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Phenotypic and molecular (RAPD) differentiation of four infraspecific groups of cultivated flax (*Linum usitatissimum* L. subsp. *usitatissimum*)

Axel Diederichsen* and Yong-Bi Fu
*Plant Gene Resources of Canada, Saskatoon Research Centre, Agri-culture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada; *Author for correspondence (e-mail: diederichsena-@agr.gc.ca; phone: ++1-306-9562839; fax: ++1-306-9567246)*

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Abstract

Based on agro-botanical characterization data, 3101 accessions of cultivated flax (*Linum usitatissimum* L. subsp. *usitatissimum*) from the flax collection held by Plant Gene Resources of Canada (PGRC) were grouped into four infraspecific groups according to the classification proposed by Kulpa and Danert (1962) . The objective of this study was to investigate phenotypic and RAPD variation within and among the four groups to better understand phenotypic and genotypic differentiation within the genepool of cultivated flax. The results of the phenotypic characterization of characters defining the convarieties (capsule dehiscence, plant height, technical stem length and 1000 seed weight) and of other quantitative (petal width, oil content in seeds) and qualitative (RAPD, petal colour, anther colour, petal longitudinal folding and margin folding, ciliation of capsule septa, seed colour) are presented using descriptive statistics. The most frequent convariety in the PGRC genebank was intermediate flax (convar. *usitatissimum*; 80.7 %), followed by fibre flax (convar. *elongatum* Vav. et Ell. in Wulff; 13.4%), large-seeded flax (convar. *mediterraneum* [Vav. ex Ell.] Kulpa et Danert; 5.6%) and dehiscent flax (convar. *crepitans* [Boenningh.] Kulpa et Danert; 0.3%). Analyses of RAPD data and two qualitative characters (longitudinal and marginal folding of petals) did not show marked differences among the proposed convarieties. However, differences among the convarieties in quantitative traits defining them (plant height, technical stem length and seed size) were considerable. Patterns of variation among the convarieties for other quantitative characters (petal width and seed oil content), as well as the frequencies of character expressions of four qualitative characters (petal colour, anther colour, ciliation of capsule septa and seed colour) were significantly associated with the four proposed convarieties, underlining the phenotypic and genotypic validity of this grouping. The patterns of geographic distribution of the convarieties and important characters showed that certain convarieties dominate in some areas of origin. The infraspecific classification and the presented characterization data increase the transparency of genetic diversity available in cultivated flax and in particular in the PGRC flax collection.

Introduction

In cultivated flax (*Linum usitatissimum* L. subsp. *usitatissimum*), disruptive selection during domestication has resulted in distinct types for fibre and seed use. Informal infraspecific distinctions are often made by using a combination of a defining noun (fibre, textile, oil, seed, or dual purpose) in combination with the base noun flax to name different infraspecific groups. The term linseed refers to *L. usitatissimum* grown for seed and is used commonly in Europe. However, in North America, the base noun flax names mostly *L. usitatissimum* grown for seed. Commercial fibre use based on the tall growing types has nearly disappeared from North America. Such naming of infraspecific groups in common language (informal classification) is widely used, but lacks the clear definition and exact references necessary for a scientific (formal) classification as outlined in the International Code for Botanical Nomenclature (Greuter et al. 2000). The need to effectively communicate about crop diversity based on retraceable definitions occurs in different contexts (Diederichsen 2004). The infraspecific diversity is of particular importance in many crop species, as the loss of biodiversity (gene-erosion) occurs at the infraspecific level, and appropriate taxonomic systems on this diversity are useful. Breeding focuses usually also on certain infraspecific groups, and a taxonomical reference system facilitates communication. The recently introduced 'culton concept' for classification of cultivated plants into crop groups is not complementary; it is suitable for recent cultivars only (Hetterscheid and Brandenburg 1995). Therefore, it cannot be applied to structure the whole range of diversity within a crop species (Diederichsen 2004).

Based on investigations of flax germplasm collections, several proposals for infraspecific groupings of flax have been made (see review in Diederichsen and Richards 2003). The formal classification suggested by Kulpa and Danert (1962), as well as the informal classifications by Sizov (1955) and Dillman (1953), have been used to classify large genebank collections in Germany (Knüpffer 2000; IPK website: http://fox-serv.ipk-gatersleben.de/), Russia (Zhuchenko and Rozhmina 2000) and the United States of America (Dillman 1953; USDA website: http://www.ars-grin.gov/), respectively. Although classifications are meant to facilitate communication among all researchers interested in infraspecific diversity, none of the classifications has been applied to more than one genebank collection so far, and the formal names are rarely used by plant breeders. Only a few experimental studies were made on the consistency, usefulness or biological relevance of infraspecific groupings; examples are the comparison of numerical and classical infraspecific taxonomy in wheat by Schultze-Motel (1987) and in maize by Gutiérrez et al. (2003). Genebank collections offer opportunities to investigate the diversity within a crop species. Categorizing this diversity is useful for efficient collection management of genetic resources (Hanelt 1988; Hazekamp and Guarino 1999; Diederichsen 2004).

Kulpa and Danert (1962) suggested a formal distinction of four major infraspecific groups (convarieties) of cultivated flax: (1) Dehiscent flax (convar. *crepitans*), a primitive flax with spontaneously opening capsules; (2) fibre flax (convar. *elongatum*), exclusively grown for fibre use with tall plants and branching confined to the upper stem, i.e. a high technical stem length (taller than 70 cm and only upper 1/4 or less of the stem with side branches; if shorter, then only upper 1/5 or less with side branches; see Figure 1); (3) large-seeded flax (convar. *mediterraneum*) with large seeds (1000 seed weight >9 g); and (4) intermediate flax (convar. *usitatissimum*) which is grown for fibre and/or seed production. Kulpa and Danert (1962) also suggested a classification of 28 botanical varieties under these four convarieties. In cultivated flax, the convariety concept has also been applied by Hammer (2001). The category convariety, i.e. convarietas, is not synonymous to the category cultivar group suggested for crops by the International Code of Nomenclature for Cultivated Plants (Trehane et al. 1995), because the convariety is a formal category and based on a complementary classification which allows to name landraces and other accessions without cultivar names. Maintaining the formal category convarietas has been suggested by Hanelt (2001) and more information on this controversial issue in cultivated plant classification can be found in Knüpffer and Ochsmann (2003).

The objective of this investigation was to analyze phenotypic and random amplified polymorphic DNA (RAPD) variation within and among the four major infraspecific groups (dehiscent flax, fibre flax, large-seeded flax and intermediate flax), formally described as convarieties by Kulpa and Danert (1962). In this paper, we discuss the biological relevance of the classification by considering how variation of phenotypic characters and genetic variation as revealed by the RAPD technique relate to the four proposed convarieties. The geographic distribution of the convarieties and of

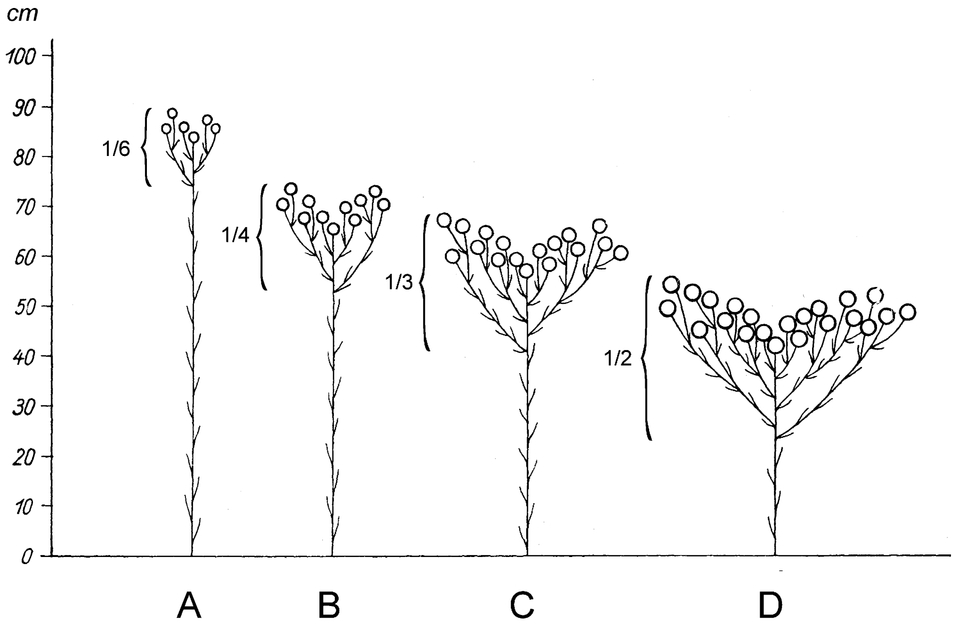

Figure 1. The technical stem length in flax is defined by the length of the stem without side branches. It can be measured in cm or as a fraction of the stem with branching compared to the entire stem length (see figures beside branches). Basal branches are not considered. (A) and (B) typical fibre flax with less than 1/4 of the stem branched; (C) intermediate flax; (D) large-seeded flax. (Adapted from Kulpa and Danert 1962).

important characters are also used to discuss the relevance of the classification.

Materials and methods

A total of 3101 flax accessions of the Canadian national genebank, Plant Gene Resources of Canada (PGRC), were categorised by using the classification suggested by Kulpa and Danert (1962). The field observations were conducted in Saskatoon, Saskatchewan, Canada (52°10′N, 106°41′W; elevation 501 m) on loamy, dark chernozemic soil during the growing seasons 1998–2002. From each accession, 2 g of seed were planted in 3 m long single rows spaced 30 cm between rows. Characterization was completed using a standardised descriptor list (Diederichsen and Richards 2003) including the diagnostic characters for determination of the convarieties according to Kulpa and Danert (1962). The flax germplasm originated from 71 countries across the world (Table 1) and, therefore, should represent the diversity available in the cultivated flax gene pool.

The large number of germplasm accessions grown in each year did not allow for replicated field trials. Thus, variance due to environmental influence could not be eliminated. The variation of seed oil concentration was measured by Dr. J.P. Raney (Saskatoon Research Centre, Saskatoon) using Continuous Wave Nuclear Magnetic Resonance Spectroscopy (NMR) based on a seed sample of 10 g at 3–7 % water content (Firestone 1998).

Kulpa and Danert (1962) defined flax belonging to convar. *mediterraneum* (large-seeded flax) as having a 1000 seed weight of more than 9 g. The determination consistency of 88 flax accessions which PGRC received with authentic convariety assignment by Dr S. Danert himself from the Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Gatersleben, Germany, was optimised by changing this threshold value to 8 g when considering seeds from field regeneration at Saskatoon. Such modification when categorizing the PGRC collection seemed justified, because the growing season at Saskatoon is about 30 days shorter than at Gatersleben, which did not allow all genotypes to realise their genetic potential for seed size. The grand average over 5 years for 1000 seed weight at Saskatoon was 5.9 g ($n = 3089$) while at Gatersleben the grand mean observed on a random selection of flax accessions ($n = 63$) from 26 botanical varieties over 5 years was 6.3 g

Table 1. Geographic origin of flax accessions with determinated convariety at Plant Gene Resources of Canada.

Area of origin[a]	*n*	Breakdown by country of origin (number of accessions)
Africa	70	Africa (4), Eritrea (1), Ethiopia (61), Kenya (1), South Africa (2), Zimbabwe (1)
America (North)	894	Canada (265), United States (629)
America (Central and South)	219	Argentina (194), Brazil (7), Bolivia (1), Chile (1), Costa Rica (1), Guatemala (2), Mexico (1), Nicaragua (1), Uruguay (10), Venezuela (1)
Asia (Central)	62	Unknown (2), Afghanistan (51), Kazakhstan (6), Tajikistan (1), Uzbekistan (2)
Asia (East and Southeast)	72	China (55), Japan (15), Democratic People's Republic of Korea (1), Taiwan (1)
Australia and New Zealand	43	Australia (42), New Zealand (1)
Europe (Central, North, West)	255	Austria (1), Belgium (2), Finland (2), France (125), Germany (31), Great Britain (4), Ireland (11), Netherlands (72), Sweden (7)
Europe (East)	682	Belarus (12), Bulgaria (13), Czechoslovakia (4), Czech Republic (80), Estonia (1), Former Soviet Union (50), Hungary (137), Lithuania (12), Poland (13), Romania (41), Russia (285), Ukraine (24), Yugoslavia (10)
Indian Subcontinent	410	India (265), Pakistan (145)
Mediterranean	129	Algeria (3), Balkans (1), Cyprus (3), Egypt (24), Greece (28), Italy (8), Malta (1), Morocco (38), Portugal (8), Spain (7), Tunisia (8)
Middle East	238	Armenia (2), Azerbaijan (2), Georgia (10), Iran (52), Iraq (12), Israel (8), Syria (10), Turkey (142)
Unknown	27	
Total	3101	

[a]Countries assigned to areas according to the textual descriptions of Zhukovskij (1971).

(Diederichsen and Hammer 1995). Kulpa and Danert (1962) reported 1000 seed weights of more than 11.5 g in many accessions grown at Gatersleben, while such a seed weight was the absolute maximum observed during the 5 years at Saskatoon. These observations indicated that flax generally produces smaller seeds under Saskatoon environmental conditions. Using the modified classification, the re-determination at the convariety level of the 88 accessions received from IPK had a consistency of 77.3%. The inconsistent determinations occurred in intermediate flax being re-determined as fibre flax and the reverse, and also in both directions between large-seeded flax and intermediate flax. There was no confusion in determinations between the extreme groups convar. *elongatum* and convar. *mediterraneum*.

Descriptive statistics of phenotypic variation of the quantitative characters (1000 seed weight, plant height, technical stem length [Figure 1], oil content in seed and petal width) within the four convarieties of flax were calculated. An analysis of variance was conducted to assess the significance of the differences among the groups. For qualitative floral and fruit characters, such as petal colour, anther colour, longitudinal folding of petals (Figure 2), marginal folding of petals (Figure 2), ciliation of septa and seed colour, the frequencies within the convarieties were observed and tested (χ^2 test) against the expected frequency based on the observation in the entire gene-pool to detect significant differences among the convarieties. The frequencies of the four convarieties in germplasm from different geographic areas were tested (χ^2

Figure 2. Petal shapes in flax: without folding (left), longitudinal folding (middle), marginal folding (right).

test) against the null-hypothesis of being randomly distributed.

Characterization of the PGRC flax collection by using the RAPD technique (Williams et al. 1990) was conducted during 1999–2001. Specifically, 16 informative RAPD primers were applied to assay bulk samples of about 10 individuals from each of 2741 accessions and 149 polymorphic bands were scored (Fu et al. 2002, 2003). Scoring RAPD bands was conducted independently by two evaluators as present (1) or absent (0) for all accessions. Each RAPD band was assumed to represent a unique genetic locus. The presence of a RAPD band in an individual-plant sample was interpreted as either a heterozygote or dominant homozygote and the absence of a RAPD band as a recessive homozygote for the plant. As each accession was represented with a bulk sample of 10 individuals, the absence of a RAPD band at a locus would also mean a fixation of the recessive allele in the accession. Counting the number of fixed recessive RAPD loci for each accession generated the proportion of fixed recessive RAPD loci over the 149 polymorphic loci assessed. Proportions of fixed recessive loci were used to compare the level of genetic heterogeneity among accessions. To assess within-convariety variation, summary statistics for the proportion of fixed recessive RAPD loci for all accessions of the four convarieties were first calculated. Second, pair-wise similarity in RAPD variation within each convariety was calculated by using the simple match formula (Apostol et al. 1993), and their summary statistics were generated for each convariety. These similarities measure the relative genetic relatedness among the accessions within a group, with greater similarity reflecting increased genetic relatedness among the accessions. To evaluate the RAPD variation among the four convarieties, RAPD similarities were calculated for all pair-wise combinations of individual accessions between any two convarieties, and their summary statistics were given for each pair of convarieties. To determine whether the RAPD similarity between two convarieties was different from the similarities within convarieties, a permutation test was made following Leonard et al. (1999).

Results

Representation of the four convarieties of cultivated flax in the PGRC collection

Representation of the four convarieties of cultivated flax in the Canadian genebank (PGRC) is shown in Table 2. The most frequent convariety (80.7%) was intermediate flax (convar. *usitatissimum*), followed by fibre flax (convar. *elongatum*, 13.4%), large-seeded flax (convar. *mediterraneum*, 5.6%) and dehiscent flax (convar. *crepitans*, 0.3%). The small representation of dehiscent flax is due to the rare occurrence of this type (Elladi 1940). There are no recent reports of cultivation of dehiscent flax in agriculture.

Variation of quantitative characters defining the four convarieties of flax

Phenotypic variation within and among the four convarieties for the three characters defining these convarieties is shown in Table 3. Differences among the convarieties were considerable, which could be expected, because these characters define them. There were pronounced contrasts between the convarieties *elongatum* and *mediterraneum* in plant height (means: 75.3 cm versus 49.9 cm), seed size (4.9 g versus 8.9 g) and branching (4.6 versus 2.1). The antagonism between these two cultivars can also be seen on the histograms for these characters shown in Figures 3–5. Convarietas

Table 2. Representation of the four convarieties suggested by Kupla and Danert (1962) for cultivated flax *(Linum usitatissimum* L. subsp. *usitatissimum*) in the Canadian genebank (PGRC).

Convariety	Number of accessions	Relative (%)
convar. *crepitans* (Boenningh.) Kulpa et Danert	10	0.3
convar. *elongatum* Vav. et Ell. in Wulff	417	13.4
convar. *mediterraneum* (Vav. ex Ell.) Kulpa et Danert	173	5.6
convar. *usitatissimum*	2501	80.7
Total accessions with convariety determinated	3101	

Table 3. Variation parameters of phenotypic quantitative characters defining the four convarieties of cultivated flax.

	n	Mean	Min.	Max.	SD	CV (%)	SE of *M*
Plant height (cm)							
convar. *crepitans*	7	64.3	24	92	22.7	35.3	9.25
convar. *elongatum*	417	75.3	36	130	21.5	28.5	1.05
convar. *mediterraneum*	171	49.9	29	86	11.1	22.2	0.85
convar. *usitatissimum*	2492	57.8	17	105	14.2	24.6	0.29
Total	3087	59.8	17	130	16.6	27.8	–
Technical stem length (value of x where $1/x$ = fraction of the total stem length without branches)							
convar. *crepitans*	8	2.4	2	3	0.52	21.8	0.20
convar. *elongatum*	417	4.6	4	6	0.70	15.3	0.03
convar. *mediterraneum*	171	2.1	1	4	0.78	36.6	0.06
convar. *usitatissimum*	2501	2.6	1	6	0.84	32.3	0.02
Total	3097	2.8	1	6	1.07	37.6	0.02
1000 seed weight (g)							
convar. *crepitans*	6	5.0	4.4	5.8	0.53	10.7	0.24
convar. *elongatum*	409	4.9	3.1	7.9	0.81	16.3	0.04
convar. *mediterraneum*	173	8.9	8.0	11.5	0.72	8.1	0.05
convar. *usitatissimum*	2501	5.9	2.8	8.0	0.98	16.7	0.02
Total	3089	5.9	2.8	11.5	1.23	20.8	–

Explanation: *n*, number of observed accessions; SD, standard deviation; CV, coefficient of variation; SE of *M*, standard error of mean.

usitatissimum had intermediate mean values between convar. *elongatum* and convar. *mediterraneum* for these characters. Convarietas *crepitans* was close to convar. *elongatum* in seed weight, but in plant height and technical stem length closer to convar. *usitatissimum*. Within convar. *mediterraneum*, seed weight was more uniform (lower CV) than in the other convarieties.

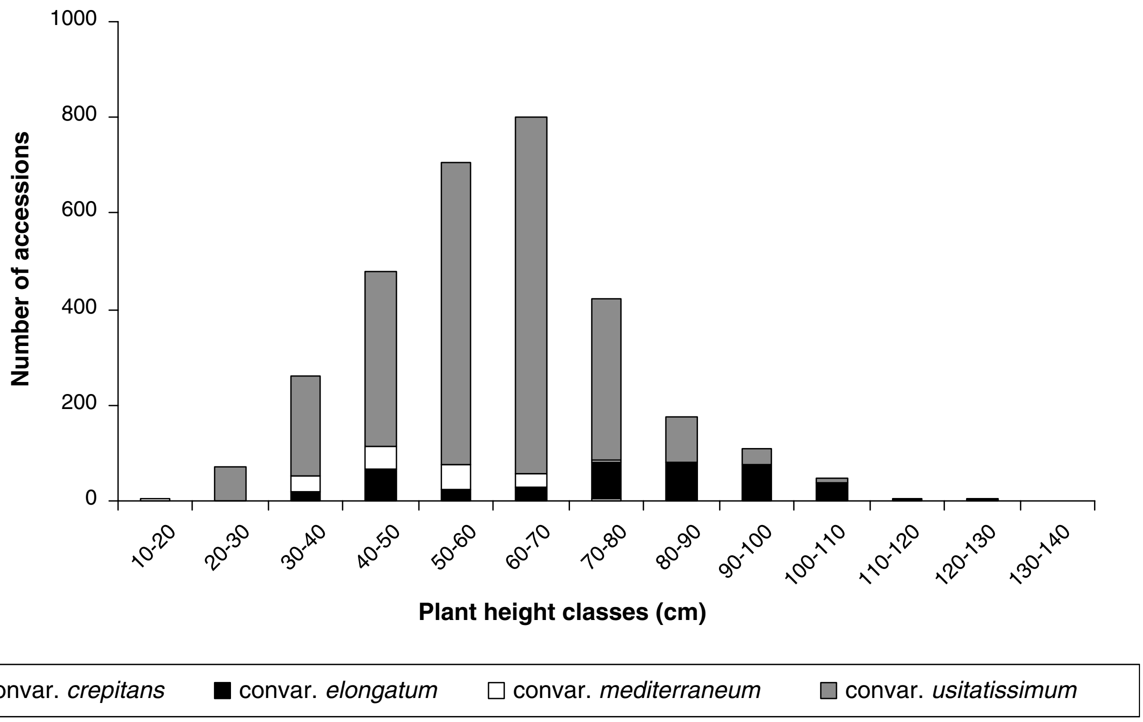

Figure 3. Histogram for plant height in flax accessions of PGRC collection with convariety determination (n = 3087).

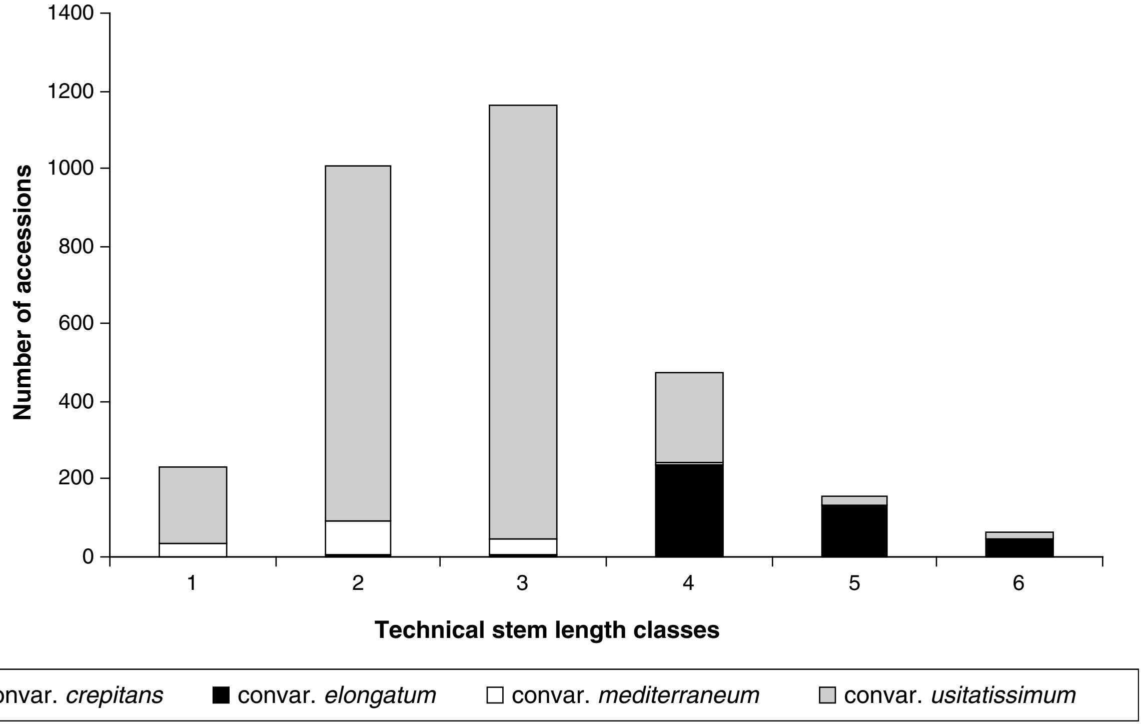

Figure 4. Histogram for technical stem length in flax accessions of PGRC collection with convariety determination (n = 3097).

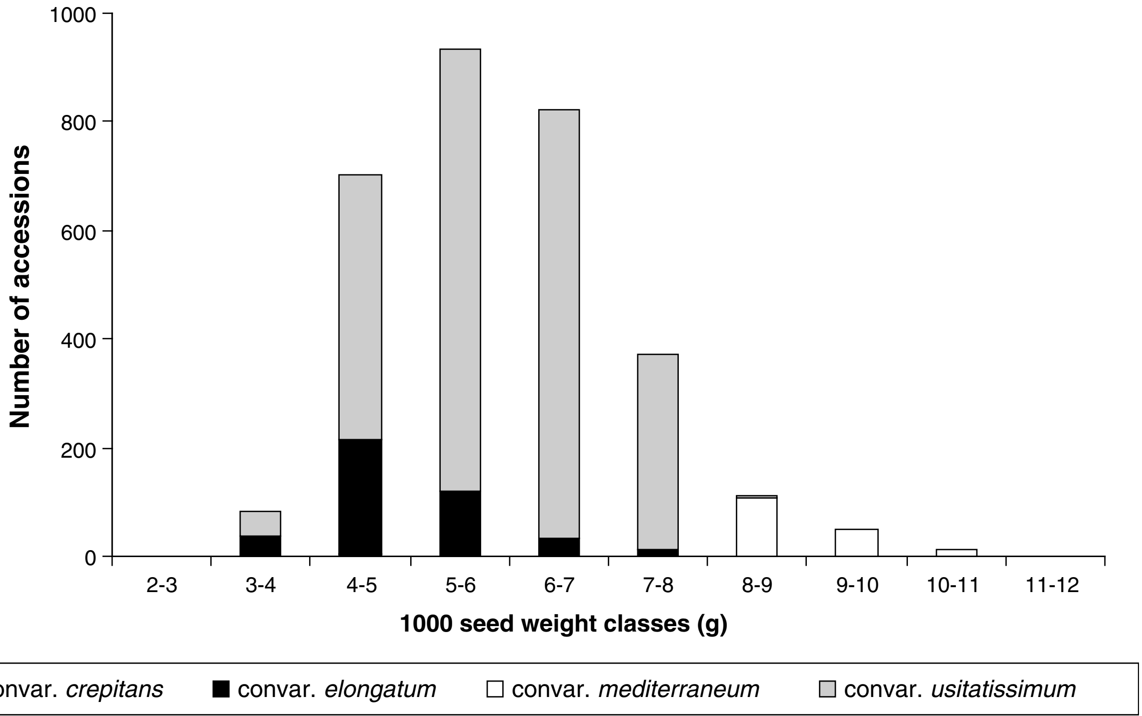

Figure 5. Histogram for 1000 seed weight in flax accessions of PGRC collection with convariety determination (n = 3089).

Table 4. Variation of phenotypic quantitative characters and 149 RAPD loci of the four convarieties of cultivated flax.

	n	Mean	Min.	Max.	SD	CV (%)	SE of *M*
Petal width (mm)***							
convar. *crepitans*	7	6.9	5.6	8.7	1.12	16.2	0.46
convar. *elongatum*	351	8.6	3.0	13.1	1.34	15.6	0.07
convar. *mediterraneum*	156	11.1	5.8	15.8	2.43	21.9	0.20
convar. *usitatissimum*	2210	9.7	4.1	15.4	1.59	16.5	0.03
Total	2724	9.6	3.0	15.8	1.71	17.8	–
Oil content in seed (%)***							
convar. *crepitans*	7	35.7	34.0	36.9	1.13	3.2	0.46
convar. *elongatum*	351	37.0	33.9	45.7	1.49	4.0	0.08
convar. *mediterraneum*	170	39.9	36.4	43.5	1.61	4.0	0.12
convar. *usitatissimum*	2379	38.4	26.2	45.6	1.66	4.3	0.03
Total	2907	38.3	26.2	45.7	1.75	4.6	–
RAPD (% fixed recessive loci)							
convar. *crepitans*	1	47.3	–	–	–	–	–
convar. *elongatum*	401	46.7	34.0	65.7	5.42	11.6	0.27
convar. *mediterraneum*	149	46.8	37.0	61.0	4.14	8.9	0.34
convar. *usitatissimum*	2190	46.7	30.9	79.2	4.92	10.5	0.11
Total	2741	46.7	30.9	79.2	4.96	10.6	–

Explanation: *n*, number of observed accessions; SD, standard deviation; CV, coefficient of variation; SE of *M*, standard error of mean, ***mean values among convarieties different at $p < 0.001$.

Variation of quantitative characters not used to define the convarieties

Parameters of phenotypic variation for quantitative characters which are not related to the definitions of the convarieties are shown in Table 4. The four convarieties displayed significant differences ($p < 0.001$) in the quantitative characters. For petal width, the variation within convar. *mediterraneum* was larger than in the

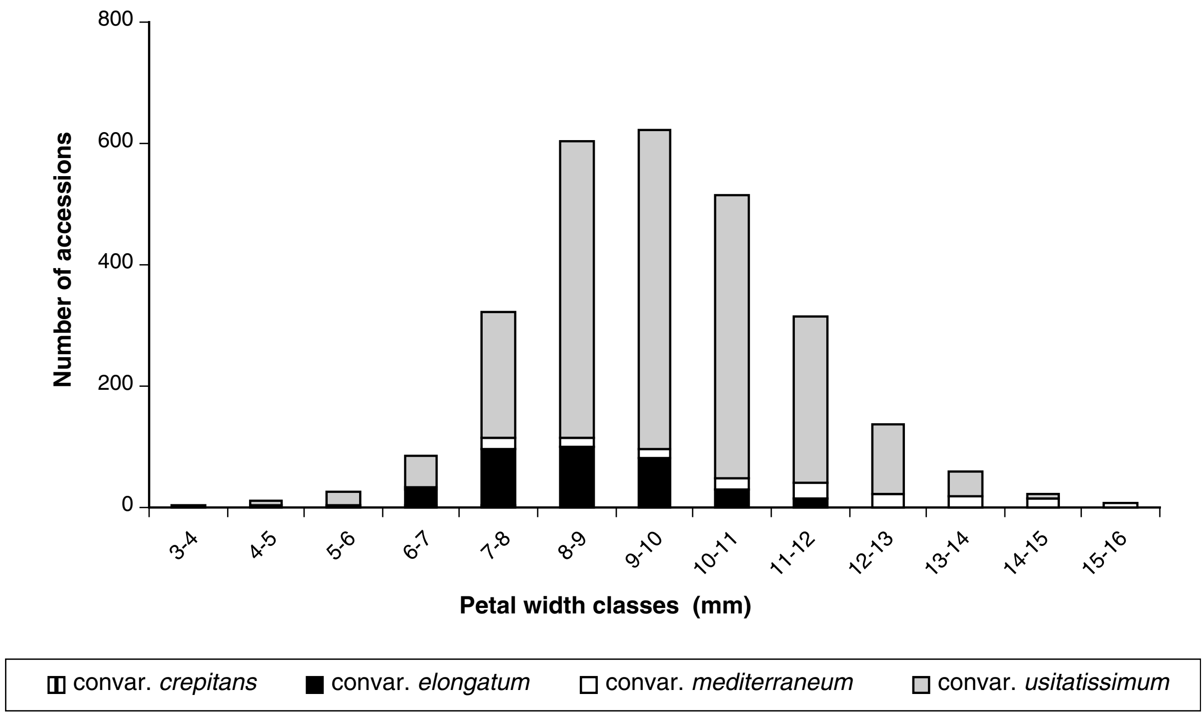

Figure 6. Histogram for petal width in flax accessions of PGRC collection with convariety determination ($n = 2724$).

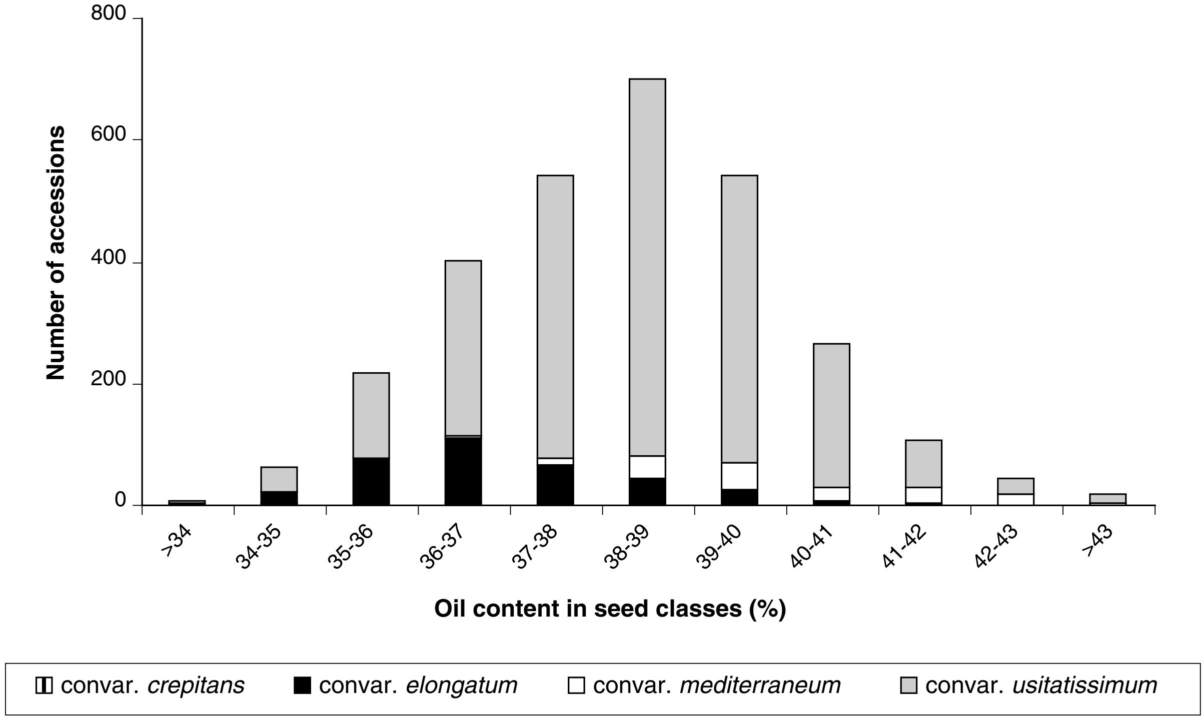

Figure 7. Histogram for oil content in seed in flax accessions of PGRC collection with convariety determination (n = 2907).

other convarieties (higher CV). The histogram (Figure 6) shows the antagonism between convar. *elongatum* and convar. *mediterraneum* in this character. The width of petals decreased from convar. *mediterraneum* to convar. *usitatissimum*, convar. *elongatum* to convar. *crepitans*. Compared to the other characters discussed, oil content showed a low variation within cultivated flax (CV = 4.6%). The oil content increased in order as follows: convar. *crepitans*, convar. *elongatum*, convar. *usitatissimum* and convar. *mediterraneum*. The histogram (Figure 7) shows this polarization between convar. *elongatum* and convar. *mediterraneum*.

RAPD variation within and among the convarieties

The level of genetic diversity within each convariety, as revealed by proportions of fixed recessive RAPD loci, was nearly the same for all four convarieties with an average of 46.7 % (Table 4). As there was only one accession analyzed for convar. *crepitans*, we consider the comparisons of this convariety with the others as preliminary. The average RAPD similarities within the three convarieties *elongatum*, *mediterraneum* and *usitatissimum* were very similar and ranged from 75.1 to 76.6% (Table 5). Also, the RAPD similarities between these three convarieties were only slightly

Table 5. Similarity (%) within and between the four convarieties of flax based on pairwise comparison of 149 RAPD loci (mean and range).

Convariety	n	Similarity within convarieties	Similarity between convarieties		
			elongatum	*mediterraneum*	*usitatissimum*
crepitans (dehiscent flax)	1	(100) (one accession)	62.6 (48.4–71.7)	63.1 (55.3–72.6)	62.9 (48.0–80.0)
elongatum (fibre flax)	401	76.6 (50.0–99.3)		72.3 (48.9–94.6)	74.1 (38.9–99.3)
mediterraneum (large-seeded flax)	149	76.3 (51.1–99.3)			74.7 (40.3–99.3)
usitatissimum (intermediate flax)	2190	75.1 (36.5–100)			

Explanations: n, number of accessions scored for RAPD bands.

Table 6. Classes and frequencies of character expression of qualitative character classes within the four convarieties of flax.

Petal colour (χ^2 = 33.71***)						
	n	White	Blue (all shades)	Pink		
convar. *crepitans*	9	–	9	–		
convar. *elongatum*	399	73 (+ +)	325 (–)	1		
convar. *mediterraneum*	160	21	138	1		
convar. *usitatissimum*	2354	214 (–)	2124	16		
Total	2922	308	2596	18		
Relative frequency (%)	–	10.5	88.8	0.6		
Anther colour (χ^2 = 17.05*)						
	n	White	Blue (all shades)	Yellow, orange		
convar. *crepitans*	10	–	10	–		
convar. *elongatum*	399	–	377	22		
convar. *mediterraneum*	163	–	138	25 (–)		
convar. *usitatissimum*	2345	5	2149	191 (+)		
Total	2917	5	2674	238		
Relative frequency (%)	–	0.2	91.7	8.2		
Petal, longitudinal folding (χ^2 = 7.44 n.s.)						
	n	Absent	Present	Mixture		
convar. *crepitans*	8	8	–	–		
convar. *elongatum*	305	289	14	2		
convar. *mediterraneum*	79	76	2	1		
convar. *usitatissimum*	1401	1278	99	24		
Total	1793	1651	115	27		
Relative frequency (%)	–	92.1	6.4	1.5		
Petal, marginal folding (χ^2 = 1.35 n.s.)						
	n	Absent	Present	Mixture		
convar. *crepitans*	8	8	–	–		
convar. *elongatum*	305	293	11	1		
convar. *mediterraneum*	79	77	2	–		
convar. *usitatissimum*	1401	1360	38	3		
Total	1793	1738	51	4		
Relative frequency (%)	–	96.9	2.8	0.2		
Capsule, ciliation of septa (χ^2 = 263.62***)						
	n	Absent	Present			
convar. *crepitans*	9	9 (+)	– (–)			
convar. *elongatum*	395	256 (+ +)	139 (–)			
convar. *mediterraneum*	172	26 (–)	146 (+ +)			
convar. *usitatissimum*	2364	642 (–)	1722 (+)			
Total	2940	933	2007			
Relative frequency (%)	–	31.7	68.3			
Seed colour (χ^2 = 27.99*)						
	n	Brown	Yellow	Olive	Mottled	Mixture
convar. *crepitans*	9	9	–	–	–	–
convar. *elongatum*	410	389	10 (–)	5	2	4
convar. *mediterraneum*	173	153	13 (+)	–	4 (+)	3
convar. *usitatissimum*	2501	2335	110	17	7 (–)	32
Total	3093	2886	133	22	13	39
Relative frequency (%)	-	93.3	4.3	0.7	0.4	1.3

Explanation: The observed frequencies within the convarieties tested (χ^2-test) against the expected frequency based on the observation in all accessions (***p = 0.001, * p = 0.05, n.s. = not significant); considerable deviations marked with (+) for higher and (–) for lower than expected.

different with the lowest similarity between convar. *elongatum* and convar. *mediterraneum* (72.3%) and the highest similarity between convar. *usitatissimum* and convar. *mediterraneum* (74.7%). Molecular within-convariety RAPD similarities were only slightly higher than those among the convarieties, but the difference was not significant based on the permutation test.

Variation of qualitative characters not used to define the convarieties

The two types of petal folding (longitudinal and marginal, Figure 2) were rare in the collection (6.4 and 2.8%, respectively), and no significant associations of these petal shapes with any convariety were found (Table 6). For all other characters, the frequencies of qualitative classes within the convarieties showed significant differences. White petal colour was more frequent in fibre flax (convar. *elongatum*). Yellow or orange anthers were less frequent in convar. *mediterraneum* and more frequent in convar. *usitatissimum*. Ciliation of septa was much more frequent in convar. *mediterraneum* and less frequent in convar. *elongatum*. Yellow and mottled seeds occurred more frequently in large seeded flax (convar. *mediterraneum*).

Geographic distribution of the four flax convarieties

The geographic distribution of the four convarieties showed significant imbalances among the areas of origin (Table 7). Convar. *elongatum* was dominating in accessions with European origin, but rarely found in germplasm from the Indian subcontinent, the Mediterranean area and the Middle East. In the Indian subcontinent and the Mediterranean area, convar. *mediterraneum* was relatively frequent. Germplasm from the Middle East mostly belonged to the intermediate group, i.e. convar. *usitatissimum*. The few accessions representing convar. *crepitans* did not allow for sound conclusions; they came from the Mediterranean area and adjacent Middle East and East Europe. The mean values for plant height, technical stem length and 1000 seed weight for germplasm from the different areas (Table 8) showed that taller flax originated from East and Southeast Asia and Europe, while flax from the Indian subcontinent was short. The technical stem length showed a similar geographic pattern with the exception that flax from Australia and New Zealand also had a high technical stem length. The distribution for 1000 seed weight pointed at flax with heavy seeds from the Mediterranean area, the Indian Subcontinent and Australia/New Zealand. Light-seeded flax was characteristic for Africa, East and Southeast Asia, and East Europe.

Discussion

Usefulness of the classification

The convariety-delimitation that Kulpa and Danert (1962) suggested is based on three quantitative traits (seed size, technical stem length, plant height) and one qualitative trait (capsule dehiscence). Although the quantitative characters have high heritabilities (Diederichsen and Hammer 1995), they are more influenced by environment than are qualitative characters such as capsule dehiscence, petal colour

Table 7. Frequency of the four flax convarieties in PGRC accessions for different geographic areas.

Area of origin	*n*	convar. *crepitans*	convar. *elongatum*	convar. *mediterraneum*	convar. *usitatissimum*
Africa	70	–	2	1	67
America (North)	894	–	110	27 (–)	757 (+)
America (Central and South)	219	–	8 (–)	2 (–)	209 (+)
Asia (Central)	62	–	2	–	60
Asia (East and Southeast)	72	–	21 (+)	1	50
Australia and New Zealand	43	–	2	4	37
Europe (Central, North, West)	255	–	73 (+)	21	161 (–)
Europe (East)	682	4	180 (+ +)	25	473 (–)
Indian Subcontinent	410	–	1 (–)	64 (+ +)	345
Mediterranean	129	5 (+ +)	3 (–)	22 (+ +)	99
Middle East	238	1	4 (–)	6	227 (+)
Total	3074	10	406	173	2485
Relative frequency (%)		0.3	13.2	5.6	80.9

Explanation: The observed frequencies deviated significantly ($p = 0.001$; $\chi^2 = 507.18$) from the distribution expected when assuming the same relative frequency in all areas; considerable deviations marked with (+) for higher and (–) for lower than expected.

Table 8. Mean values for important quantitative characters by geographic origin of the accessions.

Area of origin	Plant height (cm)			Technical stem length[a]			Weight of 1000 seeds (g)		
	n	Mean	SD[b]	*n*	Mean	SD[b]	*n*	Mean	SD[b]
Africa	69	54.6	12.3	70	2.6	0.62	70	4.9	1.20
America (North)	893	62.0	14.9	894	2.9	0.99	893	5.7	1.02
America (Central and South)	219	56.7	10.5	219	2.4	0.79	219	6.1	0.83
Asia (Central)	62	60.4	11.4	62	2.5	0.80	62	5.6	0.59
Asia (East and Southeast)	72	73.7	24.7	72	3.4	0.88	72	5.2	0.96
Australia and New Zealand	43	55.7	14.1	43	3.3	0.86	43	6.6	1.12
Europe (Central, North, West)	255	70.1	16.8	255	3.4	0.84	254	5.9	1.35
Europe (East)	681	65.5	16.4	681	3.3	1.09	677	5.5	1.28
Indian Subcontinent	407	41.8	10.2	410	2.1	0.90	410	6.6	1.35
Mediterranean	125	62.6	11.4	126	2.5	0.76	125	6.7	1.28
Middle East	234	54.2	10.6	238	2.0	0.77	238	6.4	0.90

[a]Value of x where $1/x$ = fraction of the total stem length without branches.
[b]SD = standard deviation.

or seed colour. These qualitative characters could be considered as morphological genetic markers (Kutuzova 1998). However, the advantage of the characters used by Kulpa and Danert (1962) is that they allow for distinguishing infraspecific groups which are of great relevance from a practical point of view, and they reflect the disruptive selection for fibre types and linseed types during the domestication process. Large differences in mean values for plant height, technical stem length and seed size (Table 3), as well as the geographic distribution of the convarieties (Table 8) support this delimitation. For practical genebank management, the classification of the easily distinguishable convar. *crepitans* is of great relevance, because the capsules have to be harvested before the seeds shatter.

The flax collection at PGRC

Fibre flax breeding has received little attention in North America (Kenaschuk and Rowland 1993), which may be the reason for lesser representation of this germplasm in the PGRC genebank compared to other collections (Diederichsen 2004). Most linseed cultivars grown in North America were determined as convar. *usitatissimum* (Table 7) since their 1000 seed weight was lower than 8 g and they had a short technical stem length (see also Diederichsen 2001). The convar. *usitatissimum* is very dominant in the PGRC genebank and nearly all North American cultivars released belong into this group. Some accessions in the PGRC collection were mixtures of dehiscent and indehiscent flax with a very low frequency of the dehiscent plants. According to passport data, these accessions had originally been purely dehiscent accessions. They have probably been contaminated with indehiscent flax during genebank regeneration.

Variation of phenotypic characters and RAPD within and among the four convarieties

The large differences in the mean values in the characters plant height, technical stem length and 1000 seed weight underline that the phenotypic differentiations of these groups are of practical relevance. The phenotypic antagonism between convar. *elongatum* and convar. *mediterraneum* illustrates this clearly. The characters seed oil content and width of petals were not used by Kulpa and Danert (1962) to define the four convarieties, yet the mean values for each of the four convarieties were significantly different for these characters. Although there was little variation for seed oil content in cultivated flax (CV = 4.6%), the large-seeded convar. *mediterraneum* showed a higher mean seed oil content (39.9%) than did intermediate flax (38.4%) or fibre flax (37.0%).

When comparing the mean values (Tables 3 and 4), convar. *crepitans* was close to convar. *elongatum* in oil content, 1000 seed weight, plant height, and petal width. It was closer to convar. *mediterraneum* in technical stem length. Elladi (1940) reported that this primitive and rare flax was cultivated for fibre use in the Pyrenees Mountains, Portugal, alpine southern Germany, and Ukraine. Hegi (1925) stated, it was mostly cultivated for seed use. The lack of ciliation of the capsule septa in all accessions of convar. *crepitans* indicated homogeneity for this character. Also in the other qualitative characters this group is uniform. Comparison of the passport data of the PGRC dehiscent flax accessions with those from other genebanks indicates that genebanks have exchanged this material frequently. There are no reports of recent cultivation of this type of flax.

Patterns of variation for quantitative characters not used by Kulpa and Danert (seed oil content and width of petals) confirmed the distinction of the four convarieties. The frequencies of the character expressions for the qualitative characters petal colour, anther colour, ciliation of capsule septa and seed colour also showed associations with certain infraspecific groups. The differences in quantitative and qualitative phenotypic characters, which are morphological genetic markers, support the delimitation of the four convarieties and underline the biological relevance of this classification.

However, none of the phenotypic characters with genetic marker quality showed exclusive association with only one of the four convarieties, except for the rare occurrence of white anthers in convar. *usitatissimum*, which was related to male sterility in five accessions.

The RAPD data collected at 149 loci, which were presumed to be randomly distributed over the flax genome, did not reveal any differences among the four convarieties. The alleles coding for the evident phenotypic differences observed among the four convarieties had either not been sampled in the amplified DNA or their contribution to the entire array of RAPD polymorphisms observed was very small. Thus, a grouping based on the observed RAPD polymorphisms would differ from the classification based on the phenotypic characterization. It would be desirable to find relationships between phenotypic and RAPD variations because of the practical relevance of the phenotypic groups. More accessions for convar. *crepitans* need to be analyzed with molecular methods for a better understanding of the position of this convariety.

Conclusion

The distinction of the four convarieties describing dehiscent flax, fibre flax, large-seeded flax and intermediate flax can be used to structure genebank collections and for interpretation of experimental results when investigating the gene pool of cultivated flax.

RAPD data and two qualitative characters (longitudinal folding of petals and marginal folding of petals) did not show marked differences among the proposed convarieties. Accordingly, the RAPD data could not be used for recognizing the infraspecific groups suggested by Kulpa and Danert (1962).

The geographic patterns of variation for the phenotypic characters plant height, technical stem length, seed size, seed oil content, petal width, petal colour, anther colour, ciliation of capsule septa and seed colour were significantly associated with the four proposed convarieties, which underlined the phenotypic and genotypic evidence of this grouping.

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