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Variation for agronomic characteristics in *Crambe hispanica*, a wild relative of *Crambe abyssinica*

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Summary

In order to assess the potential of *Crambe hispanica*, either in breeding programmes of *C. abyssinica* or as an oilseed crop in itself, 36 accessions of *C. hispanica* (29 of var. *hispanica* and 7 of var. *glabrata*) were grown in a greenhouse and evaluated for morphological characteristics, earliness, plant habit, seed characteristics and fatty acid composition. Four lines of *C. abyssinica* were included for reference. The 29 accessions of var. *hispanica* showed significant variation for all observed characteristics. Besides morphological characteristics, large variation was found for earliness, number of primary branches, seed yield, 1000 seed weight and volume, and linoleic, linolenic and erucic acid content. Morphological characteristics, earliness and plant habit did not show any high correlations with seed characteristics or fatty acid composition, except for seed hull (pericarp) mottling, which was related to a high oil and erucic acid content. The seven accessions of var. *glabrata* showed little variation. The large genetic variation in combination with promising figures for several characteristics, found in *C. hispanica*, may be useful in breeding programmes of the oilseed crop *C. abyssinica*, for which the available genetic variation is limited. Prospects of selection for high-erucic acid genotypes are discussed. Compared to *C. abyssinica*, both botanical varieties of *C. hispanica* are characterized by a cordate shape of the basal leaves, lack of seed retention and a lower DNA content. Plants of var. *glabrata* differed from var. *hispanica* in a sparsely hispid upper leaf surface and round stems and branches covered with a waxy layer. These clear differences and lack of success in intercrossing both varieties of *C. hispanica* strongly suggest that their taxonomic classification should be reconsidered.

Introduction

Crambe hispanica L. is an herbaceous annual species belonging to the Cruciferae. Plants are hispid, usually 40 to 80 cm in height, highly branched and bearing racemes with small white flowers. Basal leaves have a cordate terminal lobe and usually one to three pairs of lateral lobes. Fruits are composed of a small cylindrical lower joint and a large globular upper joint. The upper joint, containing one single seed, is readily separating from the lower joint when mature. The hull (pericarp) ranges in colour from tan to black. Within *C. hispanica* four botanical varieties are distinguished, var. *hispanica*, var. *glabrata* (DC.) Coss., var. *edentula* Boiss. and var. *major* Moris (Schulz, 1919; Zohary, 1966; White, 1975).

C. hispanica is a wild relative of the oilseed crop *Crambe abyssinica* Hochst. ex R.E. Fries. According to White (1975), the most obvious feature to distinguish the species is the basal leaf shape, which is ovate for *C. abyssinica*. The chromosome number of *C. abyssinica* (2n=90) differs from that of *C. hispanica* var. *hispanica* (2n=60) and var. *glabrata* (2n=30) (Manton, 1932; White, 1975; White & Solt, 1978).

Seed of *C. abyssinica*, including the surrounding hull, contains 31 to 35% oil, of which 55 to 60% is erucic acid. Products of the high-erucic acid oil and derivatives from either erucic acid or mixed fatty acids from the oil can be applied in a wide range of products, such as pharmaceuticals, detergents, cosmetics and coatings. The oil has good chemical and physical properties to be employed as a refrigerating and lubricating fluid, e.g. in steel casting and hardening. Erucamide, the most important derivative of erucic acid, is an excellent slip agent for plastic films (Nieschlag & Wolff, 1971; van Dyne et al., 1990; Leonard, 1993; Lazzeri et al., 1994).

Since the 1970s, several cultivars of *C. abyssinica* have been released: 'Prophet', 'Indy', 'Meyer' (Lessman, 1975), 'BelAnn' and 'BelEnzian' (Campbell et al., 1986). However, further improvement is restricted due to a lack of variation within *C. abyssinica* for important agricultural characteristics, such as erucic acid content and total glucosinolate content (Papathanasiou et al., 1966; Lessman & Meier, 1972; Mastebroek et al., 1994). *C. hispanica* might be useful as a new source of variation.

Various accessions of *C. hispanica* var. *hispanica* and var. *glabrata* have been evaluated to assess their possibilities, either in breeding programmes of *C. abyssinica* or as an oilseed crop in itself. The DNA content of some of these accessions was determined, to estimate the ploidy level. In this paper the observed variation for morphology, earliness, plant habit, seed characteristics and fatty acid composition, is described, together with relevant correlations between the observed characteristics. Differences found between *C. abyssinica* and *C. hispanica* var. *hispanica* and var. *glabrata* are discussed.

Materials and methods

Evaluation of variation

Thirty-six accessions of *C. hispanica* (29 of var. *hispanica* and 7 of var. *glabrata*) were grown in a greenhouse and evaluated for morphology, earliness, plant habit, seed characteristics and fatty acid composition of the seed oil. The material of var. *hispanica* included two lines selected from accession 910951. Four lines from two accessions of *C. abyssinica* were included for reference. In this paper, lines are dealt with as accessions. The accessions were obtained from the Centre for Genetic Resources in The Netherlands (CGN). The passport data of the accessions, being the species and variety names, other names and the country of origin are presented in Table 1.

Seeds were sown on 15 March 1993. Ten seedlings per accession were planted on 27 April, each in a container filled with 1 l of regular potting compost. The experiment had a randomized-block design with two replicates. The experimental units were rows of five plants. The minimum temperature during the experiment was 12°C at night (10 h) and 17°C during the day (14 h). High temperatures were prevented by mechanical cooling with outdoor air. Seed loss due to shattering was prevented by means of gauzy nylon bags, put around the plants when ripening started (June). Seeds were harvested during the last week of July and the first week of August, when plants had reached full maturity.

Observations were done on all individual plants, except for the determination of total glucosinolate content in the seeds and fatty acid composition of the seed oil, for which seeds from only one plant per row were used. The observed characteristics are described in Table 2. For the fatty acids C20:1 and C22:1 two fractions were determined, apparently the $\omega9$ and $\omega7$ isomers, which are also found in rapeseed (Appelqvist, 1975). Only the $\omega9$ isomer of C22:1 is regarded as erucic acid.

Row means were used for analysis of variance (ANOVA) and accession means in the correlation study. Seed hull pattern and seed colour were excluded from analysis of variance, as these were qualitative characteristics. The relation between these two characteristics and quantitative characteristics was assessed by analyses of variance of individual plant data (instead of averages), considering the qualitative characteristics as treatments.

Number of chromosomes and DNA content

Accessions 910941 of *C. abyssinica* and 910951 of *C. hispanica* var. *hispanica*, between which crosses have been made by Mastebroek (unpublished), were used for the determination of the chromosome numbers. Flower clusters from tips of racemes were fixed in Carnoy solution for 18 hours and flower buds were ranked according to Meier & Lessman (1973). Selected buds were stained in Snow's solution (Snow, 1963) for 16 hours at 50°C, spread and squashed in 45% acetic acid and examined under a light microscope.

The DNA contents of accessions 910941 of *C. abyssinica*, 910947, 910948, 910949 of *C. hispanica* var. *glabrata* and 910951, 910952, 921281, 921287 of var. *hispanica* were determined by means of flow cytometry. Young leaf material was chopped with a sharp razor blade in nucleus isolation buffer. The suspension was filtered through a 25 μm nylon filter and analyzed immediately (Bino et al., 1993). Accession 910951 of var. *hispanica* was used as a standard.

Table 1. Evaluated accessions and lines of *Crambe abyssinica* and *Crambe hispanica*

CGN[a] accession number	Species name and variety	Other accession number	Country of origin
910927 line 32.1[b]	*C. abyssinica*		
910927 line 32.2[b]	*C. abyssinica*		
910941 line 1[c]	*C. abyssinica*		
910941 line 2[c]	*C. abyssinica*		
910947	*C. hispanica* var. *glabrata*	PI 388783	Morocco
910948	*C. hispanica* var. *glabrata*	PI 388854	Portugal
910949	*C. hispanica* var. *glabrata*	PI 388857	Portugal
910950	*C. hispanica* var. *hispanica*	PI 378590	Spain
910951	*C. hispanica* var. *hispanica*	PI 388708	Israel
910951 line 1[d]	*C. hispanica* var. *hispanica*		
910951 line 2[d]	*C. hispanica* var. *hispanica*		
910952	*C. hispanica* var. *hispanica*	PI 388822	Cyprus
910953	*C. hispanica* var. *hispanica*	PI 388835	Italy
921262	*C. hispanica* var. *glabrata*	PI 388786	Morocco
921263	*C. hispanica* var. *glabrata*	PI 388787	Morocco
921264	*C. hispanica* var. *glabrata*	PI 388789	Morocco
921265	*C. hispanica* var. *glabrata*	PI 388853	Portugal
921266	*C. hispanica* var. *hispanica*	PI 337996	Israel
921267	*C. hispanica* var. *hispanica*	PI 380519	Hungary
921268	*C. hispanica* var. *hispanica*	PI 388706	Israel
921269	*C. hispanica* var. *hispanica*	PI 388707	Israel
921270	*C. hispanica* var. *hispanica*	PI 388709	Israel
921271	*C. hispanica* var. *hispanica*	PI 388710	Israel
921272	*C. hispanica* var. *hispanica*	PI 388711	Israel
921273	*C. hispanica* var. *hispanica*	PI 388712	Israel
921274	*C. hispanica* var. *hispanica*	PI 388713	Israel
921275	*C. hispanica* var. *hispanica*	PI 388714	Israel
921276	*C. hispanica* var. *hispanica*	PI 388715	Israel
921277	*C. hispanica* var. *hispanica*	PI 388717	Israel
921278	*C. hispanica* var. *hispanica*	PI 388718	Israel
921279	*C. hispanica* var. *hispanica*	PI 388719	Israel
921280	*C. hispanica* var. *hispanica*	PI 388720	Israel
921281	*C. hispanica* var. *hispanica*	PI 388721	Israel
921282	*C. hispanica* var. *hispanica*	PI 388722	Israel
921283	*C. hispanica* var. *hispanica*	PI 388723	Israel
921284	*C. hispanica* var. *hispanica*	PI 388724	Israel
921285	*C. hispanica* var. *hispanica*	PI 388725	Israel
921286	*C. hispanica* var. *hispanica*	PI 388726	Israel
921287	*C. hispanica* var. *hispanica*	PI 388727	Israel
921288	*C. hispanica* var. *hispanica*	PI 388729	Israel

[a] CGN-Centre for Genetic Resources, the Netherlands.

[b] Derived from 910927 (other accession number: NGB 4010 / NR 60520 F.R. Marshall; country of origin unknown).

[c] Derived from 910941 (other accession number: PI 384533; country of origin: Ethiopia).

[d] Derived from 910951.

Table 2. Characteristics evaluated for *Crambe hispanica*

Morphological characteristics
Lateral lobes of third leaf[a] (number)
Maximum tooth size of dentated leaf margin[b] (mm)
Relative width of terminal lobe[b,c]
Relative depth of cordate leaf base incision[b,c]
Relative length of leaf rachis[b,c]
Pubescence of stem base (0:glabrous - 4:dense)
Pubescence of stem top (0:glabrous - 4:dense)
Pubescence of leaf rachis[b] (0:glabrous - 3:dense)
Pubescence of leaf upper surface[b] (0:glabrous - 3:dense)
Anthocyanin intensity of hypocotyl[a] (0:absent - 3:dark)
Anthocyanin intensity of hypercotyl[a] (0:absent - 3:dark)
Anthocyanin intensity of leaf rachis[b] (0:absent - 3:dark)
Flower diameter (mm)
Seed hull colour (0:tan - 4:black)
Seed hull pattern (0:unpatterned, 1:mottled)
Seed colour (0:brown, 1:black)

Earliness
Days until development of lateral shoots
Days until anthesis

Plant habit
Plant height (cm)
Length of top branch (cm)
Length of top branch (% of plant height)
Primary branches (number)

Seed characteristics
Seed yield (g)
Seed yield (number)
1000 seed weight (g)
1000 seed volume (ml)
Test weight (g/l)
Oil content[d] (%)
Glucosinolates[e] (μmol/g)

Fatty acid composition of seed oil[f] *(%)*

C16:0	Palmitic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
C20:0	Arachidic acid
C20:1[Δ11]	Gadoleic acid
C20:1[Δ13]	Cis-13-eicosenoic acid
C22:1[Δ13]	Erucic acid
C22:1[Δ15]	Cis-15-docosenoic acid
C22:2	Docodienoic acid
C24:0	Lignoceric acid
C24:1	Nervonic acid
C16:0 + C18:0 + C20:0 + C22:0 + C24:0	
C18:1 + C18:2 + C18:3	

[a] Observed during the 8th week after sowing.
[b] Observed on third leaf, during the 8th week after sowing.
[c] Related to the maximum length of the terminal lobe.
[d] Determined by means of NMR (nuclear magnetic resonance) on the basis of 6% moisture (Tiwari & Burk, 1980).
[e] Determined by the Palladium test (Thies, 1989).
[f] Determined by GLC (gas-liquid chromatography) conform IUPAC methods 2.301 and 2.302 (Paquot, 1979).

Results

Variation within C. hispanica

Morphological characteristics

Cordate terminal lobes of the basal leaves, pubescence and seed shattering were characteristic for both botanical varieties of *C. hispanica*. Within var. *hispanica* every quantitatively observed morphological characteristic showed significant variation (data not presented). The largest variation was found for tooth size and cordateness of the terminal lobe of the basal leaves, the number of lateral lobes and the relative leaf rachis length of the basal leaves, and seed hull colour (F-values of 24.5, 23.0, 10.0, 8.1 and 38.1 respectively). The number of lateral lobes of the basal leaves ranged from one to four. Seed hulls of var. *hispanica* ranged in colour from tan to black, whereas seeds were black. Seed hull pattern was the only qualitative characteristic showing variation.

Accessions of var. *glabrata* were characterized by round stems and branches which were slender and covered by a waxy layer, in contrast to accessions of var. *hispanica* or *C. abyssinica* which had angular stems without a waxy layer. Compared to var. *hispanica*, plants of var. *glabrata* had a sparse pubescence of the stem base and the upper leaf surface, and the terminal lobes of basal leaves were more cordate and less ovate. Seed hulls of var. *glabrata* were tan, without pattern and contained brown seeds. Within var. *glabrata*, despite the low number of accessions, significant variation ($P \leq 0.05$) was found for cordateness and relative

Table 3. Variation among 29 accessions of *Crambe hispanica* var. *hispanica* and 7 accessions of var. *glabrata* for earliness and plant habit. Means and ranges of *C. abyssinica* are also included

Characteristic	Variety/species	Mean	Range	F-value[a]
Days until development of lateral shoots	var. *hispanica*	48.7	45.0–72.8	30.5**
	var. *glabrata*	50.9	49.3–54.3	1.25
Days until anthesis	var. *hispanica*	57.8	45.9–85.5	25.2**
	var. *glabrata*	61.0	58.2–65.3	7.03*
	C. abyssinica	61.3	60.4–62.6	
Plant height (cm)	var. *hispanica*	121	71–168	5.75**
	var. *glabrata*	138	123–156	2.94
	C. abyssinica	97.7	97–100	
Length top branch (cm)	var. *hispanica*	48.9	1.9–68.6	6.54**
	var. *glabrata*	50.9	34.5–61.0	6.03*
	C. abyssinica	32.2	30.6–33.6	
Length top branch (% of plant height)	var. *hispanica*	41.2	1.4–54.5	6.81**
	var. *glabrata*	37.5	25.0–45.1	16.7**
	C. abyssinica	33.1	31.7–34.7	
Primary branches (number)	var. *hispanica*	7.8	4.0–26.4	21.0**
	var. *glabrata*	10.5	9.0–12.7	8.91**
	C. abyssinica	15.1	13.4–16.2	

[a] * and ** significant at $P \leq 0.05$ and 0.01, respectively.

width of the terminal lobe of the basal leaves, relative length of the leaf rachis of the basal leaves, and anthocyanin intensity of the leaf rachis, hypocotyl and hypercotyl.

In contrast to *C. hispanica*, plants of *C. abyssinica* showed very little or no anthocyanin and were glabrous, except for the base of the stem, which was densely pubescent. Basal leaves had an ovate terminal lobe and practically no lateral lobes. Seed hulls were tan and seeds black.

Earliness and plant habit

The observed variation for earliness and plant habit is presented in Table 3. Within var. *hispanica*, most significant variation between accessions was found for the number of days until development of lateral shoots, the number of days until anthesis and the number of primary branches. The number of days until anthesis ranged from 46 days for accession 921268 to 86 days for accession 910953. Plant height ranged from 71 cm for accession 921269 to 168 cm for accession 921276. In cases that low numbers of primary branches were found, often some of the primary branches acted as stems, while the main stem remained short. Accession 910953 was distinct from other accessions of var. *hispanica* in its extreme lateness and high number of primary branches.

Within var. *glabrata*, significant variation ($P<0.05$) was found for the number of days until anthesis, the (absolute and relative) length of the top branch and the number of primary branches. Accessions of var. *glabrata* did not flower earlier than 58 days after sowing. Plant heights were more than 123 cm but did not exceed the maximum plant height found for var. *hispanica*.

The four evaluated lines of *C. abyssinica* showed their first lateral shoots before 43 days after sowing, when observations started, but reached anthesis after 61 days. Plants reached an average height of 98 cm with about 15 primary branches and a top branch of 30 cm.

Seed characteristics

In Table 4 the observed variation for seed characteristics (seed plus hull) is shown. Plants of var. *hispanica* produced on average 5.3 g seed, with a maximum of 9.6 g for accession 910953. The 1000 seed weight and 1000 seed volume showed most variation. In Figure 1 the accession means for oil content are presented. Seeds of var. *hispanica* had an average oil content of

Table 4. Variation among 29 accessions of *Crambe hispanica* var. *hispanica* and 7 accessions of var. *glabrata* for seed characteristics. Means and ranges of *C. abyssinica* are also included

Characteristic	Variety/Species	Mean	Range	F-value[a]
Seed yield (g)	var. *hispanica*	5.34	1.23–9.60	5.03**
	var. *glabrata*	6.92	5.41–8.02	0.79
	C. abyssinica	6.80	5.81–8.21	
Seed yield (number)	var. *hispanica*	637	194–1345	4.80**
	var. *glabrata*	594	469–803	1.55
	C. abyssinica	889	724–1153	
1000 seed weight (g)	var. *hispanica*	8.5	5.7–13.2	16.2**
	var. *glabrata*	12.0	9.9–14.2	4.61*
	C. abyssinica	7.8	6.9–8.7	
1000 seed volume (ml)	var. *hispanica*	30.3	20.2–46.6	22.8**
	var. *glabrata*	48.6	36.7–54.9	3.52
	C. abyssinica	32.2	28.3–35.8	
Test weight (g/l)	var. *hispanica*	286	233–327	4.52**
	var. *glabrata*	250	233–268	2.67
	C. abyssinica	243	236–248	
Oil content[b] (%)	var. *hispanica*	26.4	22.9–29.5	4.75**
	var. *glabrata*	26.1	24.6–27.5	5.85*
	C. abyssinica	27.5	25.7–29.0	

[a] * and ** significant at $P \leq 0.05$ and 0.01, respectively.
[b] On the basis of 6% moisture.

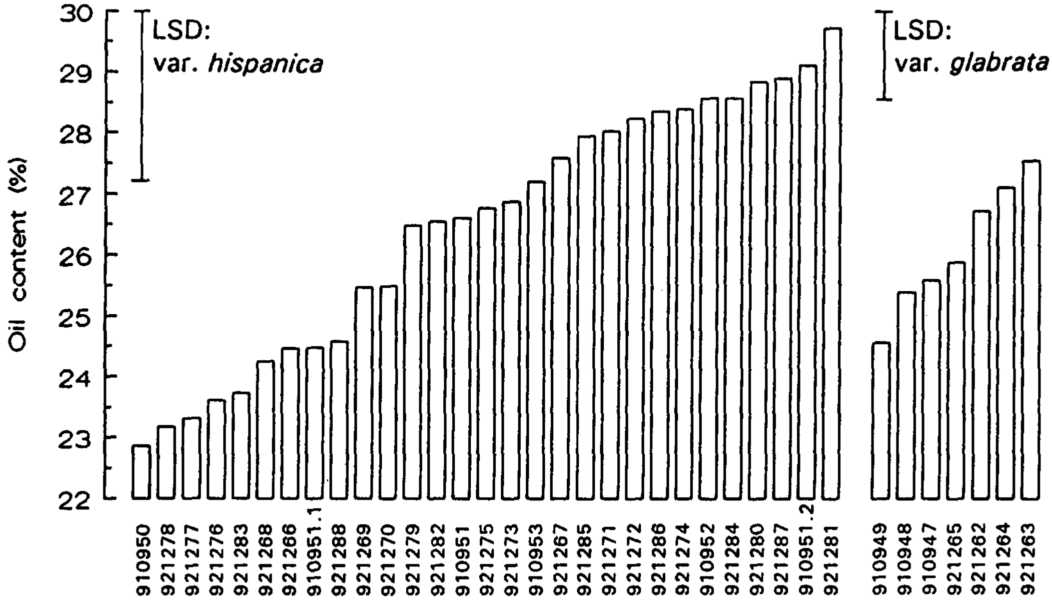

Fig. 1. Average oil content (%) of the hulled seed, per accession for var. *hispanica* (left) and var. *glabrata* (right).

26.4%, with a maximum of 29.5% in seeds of accession 921281.

On average, plants of var. *glabrata* yielded 6.9 g seed. The 1000 seed volume and 1000 seed weight were higher than found for seeds of var. *hispanica* but the seed oil content was similar.

On average, plants of *C. abyssinica* yielded 6.8 g with an oil content of 27%. The 1000 seed weight

Table 5. Variation among 29 accessions of *Crambe hispanica* var. *hispanica* and 7 accessions of var. *glabrata* for fatty acid composition of seed oil. Means and ranges of *C. abyssinica* are also included

Characteristic	Variety/Species	Mean	Range	F-value[a]
Sum saturated fatty acids (%)	var. *hispanica*	5.43	4.15–6.80	4.51**
	var. *glabrata*	5.46	4.50–6.40	6.46*
	C. abyssinica	6.23	6.05–6.60	
C18:1 Oleic acid (%)	var. *hispanica*	18.9	14.5–23.8	3.34**
	var. *glabrata*	22.2	20.3–24.4	5.57*
	C. abyssinica	14.8	13.3–15.6	
C18:2 Linoleic acid (%)	var. *hispanica*	7.53	5.25–10.6	5.15**
	var. *glabrata*	4.92	2.95–5.90	4.35*
	C. abyssinica	8.89	8.45–9.40	
C18:3 Linolenic acid (%)	var. *hispanica*	5.23	2.70–7.25	5.81**
	var. *glabrata*	4.41	3.20–5.45	2.72
	C. abyssinica	6.80	6.00–8.00	
C18:1 + C18:2 + C18:3 (%)	var. *hispanica*	31.6	30.4–33.6	3.72**
	var. *glabrata*	31.5	30.5–33.3	1.71
	C. abyssinica	30.5	30.2–30.9	
C20:1 Gadoleic acid (%)	var. *hispanica*	2.47	1.25–4.30	2.22**
	var. *glabrata*	3.11	1.60–4.15	1.92
	C. abyssinica	1.46	1.20–1.55	
C22:1 Erucic acid (%)	var. *hispanica*	56.9	53.1–60.1	5.03**
	var. *glabrata*	56.2	53.6–59.9	2.47
	C. abyssinica	56.5	55.5–57.3	
C24:1 Nervonic acid (%)	var. *hispanica*	1.74	1.20–2.60	3.83**
	var. *glabrata*	2.04	1.60–2.45	3.12
	C. abyssinica	2.00	1.80–2.25	

[a] * and ** significant at $P \leq 0.05$ and 0.01, respectively.

and 1000 seed volume were similar to those of var. *hispanica*

The content of glucosinolates in seeds of the *C. hispanica* accessions was equal or higher than 100 μmol/g, what was found for the *C. abyssinica* lines. The total glucosinolate content of several *C. hispanica* accessions was higher than the samples used for calibration, which are normally used for *C. abyssinica*, and could not be determined reliably.

Fatty acid composition

The results of the analyses of fatty acid composition are summarized in Table 5. Within var. *hispanica*, only moderate variation in fatty acid composition of the seed oil was observed. Within var. *glabrata*, significant variation ($P \leq 0.05$) was only found for the total content of saturated fatty acids and for oleic and linoleic acid content.

Differences in fatty acid composition between *C. abyssinica* and *C. hispanica* and between both varieties of the latter species, were mainly observed in oleic and linoleic acid content. The average contents of oleic acid for var. *hispanica*, var. *glabrata* and *C. abyssinica* were 18.9, 22.2 and 14.8%, respectively, the average linoleic acid contents were 7.5, 4.9 and 8.9%, respectively.

Within *C. hispanica*, the total content of oleic, linoleic and linolenic acid (ranging from 30.4% to 33.6%) showed less variation than found for the contents of these fatty acids individually. The percentage of saturated fatty acids in seed oil ranged from 4.2% to 6.8%. Figure 2 presents the average erucic acid content per accession. The highest erucic acid content (60.1%) was found in seed oil of accession 921286. For *C. abyssinica* an average erucic acid content of 56.5% was found.

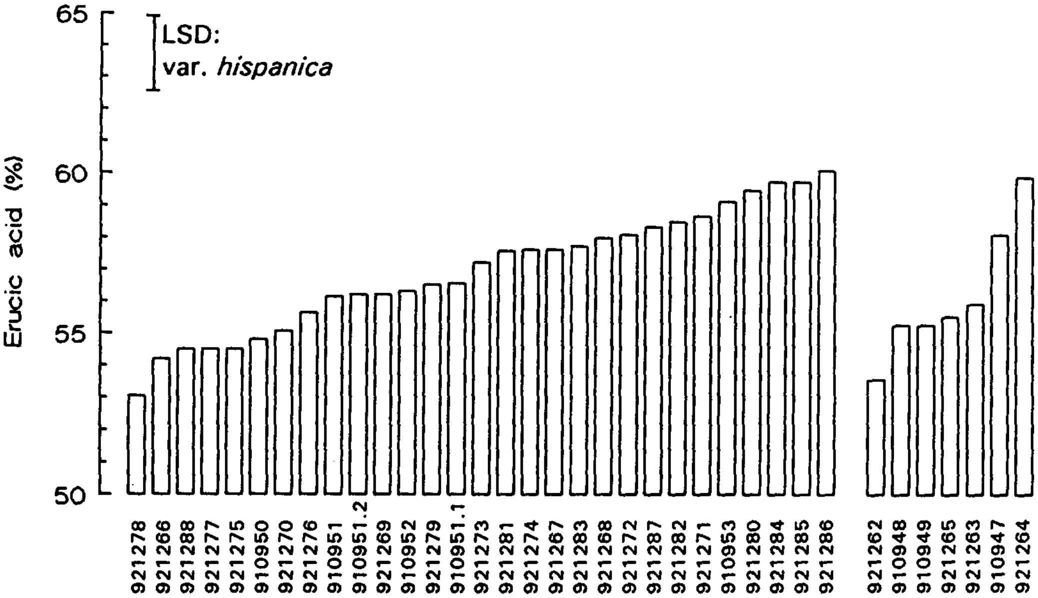

Fig. 2. Average erucic acid contents (%) of the seed oil, per accession for var. *hispanica* (left) and var. *glabrata* (right). The LSD for var. *glabrata* is not shown, as no significant variation was observed.

Correlations

Of 66 combinations of morphological characteristics within *C. hispanica* var. *hispanica*, only anthocyanin intensity of the hypercotyl and leaf rachis showed a high and significant correlation ($r>0.70$). No significant correlations higher than 0.70 were found between morphological characteristics on one hand and seed characteristics and fatty acid composition on the other hand. Within var. *glabrata*, anthocyanin intensity of the hypocotyl showed a high and negative correlation ($r=-0.97$) with the percentage of saturated fatty acids and a positive correlation ($r=0.82$; $P<0.05$) with erucic acid content.

In Table 6 correlations between days until anthesis, plant habit and seed characteristics are presented. For both varieties of *C. hispanica*, high correlations ($r>0.90$) were found between 1000 seed weight and 1000 seed volume. No significant correlation ($P\leq 0.01$) was observed between 1000 seed volume and oil content. In var. *hispanica*, the number of days until anthesis was highly correlated with plant height. In var. *glabrata* the relative length of the top branch showed significant and negative correlation with the number of primary branches ($P<0.01$) and with the oil content of hulled seed ($P<0.05$). In both varieties, days until anthesis and days until lateral shoot development showed a strong relation ($r=0.80$ and 0.88 for var. *hispanica* and var. *glabrata*, respectively).

Correlations between percentages of fatty acids in the seed oil are given in Table 7. For both varieties of *C. hispanica*, the content of erucic acid showed high and negative correlations with the content of gadoleic acid and with the total content of oleic, linoleic and linolenic acid. The correlations between erucic acid content and the percentages of the unsaturated C18 fatty acids, individually, differed considerably and were less pronounced (data not shown). The relation between oleic, linoleic and linolenic acid content differed remarkably between the two varieties of *C. hispanica*. Besides the correlation with erucic acid, the total content of oleic, linoleic and linolenic acid in var. *hispanica* was also correlated with the oil content ($r=-0.69$), the correlation between erucic acid content and oil content was 0.62. In var. *glabrata*, the gadoleic acid content not only showed negative correlation with erucic acid content but also with nervonic acid content ($r=-0.81$).

Within var. *hispanica*, analyses of variance with seed hull pattern as treatment resulted in F-values of 39 and 9.2 for oil content (228 plants) and erucic acid content (54 plants), respectively. Plants with mottled seed hulls (54 for oil content and 14 for fatty acid composition) exceeded plants with uni-coloured seed hulls in oil content and erucic acid content with 2.4% and 1.8%, respectively. Mottling was observed in ten accessions, which exceeded the other accessions in oil content and erucic acid content with 1.9% and 2.0%, respectively.

Table 6. Correlations between earliness, plant habit and seed characteristics of *C. hispanica* var. *hispanica* and var. *glabrata*[a]

Characteristics	2	3[b]	4[b]	5	6	7	8
1. Days until anthesis[b]							
hispanica	0.74	0.54	- 0.67	0.57	0.52	0.41	0.30
glabrata	0.27	- 0.42	0.47	- 0.07	0.35	0.33	- 0.74
2. Plant height (cm)							
hispanica		0.41	- 0.42	0.65	0.53	0.37	0.10
glabrata		0.15	- 0.29	0.36	0.63	0.38	0.22
3. Primary branches (number)[b]							
hispanica			- 0.49	0.35	0.64	0.57	- 0.07
glabrata			- 0.89	0.33	0.25	0.31	0.73
4. Rel. length top branch[b]							
hispanica				- 0.39	- 0.37	- 0.39	0.07
glabrata				- 0.21	- 0.13	- 0.22	- 0.86
5. Seed yield (g)							
hispanica					0.25	0.02	0.21
glabrata					- 0.06	- 0.37	0.22
6. 1000 Seed weight (g)							
hispanica						0.93	- 0.26
glabrata						0.91	0.07
7. 1000 Seed volume (ml)							
hispanica							- 0.44
glabrata							0.10
8. Oil content (%)							

[a] Significance level at P<0.01 is 0.47 for var. *hispanica* and 0.87 for var. *glabrata*.

[b] Accession 910953 excluded; significance level at P<0.01 is 0.48 for var. *hispanica*

Table 7. Correlations between oil content and percentages of fatty acids in the seed oil of *C. hispanica* var. *hispanica* and var. *glabrata*[a]

Characteristics	2	3	4	5	6
1. Oil content (%)					
hispanica	- 0.06	- 0.69	- 0.52	0.62	0.03
glabrata	- 0.12	0.03	- 0.12	0.23	- 0.40
2. % Saturated fatty acids					
hispanica		0.11	0.33	- 0.49	0.01
glabrata		0.40	0.50	- 0.74	- 0.35
3. % C18:1 + C18:2 + C18:3					
hispanica			0.82	- 0.90	- 0.02
glabrata			0.86	- 0.87	- 0.70
4. % C20:1[Δ11] (gadoleic acid)					
hispanica				- 0.84	- 0.29
glabrata				- 0.91	- 0.81
5. % C22:1[Δ13] (erucic acid)					
hispanica					- 0.05
glabrata					0.63
6. % C24:1 (nervonic acid)					

[a] Significance level at P<0.01 is 0.47 for var. *hispanica* and 0.87 for var. *glabrata*.

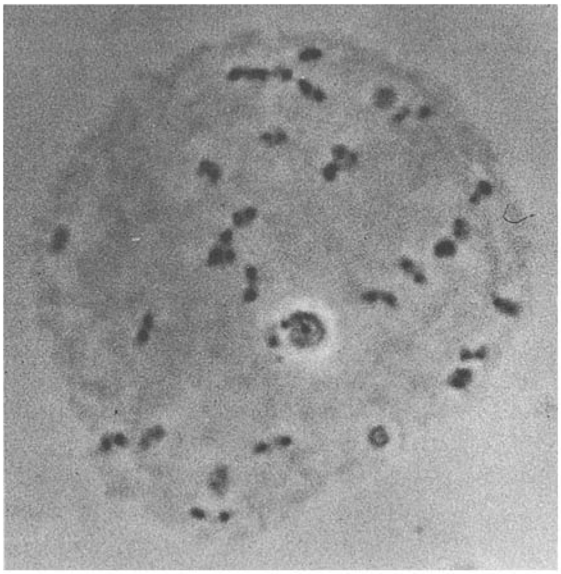

Fig. 3. Diakinesis of meiosis in accession 910951 of *C. hispanica* var. *hispanica*, showing 30 bivalents.

Chromosome number and DNA content

Chromosome counts of pollen mother cells at diakinesis succeeded only in flower buds of one plant of accession 910951 of *C. hispanica* var. *hispanica*. In four different cells, 30 bivalents (Figure 3), 29 bivalents and 2 univalents, 27 bivalents and 2 univalents, and 27 bivalents were counted. These results indicate a chromosome number of 2n=60 for accession 910951 of var. *hispanica*. Due to clustering of the large number of chromosomes, which were difficult to spread, chromosome counts of *C. abyssinica* (910941) were not successful.

The DNA content of the tetraploid *C. hispanica* accession 910951 was used as 4.0 C level in flow cytometric studies. Flow cytometric determinations of leaf material resulted in DNA contents ranging from 3.7 C up to 4.2 C with an average of 4.0 C for var. *hispanica*, and DNA contents ranging from 3.7 C up to 4.3 C with an average of 3.9 C for var. *glabrata*. For accession 910941 of *C. abyssinica*, DNA contents ranging from 6.9 C up to 7.6 C were found, with an average of 7.4 C.

Discussion

The large variation found for morphological characteristics and the lack of high correlations between these characteristics indicate that the evaluated accessions of *C. hispanica* represent a wide range of genotypes. The considerable variation within *C. hispanica* for earliness, seed yield, oil content, 1000 seed weight, test weight, erucic acid content, saturated fatty acid content and the contents of unsaturated C18 fatty acids indicates that *C. hispanica* might be a promising source of variation for improvement of *C. abyssinica*. High or moderate F-values for variation suggest that selection for these characteristics might be successful. The maximum erucic acid content of 60.1% equals levels found for *C. abyssinica* (Earle et al., 1966; Pokorny & Zeman, 1967; Nieschlag & Wolff, 1971; Mastebroek et al., 1994). The low contents of linoleic and linolenic acid in the seed oil of *C. hispanica*, compared to *C. abyssinica*, offer good prospects for its application as a lubricant or refrigerant. Low contents of these fatty acids are desired for high oxidative and temperature stability (Lazzeri, personal communication). Chances for introducing desirable characteristics from *C. hispanica* into *C. abyssinica* look promising, as crosses between these species were successful (Mastebroek, unpublished). Unfortunately, no seed retaining genotypes were found in *C. hispanica*, reducing the possibility of immediate domestication of this species.

Accession 910953, originating from Italy, was much taller and later than other accessions of var. *hispanica*. Probably, this accession could be interesting in broadening the genetic variation in *C. abyssinica* for physiological characteristics. The results presented in this paper were obtained under greenhouse conditions, hampering comparison with results obtained under field conditions. The application of gauzy nylon bags and/or high temperatures in the greenhouse might have influenced the oil content of hulled seed, which was low compared to averages of 32% (Earle et al., 1966) and 35% (Carlson & Tookey, 1983). The greenhouse conditions might also have influenced plant heights of *C. hispanica* (71 to 168 cm), which larger than those in taxonomic descriptions by Schulz (1919) and Zohary (1966).

Significant correlations of late flowering with a tall plant height, a short top branch, a high number of primary branches (var. *hispanica*) and a high 1000 seed weight have also been reported for *C. abyssinica* (Mastebroek et al., 1994).

The interpretation of correlations between contents of various fatty acids is complicated as percentages are used. Due to interdependence, the correlations will be affected negatively. A negative interrelationship will be stronger when large fractions are related. This may explain the high and negative correlation between the total content of unsaturated C18 fatty acids and erucic acid (Table 7), which amount together for about 90% of the total fatty acid content. Strong competition between these fatty acids is not expected since the C2 position of triacylglycerols is occupied almost entirely by oleic, linoleic and linolenic acid (Mattson & Volpenhein, 1961; Gurr et al., 1972), the total content of the unsaturated C18 fatty acids hardly exceeded 33%, and erucic acid is esterified exclusively at the C1 and C3 positions (Mattson & Volpenhein, 1961; Gurr et al., 1972).

Appleby et al. (1974) demonstrated that in *C. abyssinica* erucic acid is produced by elongation of oleic acid through gadoleic acid (C20:1), rather than by total *de novo* biosynthesis. The content of erucic acid could be affected by the successive elongation steps from oleic acid to nervonic acid (C24:1). The elongations of oleic acid to gadoleic acid and of gadoleic acid to erucic acid, in rapeseed (Downey & Craig, 1964) and *Brassica juncea* (Agrawal & Stumpf, 1985), were demonstrated to be controlled by different elongation systems. For *Crambe* this is not yet clear (Appleby et al., 1974). The negative correlation between gadoleic acid content and erucic acid content (Table 7) is a logical result of the gadoleic acid elongation system. The lack of significant correlation between erucic acid content and nervonic acid content suggests that the elongation of erucic acid to nervonic acid is controlled by another elongation system. Correlations of oil content with the total content of unsaturated C18 fatty acids, gadoleic acid and erucic acid (Table 7) might be the result of different maturity rates. Gurr et al. (1972) demonstrated that oil content and erucic acid content increased, and the total content of oleic, linoleic and linolenic acid decreased, during maturation. Therefore, high erucic acid contents can be the result of better ripening and do not automatically indicate high erucic acid genotypes.

Moderate negative correlations between the total content of saturated fatty acids and erucic acid, suggests some competition between these fatty acids. This competition concerns position C1, at which the saturated fatty acids are preferentially esterified (Mattson & Volpenhein, 1961; Gurr et al., 1972). Selection for high erucic acid genotypes might be supported by selection for low contents of saturated fatty acids, gadoleic acid and nervonic acid. The total content of unsaturated C18 fatty acids should not exceed 33%, lesser contents are not expected as the C2 position is occupied almost entirely by these fatty acids. The variation in *C. hispanica* observed for the total content of saturated fatty acids and the contents of gadoleic and nervonic acid may allow an increase in erucic acid content in *C. abyssinica* after recombination and selection.

The correlation of seed hull mottling with high oil and erucic acid contents needs further investigation.

The chromosome number of 2n=60, found for accession 910951 of var. *hispanica*, is in agreement with results obtained by White & Solt (1978). The DNA content of *C. abyssinica* (7.5 C) is much higher than the expectation based on a chromosome number of 90, the number reported by Manton (1932), Meier & Lessman (1973), White (1975) and White & Solt (1978). The DNA content of var. *glabrata* (3.9 C) suggests a similar chromosome number in var. *glabrata* as in var. *hispanica* (2n=60). White & Solt (1978) observed chromosome numbers of 2n=30 for PI 388784, 388785, 388850 and 388855 var. *glabrata*. These accessions have been collected in the same expeditions as accessions 910947, 910948 and 910949 of var. *glabrata*. It is therefore remarkable but not impossible that these accessions may differ in chromosome number.

Fahleson et al. (1988) found a correlation of 0.91 between the DNA content measured by means of flow cytometry and chromosome number in somatic hybrids of *Brassica* and *Eruca*. This relation might also be true for different *Crambe* species. The difference between the expected chromosome number of *C. abyssinica*, based on the DNA content, and the reported chromosome number needs further investigation.

As noted by White (1975), cordate terminal lobes of the basal leaves and pubescence were characteristics which distinguished the observed accessions of *C. hispanica* from *C. abyssinica*, which had ovate terminal lobes and were glabrous. However, since pubescent accessions of *C. abyssinica* are also known (White, 1975), pubescence can not be used to distinguish these species. On the other hand, seed shattering, chromosome number (2n=60) and DNA content seem to be characteristic for *C. hispanica* in comparison to *C. abyssinica*.

The observed slender stems of var. *glabrata* and the sparsely hispid upper leaf surface are not in line with the taxonomic description of var. *glabrata*, having thick stems and leaves with a glabrous upper surface (Zohary, 1966). Schulz (1919) and Zohary (1966) do not mention the round stem and branches and the presence of a waxy layer, which were obvious characteristics for the evaluated accessions of var. *glabrata*. It is questionable whether the accessions of var. *glabrata* studied and described here are correctly identified and should be considered as a variety of *C. hispanica*, since var. *hispanica* and the studied accessions of var. *glabrata* have only the shape of the basal leaves and DNA content in common. Mastebroek (unpublished) made crosses between var. *hispanica* and var. *glabrata* and observed embryo abortion after crossing. This fact and the clear morphological differences between the two botanical varieties strongly suggest that the taxonomic classification of these varieties should be reconsidered.

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