BIOLOGIA PLANTARUM 45 (3): 327-336, 2002

Identification of flax and linseed cultivars by isozyme markers

K. KRULÍČKOVÁ*, Z. POŠVEC** and M. GRIGA**

*Department of Botany and Plant Physiology, Mendel University of Agriculture and Forestry, Zemědělská 1, CZ-613 00 Brno, Czech Republic**
*Department of Plant Biotechnology, AGRITEC Ltd., Zemědělská 16, CZ-787 01 Šumperk, Czech Republic***

Abstract

A set of 28 fibre flax and linseed cultivars differing in plant morphology and technological parameters were analysed by isozyme markers in five ontogenetic phases. Relatively high isozyme polymorphism was observed using polyacrylamide gel electrophoresis. Altogether 18 isozyme systems produced 145 different bands; 66 of them (45.52 %) have been found to be polymorphic. The highest level of polymorphism was found in acid phosphatase and esterase, polymorphism was detected in aconitase, diaphorase, glutamate dehydrogenase, peroxidase and superoxide dismutase as well. The highest number of unique isozymic spectra (cultivar × enzyme × ontogenetic phase) was detected in the phase of shoot with removed cotyledons. Electrophoretic analyses of all polymorphic isozymes enabled to distinguish 20 cultivars (71 %) in the screened cultivar set.

Additional key words: electrophoresis, *Linum usitatissimum* L., native-PAGE, protein polymorphism.

Introduction

Various aspects of flax isozymes have been concerned during last three decades. Since early seventies, the laboratory of M.A. Fieldes in Canada has been systematically studying biochemistry and genetics of number of enzymes in flax genotrophs, *i.e.* genotypes with environmentally induced heritable changes (*e.g.* Fieldes and Tyson 1972, Fieldes 1988, Fieldes and Ross 1991, Fieldes and Gerhardt 1994, Gerhardt and Fieldes 1999, and other related papers). A group of C. Morvan in France published a series of papers concentrated on the effect of various factors on pectin methylesterase and peroxidase in flax seedlings and cultured cells (Schaumann *et al.* 1993, Burel *et al.* 1994, Mareck *et al.* 1995, Bruyant *et al.* 1996, Alexandre *et al.* 1997). McDougall *et al.* (1992, 1993) used flax tissue culture to study surface-associated peroxidases during callus formation and root and shoot regeneration. Gorman *et al.* (1993) studied the inheritance and linkage relationships of five flax enzyme systems (DIA, PGM, ACP, LAP, PGD).

Surprisingly, there is a very limited number of reports on the use of protein markers for commercial flax cultivar identification. Sammour (1988) tested several PAGE-techniques for their suitability to discriminate fiber flax cultivars based on total seed proteins. SDS-PAGE of globulins extracted with Tris/borate buffer was the method which revealed the most usable polymorphisms. Lapina (1989) successfuly used seed protein analysis

Received 7 February 2001, *accepted* 15 November 2001.

Abbreviations: AAP - alanine aminopeptidase (EC 3.4.11.2); AAT - aspartate aminotransferase (EC 2.6.1.1); ACO - aconitase (EC 4.2.1.4); ACP - acid phosphatase (EC 3.1.3.2); ADH - alcohol dehydrogenase (EC 1.1.1.1); AMY - α-amylase (EC 3.2.1.1); BPB - bromophenol blue; DIA - diaphorase (EC 1.6.4.3); DS - dry seeds; GDH - glutamate dehydrogenase (EC 1.4.1.2-4); GOT - glutamate oxaloacetate transaminase (EC 2.6.1.1); GC - green cotyledons; EST - esterase (EC 3.1.1...); FTL - first true leaf; IS - imbibed seed; IDH - isocitrate dehydrogenase (EC 1.4.1.41); LAP - leucine aminopeptidase (EC 3.4.11.1); LPX - lipoxygenase (EC 1.13.11.12); MDH - malate dehydrogenase (EC 1.1.1.37); 2-ME - 2-mercaptoethanol; PRX - peroxidase (EC 1.11.1.7); SDH - shikimate dehydrogenase (EC 1.1.1.25); SWRC - shoot with removed cotyledons; SOD - superoxide dismutase (EC 1.15.1.1); URE - urease (EC 3.5.1.5).

Acknowledgement: This research was financially supported by NAAR - Ministry of Agriculture CR (project No. 6049) and Ministry of Education CR (project No. OC 837.20).

**Corresponding author; phone: (+420) 649 382 126, fax: (+420) 649 382 999, e-mail: griga@agritec.cz

(PAGE in nondenaturing conditions) to discriminate eight fiber flax cultivars differing in their resistance to lodging, fungal diseases and some technological parameters. Both optimum sowing date and seed storage period affected the resulting spectra of total seed proteins. The storage period of the seed not exceeding a year was needed to obtain the whole (reachest) spectrum of seed proteins proper for cultivar identification. The same group (Lapina and Kelner 1990) later studied seed storage proteins of four fiber flax cultivars by SDS-PAGE in denaturing conditions. The method generated 62 to 71 bands. Via single-seed analysis, the intravarietal variation in seed storage proteins was revealed. Only one report was concentrated on utilization of isozymes for commercial fiber flax cultivar discrimination (Yurenkova *et al.* 1992). The authors studied polymorphism of five enzyme systems (EST, AAT, 6-PGD, MDH, SOD) in 6 flax cultivars/lines and one wild sample from Pamir. Only AAT and MDH exhibited exploitable polymorphism, while EST, SOD and 6-PGD disposed uniform spectra. There was a difference in 6-PGD spectra obtained by analysis of leaves and roots. Later, the same group reported significant differences in EST spectra from various plant parts in various stages of flax ontogenesis (Yurenkova *et al.* 1995).

The objective of our research was to find isozyme markers, which would be used *1*) for discrimination and identification of flax and linseed cultivars/genotypes used in breeding programmes, *2*) for testing cultivar purity in commercial lots, *3*) for identification of cultivars and redundant flax germplasm in national collections and International Flax Data Base (Pavelek 1995).

Materials a methods

Plants: The following 28 genotypes of *Linum usitatissimum* L. from *Agritec Flax Collection* were tested: flax genotypes - 1288/12, Ariane, Jitka, Jordan, SU 19-1/93, SU 19-3/93, SU 29-3/93, Texa, V-12/446, Vera, Viking, Zarja; linseed genotypes - Amazon, Areco K119, Atalante, C 1/7, C12/5, C 15/5, ED 30, Ica 44, Jupiter, Liflora, Mikael, Minerva, NLN 245-OR, NLN 245-P, Ocean, Oliver.

Sample preparation: A mixed seed sample (150 seeds per genotype as a minimum) was used to eliminate/cover potential intravarietal variation. Several types/ontogenetic phases of samples were analysed: *1*) dry seeds (DS; dry seeds were ground to fine powder which was used for extraction, *2*) imbibed seeds (IS; seeds were imbibed for 24 h on wet filter paper at dark and room temperature), *3*) shoots with removed cotyledons (SWRC; seeds were germinated for 48 h on wet filter paper at dark and room temperature and cotyledons and radicula were removed), *4*) green cotyledon (GC; seeds were cultivated on wet filter paper at 23 °C and 18 h-photoperiod with irradiance of 20.4 μmol m^{-2} s^{-1} for 5 d and only cotyledons were used for extraction), and *5*) first true leaf (FTL; seeds were aseptically grown (1/2 MS solution - mineral part) at above mentioned conditions for 11 d and green true leaves were used for extraction).

Extraction: 1 g of tissue was homogenised with extraction buffer (125 mM Tris-HCl, pH 6.8, 10 % 2-ME, 20 % glycerol, 0.02 % BPB) (Vallejos 1983, Acquaah 1992) and extracted for 1 h at -20 °C. Samples were centrifuged at 30 000 *g* (4 °C) for 10 min. Supernatant was stored at -20 °C until electrophoresis.

PAGE electrophoresis and staining: Enzymes were separated using native discontinuous polyacrylamide electrophoresis (7.5 % running gel and 5 % stacking gel) according to Samec *et al.* (1998), Pošvec and Krulíčková (1999), and Pošvec and Griga (2000). Gels were stained as described elsewhere (Vallejos 1983, Acquaah 1992, Manchenko 1994, Rothe 1994).

Data processing: The gels were scanned and saved as electronic files. Schematic figures of typical zymograms of individual enzyme systems were made and labelled by numbers, which constituted particular numerical code (Pošvec and Griga 2000).

Studied enzymes: The following 18 isozyme systems have been studied: AAP, AAT, ACO, ACP, ADH, AMY, DIA, GDH, GOT, EST, IDH, LAP, LPX, MDH, PER, SDH, SOD, URE.

Results

The highest polymorphism was found in ACP and EST, which were polymorphic in all studied phases of plant development. Lower polymorphism, only in some phases, was observed in ACO (FTL), DIA (SWRC), GDH (GC), PRX and SOD (FTL). All other analysed enzymes exhibited uniform spectra or their activity was undetectable (Table 1).

Aconitase (ACO): Enzyme consisted of one isozyme zone (Fig. 1). Polymorphism was found only in the phase of first true leaves (Table 2). One uniform isozyme zone ACO1 was detected in the phase of dry seed. Isozymes in the ACO1 zone consisted of two bands in all surveyed genotypes. Activity of this enzyme was not determined in

the phase of imbibed seed, shoot without cotyledons neither in the phase of green cotyledons. Two polymorphic bands of isozyme activity were observed in the ACO1 in the phase of first true leaves. Genotype Minerva with unique spectrum was identified.

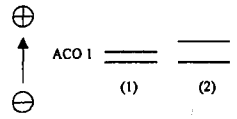

Fig. 1. Complex diagram of ACO isozymes.

Table 1. Isozymic activity and polymorphism of flax in the ontogenetic phases under study (n.a. - isozyme not analysed, N - isozyme showed very low or no activity, U - isozyme was uniform, P* - isozyme with polymorphic character, DS - dry seed, IS - imbibed seed, SWRC - shoot with removed cotyledons, GC - green cotyledon, FTL - first true leaf).

	AAP	AAT	ACO	ACP	ADH	AMY	DIA	GDH	GOT	EST	IDH	LAP	LPX	MDH	PER	SDH	SOD	URE
DS	N	U	U	P*	U	U	N	U	N	P*	N	N	N	U	U	U	U	N
IS	N	N	N	P*	U	n.a.	N	U	N	P*	N	U	N	U	U	U	U	n.a.
SWRC	N	N	N	P*	N	n.a.	P*	N	N	P*	N	N	N	U	U	U	U	n.a.
GC	N	N	N	P*	U	n.a.	N	P*	N	P*	N	N	N	N	U	N	U	n.a.
FTL	N	U	P*	P*	U	n.a.	N	U	N	P*	N	N	N	N	P*	U	P*	n.a.

Table 2. Combination of isozyme ACO, DIA, GDH, PER, SOD in the cultivars (n.a. - isozyme not analysed, * - cultivar with unique band pattern, FTL - first true leaf, SWRC - shoot with removed cotyledons, GC - green cotyledon).

Cultivar	ACO (ACO1) FTL	DIA (DIA1, DIA2, DIA3, DIA4) SWRC	GDH (GDH1, GDH2) GC	PRX (PRX1, PRX2) FTL	SOD (SOD1, SOD2) FTL
1288/12	1	1,0,1,1	1,2	1,1	1,1
Amazon	1	1,0,1,1	1,2	1,1	2,1*
Areko K119	1	1,0,1,1	1,2	n.a.	n.a.
Ariane	1	1,0,1,1	1,2	1,1	1,1
Atalante	1	1,2,1,1	1,1	1,1	1,1
C 1/7	1	1,0,2,1	1,1	1,1	n.a.
ED 30	1	1,0,1,1	1,1	2,1	1,1
Ica 44	1	1,0,2,1	1,3	2,1	1,1
Jitka	1	1,0,1,1	1,3	2,1	1,2*
Jordan	1	1,0,1,1	1,3	1,1	1,1
Jupiter	1	1,0,1,1	1,3	1,2	n.a.
Liflora	1	1,0,1,1	1,3	1,2	1,1
Mikael	1	1,2,1,1	1,3	1,2	1,3
Minerva	2*	1,0,1,1	1,2	1,1	1,1
NLN 245-OR	1	1,1,1,1*	n.a.	1,1	1,1
NLN 245-P	1	1,1,2,1*	n.a.	1,1	1,1
Ocean	1	1,3,1,1	1,2	1,2	1,3
Oliver	1	1,2,1,1	1,3	1,1	1,1
SU 19-1/93	1	1,3,1,1	3,3*	1,1	1,1
SU 19-3/93	1	1,0,1,1	1,2	1,1	1,1
Texa	1	1,0,2,1	1,2	1,1	1,3
V-12/446	1	1,0,1,1	1,3	2,1	1,1
Vera	1	1,0,1,1	1,3	1,1	1,1
Viking	1	1,0,1,1	3,1*	1,1	1,1
Zarja	1	1,0,1,1	1,2	1,1	1,1

Acid phosphatase (ACP): Enzyme was composed of six isozyme zones (Fig. 2). Polymorphism was discovered in all types of studied extracts (Table 3). Five isozyme zones were detected in the phase of dry seed, but only three of them were polymorphic (ACP2 - two bands in anodal zone, ACP4 - one band, ACP5 - three bands), zones ACP3 (two bands) and ACP6 (two bands in cathodal zone) were uniform. Zone ACP1 was absent in DS sample. After detail analysis of bands four genotypes with unique spectra (ED 30, Jupiter, NLN 245-OR, Texa)

were recognized. Three isozyme zones (ACP2 - two bands, ACP3 and ACP5 - three bands) were determined in the phase of imbibed seeds, all of them were polymorphic. Zones ACP1, ACP4 and ACP6 were absent. Three genotypes with unique spectra (1288/12, ED 30, Jordan) were distinguished. Five isozyme zones were discovered in the phase of shoot with removed cotyledons, four of them (ACP1 - five bands,

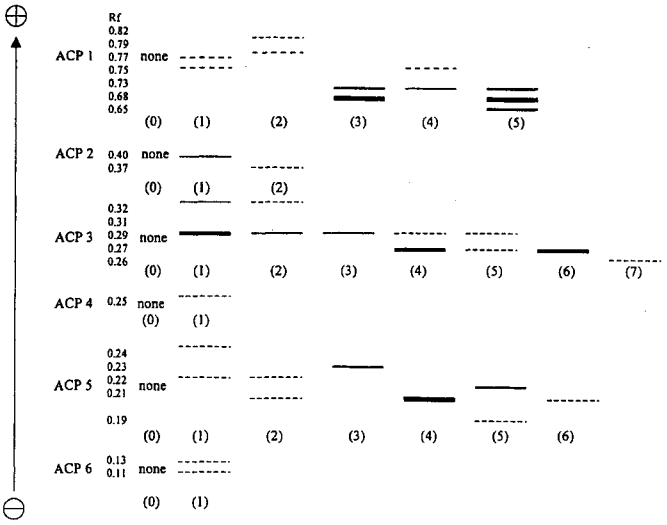

Fig. 2. Complex diagram of ACP isozymes.

Table 3. Combination of isozymes ACP in the cultivars (n.a. - isozyme not analysed, * - cultivar with unique band pattern, DS - dry seed, IS - imbibed seed, SWRC - shoot with removed cotyledons, GC - green cotyledon, FTL - first true leaf).

Cultivar	ACP (ACP1, ACP2, ACP3, ACP4, ACP5, ACP6)				
	DS	IS	SWRC	GC	FTL
1288/12	n.a.	0,2,2,0,1,0*	3,0,3,1,3,0	1,0,0,0,3,0	1,0,6,0,4,1
Amazon	n.a.	n.a.	3,0,3,1,3,1	1,0,0,0,3,0	1,0,6,0,4,1
Areko K119	0,2,1,0,1,1	0,2,1,0,1,0	3,0,3,1,3,1	1,0,0,0,3,0	1,0,6,0,4,1
Ariane	n.a.	0,2,1,0,1,0	3,0,3,1,3,1	1,0,0,0,3,0	1,0,6,0,4,1
Atalante	0,2,1,0,1,1	0,2,1,0,1,0	3,0,3,1,3,1	1,0,0,0,3,0	1,0,6,0,4,1
C 1/7	0,2,1,0,1,1	0,2,1,0,1,0	4,0,3,1,0,0*	2,0,3,1,3,0*	1,0,6,1,0,0*
C 12/5	0,2,1,0,1,1	n.a.	n.a.	n.a.	n.a.
C 15/5	0,2,1,0,1,1	n.a.	n.a.	n.a.	n.a.
ED 30	0,2,1,0,2,1*	0,2,3,0,2,0*	5,0,3,1,4,1*	1,0,0,0,3,0	1,0,6,0,4,0
Ica 44	n.a.	0,2,1,0,1,0	3,0,3,1,3,0	1,0,0,0,3,0	1,0,6,0,4,0
Jitka	0,2,1,0,1,1	0,2,1,0,1,0	3,0,3,1,4,0	1,0,0,0,3,0	1,0,6,0,4,1
Jordan	n.a.	0,2,5,0,1,0*	3,0,3,1,3,0	1,0,0,0,0,0	1,0,7,1,0,0*
Jupiter	0,1,1,0,1,1*	0,1,1,0,1,0	3,0,3,1,3,1	1,0,0,0,3,0	1,0,6,0,4,0
Liflora	n.a.	0,2,4,0,1,0	3,0,6,1,0,0	1,0,0,0,0,0	1,0,7,0,0,0*
Mikael	n.a.	0,2,4,0,1,0	3,0,6,1,3,1*	1,0,0,0,3,0	1,0,7,1,4,1
Minerva	n.a.	0,2,4,0,1,0	3,0,6,1,0,0	1,0,0,0,0,0	1,0,7,1,6,0
NLN 245-OR	0,2,1,1,1,1*	0,2,1,0,1,0	3,0,3,1,3,1	n.a.	1,0,6,0,4,0
NLN 245-P	n.a.	0,2,1,0,1,0	3,0,3,1,3,1	n.a.	1,0,6,0,4,0
Ocean	n.a.	0,2,1,0,1,0	3,0,3,1,5,1*	1,0,3,0,3,0	1,0,6,1,4,1
Oliver	0,2,1,0,1,1	0,2,1,0,1,0	3,0,3,1,3,1	1,0,0,0,0,0	1,0,6,1,0,1*
SU 19-1/93	0,2,1,0,1,1	0,2,1,0,1,0	3,0,3,1,3,1	1,0,3,0,3,0	1,0,6,1,4,1
SU 19-3/93	0,2,1,0,1,1	0,2,1,0,1,0	3,0,3,1,3,1	1,0,0,0,0,0	1,0,6,0,6,1
SU 29–3/93	0,2,1,0,1,1	n.a.	n.a.	n.a.	n.a.
Texa	0,1,1,0,2,1*	0,1,1,0,1,0	3,0,3,1,3,1	1,0,0,0,3,0	1,0,6,0,4,0
V-12/446	n.a.	0,2,4,0,1,0	3,0,6,1,3,0*	1,0,0,0,3,0	1,0,7,1,4,1
Vera	n.a.	0,2,1,0,1,0	3,0,3,1,0,0*	1,0,0,0,0,0	1,0,6,1,6,0
Viking	0,2,1,0,1,1	0,1,1,0,1,0	3,0,3,1,4,0	1,0,3,1,3,0*	1,0,6,1,6,1
Zarja	n.a.	0,2,1,0,1,0	3,0,3,1,3,0	1,0,0,0,3,0	1,0,6,0,4,1

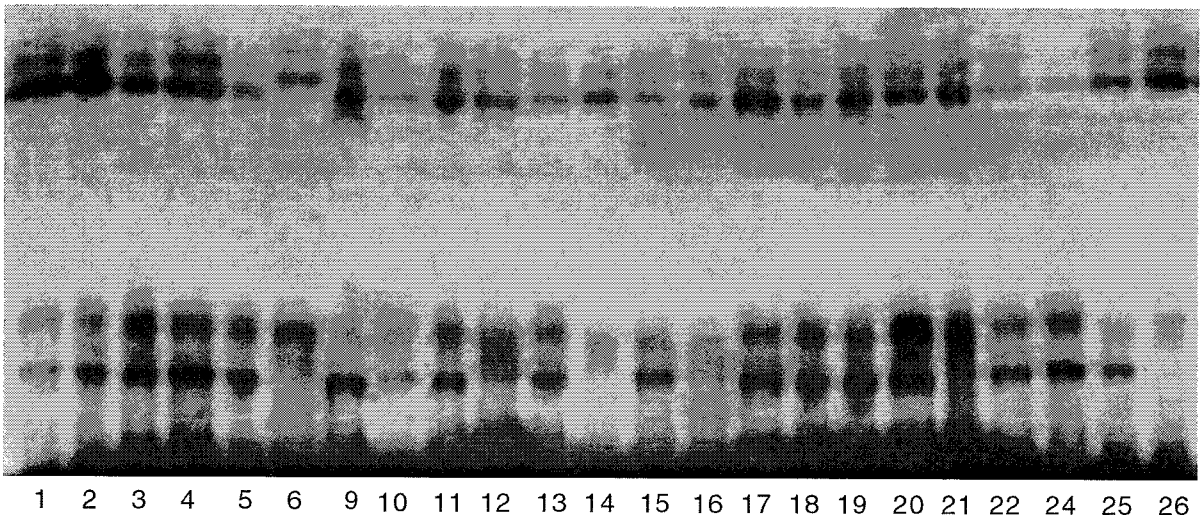

Fig. 3. ACP isozyme electrophoretic gel of shoots with removed cotyledons.

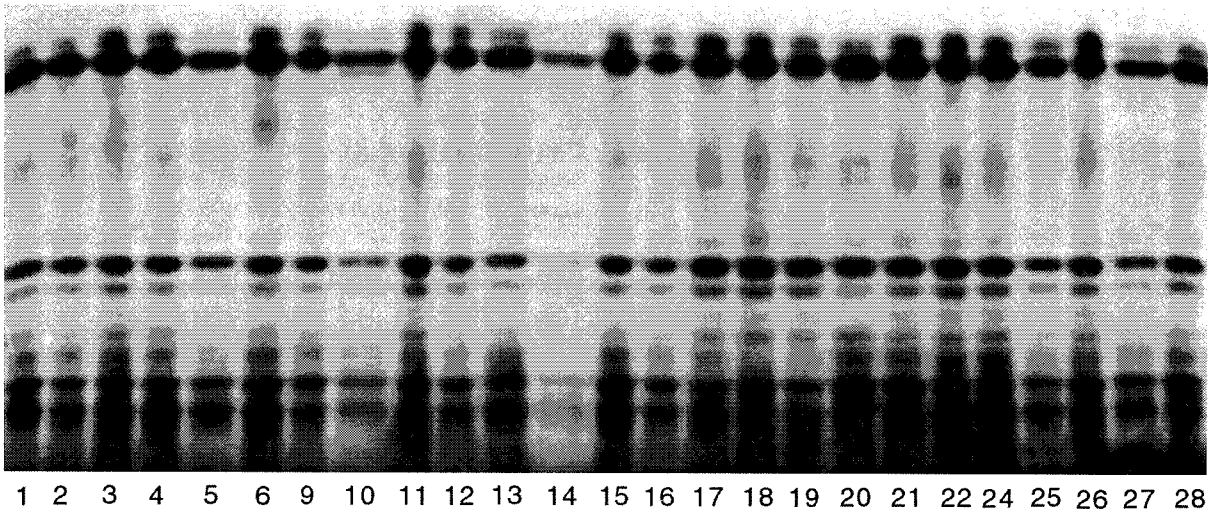

Fig. 4. EST isozyme electrophoretic gel of shoots with removed cotyledons.

ACP3 - two bands, ACP5 - three bands, ACP6- two bands) were polymorphic, zone ACP4 (one band) was uniform (Fig. 3), zone ACP2 was missing. Six genotypes with unique spectra (C 1/7, ED 30, Mikael, Ocean, V-12/446, Vera) were differentiated. Four polymorphic isozyme zones ACP1 (four bands), ACP3 (one band), ACP4 (one band) and ACP5 (one band) were observed in the phase of green cotyledons. Zones ACP2 and ACP6 were missing. Two genotypes with unique spectra (C 1/7, Viking) were established. Five isozyme zones (ACP1 - two bands, ACP3 - two bands, ACP4 - one band, ACP5 - one band, ACP6 - two bands) were described in the phase of first true leaves, but only zones ACP3, ACP4, ACP5 a ACP6 were polymorphic. Zone ACP2 was absent. All isozyme systems showed lower activity in this phase. Four genotypes with unique spectra (C 1/7, Jordan, Liflora, Oliver) were located.

Diaphorase (DIA): Enzyme diaphorase was created from four isozyme zones DIA1, DIA2, DIA3 a DIA4 (Fig. 5). Polymorphism was proved only in the phase of shoots with removed cotyledons, polymorphic zones DIA2 (two bands) and DIA3 (three bands) were detected. Two genotypes with unique spectra NLN 245-OR and NLN 245-P were distinguished in this phase (Table 2).

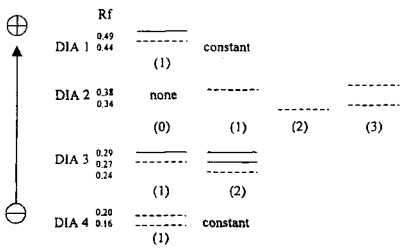

Fig. 5. Complex diagram of DIA isozymes.

Esterase (EST): Enzyme was composed of six isozyme zones (Fig. 6). Polymorphism was detected in all analysed phases (Table 4). All six isozyme zones were described in the phase of dry seeds, but only two of them EST3 (two bands) and EST6 (two bands) were polymorphic. Two genotypes with unique spectra ED 30

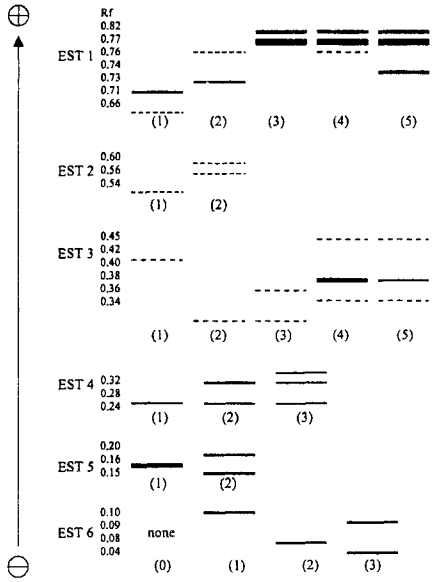

Fig. 6. Complex diagram of EST isozymes.

and Ica 44 were characterized. All six isozyme zones were found in the phase of imbibed seeds, but only EST 4 (two bands) and EST 6 (two bands) were polymorphic. Two genotypes with unique spectra (C 1/7, Oliver) were determined. Six isozyme zones were found in the phase of shoot with removed cotyledons (Fig. 4). Three of them EST1 (three bands), EST4 (three bands) and EST6 (two bands) were polymorphic. Two genotypes with unique spectra (Ica 44, Oliver) were observed. Three isozyme zones EST1 (three bands), EST4 (three bands) and EST6 (two bands) were polymorphic and three were uniform EST2 (one band), EST3 (three bands) and EST5 (one band) in the phase of green cotyledons. Three genotypes with unique spectra (C 1/7, Ica 44, Oliver) were confirmed. All six isozyme zones were detected in the phase of first true leaves, but only two EST1 (four bands) and EST6 (two bands) were polymorphic. Two genotypes with unique spectra (Minerva, Oliver) were located.

Glutamate dehydrogenase (GDH): Enzyme GDH was formed from two isozyme zones GDH1 and GDH2 (Fig. 7.). Polymorphism was ascertained only in the phase of green cotyledons (Table 2). Enzyme GDH was uniform in the phase of dry seeds, only isozyme zone GDH2 (one band) was observed. Uniform isoenzyme zone GDH2 (one band) was identified in the phase of imbibed seeds. Activity of enzyme GDH was not proved in the phase of shoot with removed cotyledons. Two polymorphic isozyme zones GDH1 (three bands) and GDH2 (four bands) were described in the phase of green cotyledons and two genotypes with unique spectra SU 19-1/93 and Viking were distinguished in this phase. One isozyme zone GDH2 (three bands) was found in the phase of first true leaves and it was uniform for all analysed genotypes.

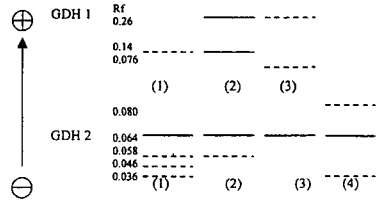

Fig. 7. Complex diagram of GDH isozymes.

Peroxidase (PRX): Enzyme peroxidase was composed of two isozyme zones PRX1 and PRX2 (Fig. 8). Enzyme PRX was uniform in the phase of dry seeds, imbibed seeds, shoots with removed cotyledons and the phase of green cotyledons. One isozyme zone PRX2 was described. PRX2 was almost constituted from two bands, only in the phase of dry seeds it was created from one band. Enzyme PRX was polymorphic in the phase of first true leaves (Table 2). Two isozyme zones PRX1 (two bands) and PRX2 (two bands) were detected. None genotype with unique spectrum was described.

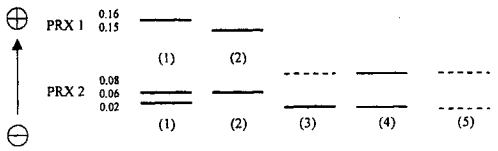

Fig. 8. Complex diagram of PRX isozymes.

Superoxide dismutase (SOD): Enzyme SOD was created from three isozyme zones SOD1, SOD2 and SOD3 (Fig. 9). Activity of this enzyme was very high in all analysed phases. Polymorphism was proved only in the phase of first true leaves. Three uniform isozyme zones SOD1 (four bands), SOD2 (three bands) and SOD3 (one band) were found in the phase of dry and imbibed seeds. Two uniform isozyme zones SOD1, SOD2 were detected in the phase of shoot with removed cotyledons (SOD1 - two bands, SOD2 - three bands) and phase of green cotyledons (SOD1 - one band, SOD2 - one band). Two isozyme zones SOD1 (three bands), SOD2 (four bands) were polymorphic in the phase of first true leaves (Table 2). Two genotypes with unique spectra Amazon and Jitka were determined.

Fourteen genotypes/cultivars with unique spectra, that is 50 % of studied cultivar set (1288/12, C1/7, ED 30, Jordan, Jupiter, Liflora, Mikael, NLN 245-OR, Ocean, Oliver, Texa, V 12/446, Věra, Viking), were distinguished based on ACP analysis. Five genotypes

(C 1/7, ED 30, Ica 44, Minerva, Oliver) were determined using enzyme EST. Cv. Minerva was identified by enzyme ACO, genotypes NLN 245-OR and NLN 245-P by enzyme DIA. Genotypes SU 19-1/93 and Viking were distinguished by enzyme GDH and cvs. Amazon and Jitka by enzyme SOD.

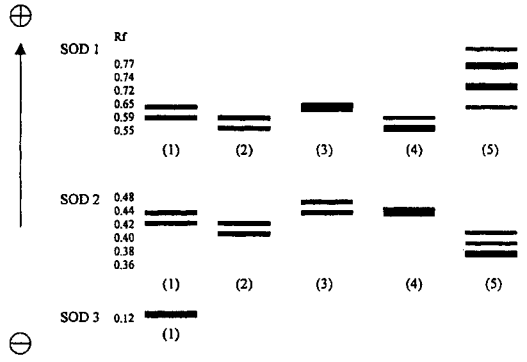

Fig. 9. Complex diagram of SOD isozymes.

Differentiation of particular genotypes using polymorphic enzymes is shown in Table 5. The best results are possible to achieve by utilising the extract from shoot with removed cotyledons. Phase of dry seeds provided 5 cultivars with unique spectrum, phase of imbibed seeds 5 cultivars, phase of shoots with removed cotyledons 10 cultivars, phase of green cotyledons 5 cultivars and phase of first true leaves 7 cultivars. Utilising all polymorphic isozymes (ACO, ACP, DIA, EST, GDH, PRX, SOD) we were able to differentiate 20 cultivars (71 %) in the screened cultivar set.

Table 4. Combination of isozymes EST in the cultivars (n.a. - isozyme not analysed, * - cultivar with unique band pattern, DS - dry seed, IS - imbibed seed, SWRC - shoot with removed cotyledons, GC - green cotyledon, FTL - first true leaf).

Cultivar	EST (EST1, EST2, EST3, EST4, EST5, EST6)				
	DS	IS	SWRC	GC	FTL
1288/12	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,2	3,1,5,2,1,1	3,1,5,3,1,1
Amazon	1,1,1,1,1,1	2,1,3,2,2,1	3,2,4,2,2,2	3,1,5,2,1,1	3,1,5,3,1,1
Areko K119	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Ariane	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Atalante	1,1,1,1,1,1	2,1,3,2,2,1	3,2,4,2,2,2	3,1,5,2,1,1	3,1,5,3,1,1
C 1/7	1,1,2,1,1,1	2,1,3,1,2,1*	3,2,4,2,2,3	3,1,5,3,1,1*	4,1,5,3,1,1
C 12/5	1,1,2,1,1,1	n.a.	n.a.	n.a.	n.a.
C 15/5	1,1,2,1,1,1	n.a.	n.a.	n.a.	n.a.
ED 30	1,1,1,1,1,2*	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	4,1,5,3,1,1
Ica 44	1,1,2,1,1,2*	2,1,3,2,2,1	4,2,4,2,2,2*	4,1,5,2,1,1*	4,1,5,3,1,1
Jitka	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,2	3,1,5,2,1,1	3,1,5,3,1,1
Jordan	n.a.	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Jupiter	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Liflora	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Mikael	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,2	3,1,5,2,1,1	3,1,5,3,1,1
Minerva	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	5,1,5,3,1,1*
NLN 245-OR	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	n.a.	4,1,5,3,1,1
NLN 245-P	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,3,2,2	n.a.	3,1,5,3,1,1
Ocean	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,3,2,2	3,1,5,2,1,1	4,1,5,3,1,1
Oliver	1,1,2,1,1,1	2,1,3,2,2,2*	3,2,4,2,2,0*	3,1,5,2,1,2*	3,1,5,3,1,2*
SU 19-1/93	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,2	3,1,5,2,1,1	3,1,5,3,1,1
SU 19-3/93	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Texa	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
V-12/446	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Vera	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Viking	1,1,2,1,1,1	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1
Zarja	n.a.	2,1,3,2,2,1	3,2,4,2,2,3	3,1,5,2,1,1	3,1,5,3,1,1

Discussion

The aim of the paper was to evaluate the possibility of utilization of isozymes for exact identification of flax and linseed genotypes for various practical purposes. Several factors seem to be important: *1*) selected enzyme system and the number of loci/alleles coding individual isoforms, whose polymorphism may be exploited, *2*) developmental expression of particular isoforms in various phases of plant ontogenesis and thus the selection of

proper tissue sample for analysis, and *3*) the choice of particular electrophoretic approach. The selection of enzymes was based on our previous experience with identification of pea cultivars (Samec *et al.* 1998, Pošvec and Griga 2000), and on published literature. In fact, only one report is available on the use of isozymes for fiber flax discrimination (Yurenkova *et al.* 1992). Of five enzyme systems analyzed by PAGE in seed/leaf/root samples only AAT and MDH were found polymorphic, EST (3.1.1.1) (with one exception), 6-PGD and SOD were uniform. This is in contrast with our data: both AAT and MDH were uniform or exhibited low/no activity in all tested sample types (Table 1). On the other hand, EST in our experiments exhibited polymorphism in all analysed sample types, and SOD in one sample type (Table 1). This situation may be explained by

Table 5. Differentiation of the cultivars via isozyme band pattern in the sample type (DS - dry seed, IS- imbibed seed, SWRC - shoot with removed cotyledons, GC - green cotyledon, FTL - first true leaf).

Cultivar	Unique isozyme band pattern in the sample type				
	DS	IS	SWRC	GC	FTL
1288/12		ACP			
Amazon					SOD
C 1/7		EST	ACP	EST, ACP	ACP
ED 30	ACP, EST	ACP	ACP		
Ica 44	EST		EST	EST	
Jitka					SOD
Jordan		ACP			ACP
Jupiter	ACP				
Liflora					ACP
Mikael			ACP		
Minerva					ACO, EST
NLN 245-OR	ACP		DIA		
NLN 245-P			DIA		
Ocean			ACP		
Oliver		EST	EST	EST	ACP, EST
SU 19-1/93				GDH	
Texa	ACP				
V-12/446			ACP		
Vera			ACP		
Viking				ACP, GDH	
Σ cultivars	5	5	10	5	7

the different cultivars tested in particular laboratories. Authors, similarly with our data, found out differences in isozyme spectra (SOD, 6-PGD, EST) of various organs in various phases of plant ontogenesis (Yurenkova *et al.* 1992, 1995). This is not surprising, as isozymes represent phenotypic traits/markers, whose particular forms may be differentially expressed during ontogenesis (tissue or organ-specific expression, *e.g.* stem-specific expression of FLAXPER1, probably involved in lignification processes - Gerhardt and Fieldes 1999, McDougall 1992) and may be even affected/induced by external environmental factors (pathogen invasion; abiotic stresses, *e.g.* heavy metal stress - Fieldes and Gerhardt 1994, Gerhardt and Fieldes 1999; changes in temperature and light conditions - Fieldes *et al.* 1987, Burel *et al.* 1994, Bruyant *et al.* 1996). It is expected, that stress-induced isozymes are forms of normal isozymes with altered post-translational modifications, mainly glycosylation, which alter molecular weight of particular isozyme and thus its electrophoretic mobility (Fieldes and Tyson 1984, Gerhardt and Fieldes 1999). Not only isozymes, but also total proteins may be affected by physiological state of the plant or seed. The changes in total proteins of flax stems in various growth stages were reported by Lapina and Rulin (1985). The storage period (effect of seed aging) may influence the electrophoretic pattern of flax seed proteins (Sammour 1989, Lapina 1989). Another aspect, which should be taken into account is the fact that some commercial flax cultivars are composed of two or more breeding lines, and thus variation in isozyme spectra may be expected. Lapina and Kelner (1990) proved this intravarietal variation via single-seed analysis of flax storage proteins. Thus, the correct isoenzyme characterization of particular cultivar should be done on the base of mixed sample involving all lines composing a cultivar.

From the practical point of view, the analysis of dry seeds is the simplest and quickest approach. Nevertheless, our results show (Table 1) that dry/imbibed seeds exhibited polymorphism only in two enzyme systems (ACP, EST), while green plant samples extended number of exploitable enzymes to seven (ACO, ACP, DIA, GDH, EST, PRX, SOD). Seed germination/plant cultivation may represent methodological complication (it requires more time and additional costs) in cases where the results should be obtained very quickly (discrimination of commercial seed lots). On the other hand, such approach should not be a significant limitation in evaluation of germplasm resources or breeding materials.

As compared to some agriculturally important crops (barley, soybean, pea), there is a limited information about genetic/linkage maps of flax based on morphological, biochemical and DNA markers exploitable in phylogenetic studies and marker-assisted selection (Gorman *et al.* 1993, Cullis *et al.* 1995, Spielmeyer *et al.* 1998, Klocke *et al.* 2000, Oh *et al.* 2000). Thus, the isozyme data obtained with our relatively broad set of flax/linseed cultivars, may be useful for such studies as reported in another crops (Havey and Muehlbauer 1989, Chase *et al.* 1991, Hoey *et al.* 1996). However, the detailed characterization of screened set by morphological and physiological data (pathogen resistance/sensitivity) as well as pedigree analysis should be done in that relation.

By the combination of seven enzyme systems and five ontogenetic phases we were able to identify twenty of total twenty eight tested flax and linseed cultivars/lines. Reported data represent first step in formulation of

cataloque of protein markers for flax and linseed genotypes. Further enzyme systems and further ELFO methods are planned to be tested to increase the information about potential use of protein markers in plant breeding, creation of core collection of *Linum usitatissimum* L., and flax genetic mapping.

References

Acquaah, G.: Practical protein electrophoresis for genetic research. - Dioscorides Press, Portland 1992.

Alexandre, F., Morvan, O., Gaffé, J., Mareck, A., Jauneau, A., Dauchel, H., Balangé A.P., Morvan C.: Pectin methylesterase patterns in flax seedlings during their development. - Plant Physiol. Biochem. **35**: 427-436, 1997.

Bruyant, P., Schaumann, A., Djiana, R., Morvan, C., Balange, A.P.: Effect of light on total proteins and peroxidase activities in the culture medium and in the cell-wall fraction of cultured flax cells. - Plant Physiol. Biochem. **34**: 417-423, 1996.

Burel, C., Berthe, T., Mery, J.C., Morvan, C., Balange, A.P.: Isoelectric focusing analysis of peroxidases in flax seedling hypocotyls grown in different light conditions. - Plant Physiol. Biochem. **32**: 853-860, 1994.

Chase, C.D., Ortega, V.M., Vallejos, C.E.: DNA restriction fragment length polymorphisms correlate with isozyme diversity in *Phaseolus vulgaris* L. - Theor. appl. Genet. **81**: 806-811, 1991.

Cullis, C.A., Oh, T.J., Gorman, M.B.: Genetic mapping in flax (*Linum usitatissimum*). - In: Proc. 3rd Meeting FAO Intern. Flax Breeding Res. Group - Breeding for Fiber and Oil Quality in Flax. Pp. 161-169. Centre Technique pour l'Étude et l'Amélioration du Lin, St. Valéry en Caux 1995.

Fieldes, M.A.: Relative mobility and activity of leaf malate dehydrogenase in flax (*Linum usitatissimum*) genotrophs and genotypes. - Biochem. Genet. **26**: 261-275, 1988.

Fieldes, M.A., Gerhardt, K.E.: Effects of Zn on flax seedlings: differences in the response of the cationic and anionic isozymes of peroxidase. - Plant Sci. **96**: 1-13, 1994.

Fieldes, M.A., Ross, J.: Peroxidase activity and relative mobility at anthesis in flax genotrophs and their F_2 progeny: developmental and genetic effects. - Genome **34**: 495-504, 1991.

Fieldes, M.A., Tyson, H.: Activity and relative mobility of peroxidase isoenzymes in genotrophs and genotypes of flax (*Linum usitatissimum* L.). - Can. J. Genet. Cytol. **14**: 625-636, 1972.

Fieldes, M.A., Tyson, H.: Possible post-translational modification and its genetic control in flax genotroph isozymes. - Biochem. Genet. **22**: 99-114, 1984.

Fieldes, M.A., Tyson, H., Gaudreault, P.-R.: Direct response of peroxidase isozyme relative mobility to short-term environmental temperature changes in flax (*Linum usitatissimum*). - Plant Cell Environ. **10**: 213-219, 1987.

Gerhardt, K.E., Fieldes, M.A.: Charge and molecular weight differences for fourteen guaiacol peroxidase isozymes in flax indicate that most are encoded by different structural genes. - Electrophoresis **20**: 1939-1945, 1999.

Gorman, M.B., Cullis, C.A., Aldridge, N.: Genetic and linkage analysis of isozyme polymorphisms in flax. - J. Hered. **84**: 73-78, 1993.

Havey, M.J., Muehlbauer, F.J.: Linkages between restriction fragment length, isozyme, and morphological markers in lentil. - Theor. appl. Genet. **77**: 395-401, 1989.

Hoey, B.K., Crowe, K.R., Jones, V.M., Polans, N.O.: A phylogenetic analysis of *Pisum* based on morphological characters, and allozyme and RAPD markers. - Theor. appl. Genet. **92**: 92-100, 1996.

Klocke, M., Frei, U., Wenzel, G.: Genome analysis of flax (*Linum usitatisssimum* L.). 2000, http://www.weihenstephan.de/pbpz/mm/mk1/mk1.htm

Lapina, G.P.: [Electrophoretic banding patterns of the seeds in different varieties of flax.] - Fiziol. Biokh. kul't. Rast. **21**: 494-500, 1989. [In Russ.]

Lapina, G.P., Kelner, E.V.: [Electrophoretic characteristics of the seed proteins in flax.] - Fiziol. Biokh. kul't. Rast. **22**: 87-93, 1990. [In Russ.]

Lapina, G.P., Rulin, V.S.: [Electrophoretic study of protein fractions of fiber flax stems at different growth stages.] - Fiziol. Biokhim. kul't. Rast. **17**: 356-360, 1985. [In Russ.]

Manchenko, G.P.: Handbook of Detection of Enzymes on Electrophoretic Gels. - CRC Press, Boca Raton 1994.

Mareck, A., Gaffé, J., Morvan, O., Alexandre, C., Morvan, C.: Characterization of isoforms of pectin methylesterase of *Linum usitatissimum* using polyclonal antibodies. - Plant Cell Physiol. **36**: 409-417, 1995.

McDougall, G.J.: Changes in cell-wall associated peroxidases during the lignification of flax fibres. - Phytochemistry **31**: 3385-3389, 1992.

McDougall, G.J., Davidson, D., Millam, S.: Alterations in surface-associated peroxidases during callus development and shoot formation in explants of *Linum usitatissimum*. - J. Plant Physiol. **140**: 195-200, 1992.

McDougall, G.J., Millam, S., Davidson, D.: Alterations in surface-associated peroxidases during *in vitro* root development of explants of *Linum usitatissimum*. - Plant Cell Tissue Organ Cult. **32**: 101-107, 1993.

Oh, T.J., Gorman, M.B., Cullis, C.A.: RFLP and RAPD mapping in flax (*Linum usitatissimum*). - Theor. appl. Genet. **101**: 590-593, 2000.

Pavelek, M.: Further development of International Flax Data Base and special descriptors for more detail evaluation of agronomic and processing characters. - In: Proc. 3rd Meeting FAO Intern. Flax Breeding Res. Group - Breeding for Fiber and Oil Quality in Flax. Pp. 1-13. Centre Technique pour l'Étude et l'Amélioration du Lin, St. Valéry en Caux 1995.

Pošvec, Z., Griga, M.: Utilization of isozyme polymorphism for cultivar identification of 45 commercial peas (*Pisum sativum* L.). - Euphytica **113**: 251-258, 2000.

Pošvec, Z., Krulíčková, K.: Identification of flax (*Linum usitatissimum* L.) cultivars by isozyme markers. - In: Abstracts XXIX Annual ESNA Meeting. Pp. 155. Wye College, Univ. London, London 1999.

Rothe, G.M.: Electrophoresis of Enzymes. Laboratory Methods. - Springer-Verlag, Berlin 1994.

Samec, P., Pošvec, Z., Stejskal, J., Našinec, V., Griga, M.: Cultivar identification and relationships in *Pisum sativum* L. based on RAPD and isoenzymes. - Biol. Plant. **41**: 39-48, 1998.

Sammour, R.H.: Flax seed proteins comparison by various PAGE-techniques in slabs. - J. Agron. Crop Sci. **160**: 271-276, 1988.

Sammour, R.H.: Effect of aging on the electrophoretic pattern of the total seed proteins of flax. - Egypt. J. Bot. **32**: 145-148, 1989.

Schaumann, A., Bruyant-Vannier, M.P., Goubet, F., Morvan, C.: Pectic metabolism in suspension-cultured cells of flax, *Linum usitatissimum*. - Plant Cell Physiol. **34**: 891-897, 1993.

Spielmeyer, W., Green, A.G., Bittisnich, D., Mendham, N., Lagudah, E.S.: Identification of quantitative trait loci contributing to *Fusarium* wilt resistance on an AFLP linkage map of flax (*Linum usitatissimum*). - Theor. appl. Genet. **97**: 633-641, 1998.

Vallejos, C.E.: Enzyme activity staining. - In: Tanksley, S.D., Orton, T.J. (ed.): Isozymes in Plant Genetics and Breeding. Part A. Pp. 469-516. Elsevier, Amsterdam 1983.

Yurenkova, S.I., Khotyleva, L.V., Tsebrikov, Y.V.: Tissue-specific expression of esterase isoenzymes in *Linum usitatissimum* L. - Biol. Plant. **37**: 375-379, 1995.

Yurenkova, S.I., Khotyleva, L.V., Zhuchenko, A.A.: [Comparative study on isoenzyme spectra of fiber flax cultivars.] - Dokl. Akad. Nauk Belarusi **36**: 473-475, 1992. [In Russ.]