *botrytis*) and Yellow Sarson (*B. rapa* ssp. *trilocularis*) were used in an earlier study for wide hybridisation to create novel rapeseed with high erucic acid content (HEAR) and which is now used as basic material for the improvement of industrial rapeseed^[12,18-20]. Similar experiments were conducted by Chen and Heneen^[13], Health and Earle^[21,22], Rahman *et al.*^[23], Lu *et al.*^[24] and Rahman^[25] using interspecific *Brassica* hybridization in order to modify the fatty acid composition of oilseed rape.

In fact, current double-low breeding material seems to be closely, related and intensive quality breeding-using the spring rapeseed cultivars LihO/Oro (low erucic acid source) and the Polish cultivar Bronowski (low glucosinolate source) as unique donors for quality improvement-has also contributed to narrow the genetic base of oilseed rape^[26,27].

The limit for low glucosinolate content in *B. napus* is 30 µmol/100 g DM in rapeseed cultivars of the EU^[28]. The glucosinolate content of the low-erucic-acid mutants are about this limit. But if we use these mutants in interspecific crosses with very low glycosinolate *B. rapa* forms, the content of the obtained hybrids will be probably under this limit.

Therefore, the discovery of low-erucic acid mutants among the mentioned *B. oleracea* accessions and the development of synthetic rapeseed forms via interspecific crosses with interesting 0-or 00-quality *B. rapa* genotypes will open further the possibility to use RS-rapeseed material as a genetic resource for example for quality and yield improvement in *Brassica napus*, perhaps in developing RS-lines with 0-or 00-quality.

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