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VARIABILITY OF *Linum usitatissimum* L. BASED ON MOLECULAR MARKERS

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ABSTRACT

Linum usitatissimum, L. accessions were analyzed using RAPD, ISSR and IRAP markers. Different primers were used for the analysis yielding 211 loci of which 87 were polymorphic. The range of polymorphism information content across all genotypes in this study was from 0, 07 to 0, 42. A dendrogram was generated based on the similarity matrix by the Un weighted Pair Group Method with Arithmetic Mean (UPGMA), wherein the flax genotypes were grouped in six major clusters. Analysis has given no evidential grouping based on straight pedigrees relationships, but wider genetic backround of landraces with breeder cultivars can be seen. The most diverse genotypes of analyzed in their intergenic space was identified and suggested their use in breeding programs and mapping the flax genetic pool.

Keywords: *Linum usitatissimum* L., cultivated flax accessions, genetic diversity, RAPD, ISSR, IRAP, REMAP.

INTRODUCTION

Flax (*Linum usitatissimum subsp. usitatissimum*) as one of the founding crops in Near Eastern agriculture is a diploid, annual plant species, which is mainly considered to be inbreeding (Durrant, 1986). All of the nearly 200 species of the genus *Linum* grow in temperate and warm temperate zones of the northern hemisphere. *Linum* has been cultivated for thousands of years for its fibre and for its oil rich seeds. Already the Egyptians, about 2400 BC, knew how to treat the flax fibre to make linen cloth, used, for example, for wrapping of mummies (Zohary, Hopf, 1993). Since flax, and in particular fibre flax, has been such an important cultivated crop, it is of great importance to conserve as widely genetic material of flax as possible for future utilisation in breeding. The largest production of fibre flax occurs presently in China, Russia, Belarus and Ukraine, while oilseed production is important in India, Canada, China, United States, Germany, Argentina, United Kingdom and Spain (FAOSTAT data, 2006). In order to maintain and exploit these genetic resources efficiently, an understanding of the amount and distribution of genetic variation within and among accessions in a collection is required (Månsby *et al*., 2000).

Due to advances in the field of molecular genetics a variety of different techniques to analyse genetic variation has emerged during the last few decades (Parker *et al*., 1998; Argawal *et al*., 2008; Kalendar *et al*., 2006). Use of genetic markers based on PCR are nowadays useful in a wide range of different applications in plant, animal and food science for systems of genotypying, identification and authentification of samples (Boiniv, Danailov, 2009; Zeleňáková, 2009; Ansari-Mahyary, 2008; Prasad *et al*., 2002).

Retrotransposons and microsatellites are some of very interesting DNA sequences from the molecular genetic point of view. Both of them can be found as a part of intergene DNA and all the methods working with intergene DNA are seemed to be very suitable for searching wide genetics relationships expressing in the concrete genome. Molecular characterization of flax germplasm has been made using various molecular techniques to assess genetic diversity of cultivated flax and to examine evolutionary relationships of wild flax species (Campbell *et al*., 1995; Everaert *et al*., 2001; Fu 2005; Vromans, 2006; Cloutier *et al*., 2009; Fu and Allaby, 2010). Rajwade *et al*., (2010) summarized the flax diversity studies as follows. Diversity assessment in flax was earlier attempted using morphological parameters (Diederichsen, 2001) and isozyme markers (Mansby *et al*., 2000). The use of DNA-based markers to study flax diversity was first reported by Oh *et al*., (2000), who compared RAPD and RFLP techniques and generated a preliminary genomic map based on these marker data. Fu *et al*., (2002) used RAPD markers to study diversity in Canadian cultivars and landraces, and reported low genetic variability. Fu *et al*., (2003) also used RAPD markers to analyze the genetic variation, genetic erosion, and relationship in North American flax cultivars. Further, Diederichsen and Fu (2006) studied phenotypic and RAPD variation within and among the infraspecific groups of flax.

The ISSR technique for flax fingerprinting was optimized by Wiesner and Wiesnerova (2003, 2004) using re-amplification method and through statistical correlation of free energy of dissociation of ISSR primers. An ISSR primer assay is reported in the study of flax germplasm by Rajwade *et al*., (2010) and Uysal *et al*., (2010). Retrotransposons as cultivated flax markers are reported in the previous study of authors (Žiarovská *et al*., 2007, 2009). The study of Smýkal *et al*., (2011) has taken advantage of the ubiquity and abundance of LTR retrotransposons in plant genomes and their role in genomic diversification and is aimed to developing and applying the flax specific IRAP method to this species.

Since their introduction, Random Amplified Polymorphic DNA (RAPD) markers (Williams *et al*., 1990, 1993) have become very popular and have been used for a variety of purposes in plant genetics: cultivar identification, parentage determination, genetic

relationships evaluation, estimation of population genetic variability, identification of interspecific hybrids and estimation of clonality. ISSR markers have been widely applied to characterize plant germplasm and demonstrated its effectiveness in assessments of plant genetic diversity (Galván *et al*., 2003; Pharmawati *et al*., 2005; Rajwade *et al*., 2010; Uysal *et al*., 2010). Retrotransposons flanked by long-terminal repeats (LTR) are abundant in plants (Pearce *et al*., 1996, 1997) and propagate within the genome via RNA intermediates by the cycle of transcription, reverse transcription, and integration (Kumar and Bennetzen, 1999). A group of techniques based on inter or internal retrotransposon polymorphism approach is now used by individual teams to discover molecular diversity of many species (Manninen *et al*., 2000; Boyko *et al*., 2002). Kalendar *et al*., (2006) suggested retrotransposon based molecular markers as cheap to establish and assay, easy to perform, reproducible and inexpensive. The advantage of application of retrotransposon-based marker systems consists in the ability to track an insertion event and its subsequent vertical radiation through a pedigree or phylogeny. Some of the plant retrotransposons are structurally intact and transcriptionaly active (Vicien *et al*., 1999) and presented in many copies throughout plant genomes.

In this study, we aimed to assess genetic diversity of cultivated flax accessions using randomly amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) markers, inter retrotransposon amplified polymorphism (IRAP) and retrotransposon microsatellite amplified polymorphism (REMAP) and finding genetically similar groups of *Linum usitatisssimum*, L. in the intergene space in regard to non-european and european assecions.

Plant material and DNA isolation

The flax (*Linum ussitatissimum*, L.) accessions listed in Table-1 were obtained from AGRITEC, Research, Breeding and Services, Ltd., Czech Republic and from The Research Institute of Plant Production in Piešťany, Slovak Republic. DNA samples were extracted from 10 randomly chosen fourteen-day-old *in vitro* regenerated plants and bulked for DNA isolation. This approach was taken in order to capture heterigeneity of accession (Fu 2005; Kwon *et al*., 2010). Plant material was stored at - 20 °C until DNA isolation. Genomic DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega). DNA obtained from approximately 90 - 110 mg fresh weight material was eluted in 150 µl of the kit´s elution buffer at a concentration of about 40 - 90 ng/µl and stored at - 20°C till use. The genomic DNA isolated was quantified spectrophotometrically by measuring absorbance at 260 nm and stock DNA was dilluted to make a working solution of 10 ng/µl for PCR analysis.

Table-1. List of *Linum usitissimum* L. genotypes used in work.

No.	Name	Origin	Characteristics
1	Albidum	IND	Landrace
2	Albocoeruleum	-	
3	Deubgrc	-	
4	Svaloef	SWE	
5	Ilona	NLD	Cultivars
6	Flanders	CAN	
7	Red Wing	USA	
8	Rembrant	NLD	
9	Renodlat Oljelin	SWE	
10	Gisa	EG	
11	Hor Nr 048	DNK	
12	Krasnoder	SUN	
13	La Plata 1938	ARG	
14	Norfolk Proncess	GBR	
15	Otofte 15/47	DNK	
16	Horan	CZE	
17	Marina	NLD	
18	Escalina	NLD	

Primer screening and design

A set of 10 RAPD and 15 ISSR primers were initially screened for their repeatable amplification. RAPD and ISSR markers were selected for further analysis based on their ability to detect polymorphism between 50-100% and polymorphic amplified products across the accessions. To the random and single sequence repeats markers were added primers of two plant retrotransposons and one flax transposon as another important parts of plant intergene space. Primers for IRAP method were designed in the first case Tst1 retrotransposon (Camirand and Brisson, 1990). This primer is complementar to bases 1-28 and 4778-4800 (accession in NCBI database X52387). IRAP setting for Cassandra retrotransposon (Kalendar and Schulmann, unpublished), were designed to bases 1-28 and 597-615 (AY860314). The last evaluated length polymorphism was established for flax transposable element dLUTE and primers were designed to the nucleotide accessions AF036935.

Table-2. List of primers, number of amplified products, polymorphism percentage and polymorphism information content

	Technique	Sequence 5´- 3´	Total amplified polymorphics levels	Percent polymorphism	PIC values	Primer Index
RP1	RAPD	CCGGCCTTAG	8	71,4	0,42	3,37
RP2	RAPD	AGGAGTGAGA	7	83,3	0,30	3,27
RP3	RAPD	CCTCCCTCTT	9	76,5	0,25	3,06
IP1	ISSR	$(GT)_6CC$	27	77,8	0,18	6,25
IP2	ISSR	$(GAG)_3GC$	7	65	0,12	2,5
IP3	ISSR	$(GA)_6GG$	17	87,5	0,37	6,21
IP4	ISSR	$(CTG)_3GC$	8	91,6	0,07	1,65
IP5	ISSR	$(CA)_6GT$	15	88,9	0,29	5,25
TP1	IRAP	ATGACTAAATCTGCCTACTCATTCAACA	10	76,9	0,28	3,68
TP2	IRAP	ACGGCGGAGCCGATCCCGGGATGTGACA	10	68,7	0,22	3,52
TP2 + IP2	REMAP	ACGGCGGAGCCGATCCCGGGATGTGACA+ $(GAG)_3GC$	16	86,4	0,28	6,21
TPF3	LP*	GCCCTGTGCTGAAATCTGA	8	80	0,37	3,0
TPR3	LP*	CCGCCCAATAACCTGTGCTG				
		Mean	11,8	79,5	0,25	3,99

LP* - simple length polymorphism based on specific primer target annealig region detected by PCR

PCR amplification

RAPD and ISSR amplification of 50 ng DNA was performed in a total volume of 20 µl reaction volume contained 1× PCR buffer (10 mmol l^{-1} Tris HCl; pH 8, 3; 50 mmol l^{-1} KCl) 3 mmol l^{-1} $MgCl_2$ (Invitrogen) for RAPD and 3 mmol l^{-1} for ISSR; 0, 15 mmol l^{-1} each dNTP (Promega); 1U Taq polymerase (Invitrogen); 400 mmol l^{-1} primers. The following cycling program was applied: at 94 °C denaturation for 1 min. 35 cycles (RAPD) and 45 cycles (ISSR) of at 94°C for 1 min, at 36°C (RAPD) and 55°C (ISSR) annealing for 2 min. and 72°C for 1 min.; and a final elongation step at 72°C for 10 min. and holding at 4°C using the Biorad MJ Mini™ Gradient Thermal Cycle.

Amplification screening the length polymorphism between retrotransposons and dLUTE transposable element was performed in a total volume of 15 µl reaction volume contained 1× PCR buffer (20 mmol l^{-1} Tris HCl; pH 8,3; 50 mmol l^{-1} KCl) 1,5 mmol l^{-1} $MgCl_2$ (Invitrogen); 0,3 mmol l^{-1} each dNTP (Promega); 1U *Taq* polymerase (Invitrogen); 400 mmol l^{-1} primers. After initial denaturation at 94°C 1 min. each cycle comprised 15 s denaturation at 94°C, 40 s annealing at 54°C and 2 min. extension at 72°C at the end of 45 cycles using the same thermocycle with a total of 30 ng DNA.

PCR products were separated in 2% (w/v) agarose gels in 1 × TBE by electrophoresis at 100V for 3, 5 h. Gels were stained in ethidium bromid (RAPD and ISSR) or GelRed™ (IRAP) and digitally photographed. All the accessions were growth and sampled through the two seasons to ensure the stability of the markers and all the PCR amplifications were repeated at least twice to establish reproducibility of polymorphic fragments and scored independently by KODAK EDAS software and two individuals to verify possible scoring errors.

Statistical analysis

All the markers bands were treated as a single locus and across the accessions were scored for their presence or absence of the band. The individual fragment of a given length was recorded in binary code. By comparing the banding patterns of genotypes for a specific primer, genotype specific bands were identified and faint or unclear bands were not considered. The binary data generated this way were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. Data were evaluated using POPGENE software version 1.32 (Yeh and Boyle, 1997) for the Shannon index (H'j, Shannon and Weaver, 1949) as defined for multilocus markers as $H'j = -\sum p_i \log p_i$, where p_i is the frequency of the *i*th fragment in the sample.

Then the primer differentiation ability index (PDAI) for IRAP and REMAP was defined based on the relative value ranged from 0 (the best distinguishion) to 0.25 and on the ability to distinguish first quartile of lowest 25% of possible unique fragments for interpretation

of the effectivity of the primers to distinguish samples of whatever size. The maximum possible considerating unique (or unique absent) fragments were calculated as a quarter of all samples and the index was calculating on the base of Kalendar´s *et al*. similarity index (1999) as: PDAI = 0, 25 - (the lowest number of unique fragments up to the maximum possible + the lowest number of unique absent fragments up to the maximum possible) / total sum of fragments.

Polymorphic information content (PIC) was calculated for each marker using the formula PICi = 1 - $\sum P2_{ij}$, where P_{ij} is the frequency of the *j*th allele in clone (*i*) according to Smith *et al*., (1997). PIC values were used to calculate the Primer index (Rajwade *et al*., 2010) generated by summing up the PIC values of all the loci amplified by the same primer.

Pair-wise dissimilarity matrices were generated by 1- Jaccard´s coefficient of similarity (Jaccard, 1908) by using the SYNTAX softwer format. A dendrogram was constructed by using the unweigted pair group method with arithmetic average (UPGMA) with the HIERCLUS module of SYNTAX to show a phenetic representation of genetic relationships as revealed by the dissimilarity coefficient.

RESULTS AND DISCUSSIONS

Polymorphism level and discriminating capacity

Linum usitatisssimum accessions were analyzed using random, microsatellite, retrotransposon and transposon markers of which all produced reproducible polymorphic banding patterns. A total of 211 band levels were obtained of which 148 (70, 1 %) were polymorphics. All the polymorphics bands produced by the used set of primers were 2664. The number of polymorphic bands generated per primer varied from 7 to 27 and minimum of 7 bands were generated by the primers RP2 and IP2 while the maximum of 27 bands were observed with primer IP1. The size of the amplified products varied from 143 to 3333 bp. Non of the used marker profiles gives the 100% polymorphism, but the individually used primers gives the polymorphism in the range from 65 to 91, 6 % and the evaluation as a whole gives the 95, 27 % polymorphic bands. The level of polymorphism detected in this study by the intergenic space markers is comparable by other authors (Oh *et al*., 2000; van Treuren *et al*., 2001; Fu *et al*., 2002; Wiesner, Wiesnerova, 2003; Diederichsen, Fu 2006).

The genomic DNA has shown enough multiple polymorphic bands with the *copia*-like IRAP primer but less with Cassandra-like IRAP primer. One of the objectives of this study was to assess the usefulness of retrotransposon-based markers IRAP and REMAP relative to two different retrotransposons, both derived from another species as the flax is. REMAP analyse a larger number of bands per assay than IRAP, which has a significant effect on the cost per assay. REMAP revealed the highest number of polymorphic bands per assay, also. On the basis of the marker index, REMAP was the most informative system when compared to IRAP with both of tested retrotransposons. Highly heterogenous populations of Ty 1-*copia* group retrotransposons are found in many higher plant genomes and Ty1-*copia*-like retrotransposons were extensively used as molecular markers plant species (Pearce *et al*., 1997; Kalendar *et al*., 1999; Yu and Wise, 2000), but LTR sequence of retrotransposons from flax as molecular markers are only at beginning, especially of those derived from flax genome itself (Smýkal, 2011).

Using the PIC value as a measure of variability at a specific locus gives the information about the probability that polymorphism will exist between two randomly selected genotypes at that locus. The mean polymorphic information content calculated from the frequency of polymorphics bands across all genotypes in this study was 0, 25 (Table-2). The primer IP4 and IP2 reveals the lowest PIC value of 0, 07 and 0, 12. The highest value 0, 42 was obtained by the RP1 primer. The law average PIC value obtained in this study underscores the nature of the used genotypes, because as the landraces and cultivars of the same species they are genetically similar what is showed when Jaccard´s coefficient range of 0, 432 - 0, 649 is considered. Polymorphisms between individuals mainly result from sequence differences in one or both of the primer binding sites and are visible as the presence or absence of a particular amplification product. These polymorphisms behave, therefore, as dominant genetic markers (Sperisen and Bücher, 1998). As the PIC provides a measure that is influenced by both the number and frequency of alleles, the maximum PIC value for markers for markers where two alleles per locus are assumed is 0,5 (Henry, 1997; Li, Nelson, 2001). The summary analysis of the intergenic length polymorphism variability was done for markers from the both sides of PIC value spectrum with the aim of whole range analysis, not only for a narrow range of low or high polymorphism. The plant intergenic space reflects the both wild and domestic evolution effects (Datwyler, Wolfe, 2004; Ammiraju *et al*., 2008; Brinegar, 2009) and the approach of "wide PIC values" markers can provide a fine and detailed differences among the genotypes. The primer index as an indicator of the efficiency of marker system (Rajwade *et al*., 2010) ranged from 2, 5 to 6, 5 with an average of 3, 99. The ISSR primers IP1 and IP3, having the highest primer index values of 6, 25 and 6, 21, respectively, were found to be the best primers from the used range for detecting polymorphism in flax.

Linum usitatissimum L. genotyping by itergenic space markers

The 12 intergenic primer pairs applied in this study generated a total of 3798 fragments across the 18 plant samples, of which 2664 bands were polymorphics. A genetic distance matrix was generated based on these polymorphic loci. The range of PIC scores obtained in this study was 0, 07- 0, 42, with an average of 0, 25. The primer index revealing the information content of the primer per assay ranged from 1, 65 - 6, 25 (Table-2). The Shannon index varies both among the technigues and

individually primers with the range from 0,214 to 0,689 what corresponds to the reached polymorphism level.

The Nei similarity coefficient (1978) for all the used marker systems ranged through the whole possible spectrum with very little differences for individual analyzed techniques. For RAPD markers the range was 0, 36 - 1; for ISSR 0 - 1 and for IRAP and REMAP 0, 32 - 1.

Absolute certainty that two samples are genetically identical can only be obtained by comparison of their complete genomes. However, samples do not necessarily have to be completely identical in order to be considered redundant. For example, two samples collected from the same out crossing population will have a very small probability to be completely identical, yet they will share a similar genetic background. Therefore, we can consider redundancy by quantifying the genetic diversity between samples based on the screening of germplasm for a large number of polymorphic markers. Statistical tools may then be used to evaluate whether samples display sufficient genetic variation in order to consider them distinct (van Treuren *et al*., 2001).

Of the ten primers we tested to evaluate the polymorphism the analyzed genotypes of flax five was appropriate. Mattioni *et al*., (2002) states that not all random primers are suitable for RAPD analysis, while of the 21 primers used products were rate only with the six primers.

All primers used for RAPD analysis were decamers differing in CG content Primers RP1 and RP3 contain 80% GC, primer PR2 70% GC. RAPD primers provided 1558 fragments with an average of 8.65 fragment of genotype. The fragments size ranged from 271 to 1821 bp which means the range allowed by PCR.

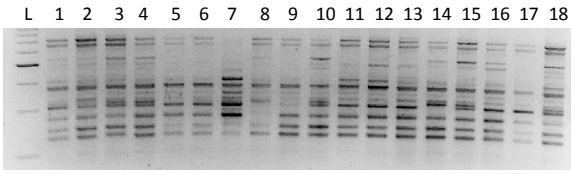

Figure-2. RAPD profiles of the primer RP2 (Samples 1-18 as in Table-1; L - DNA size Marker).

ISSR primers provided a total of 1854 fragments dividing into 16 to 36 levels. On average, 50 amplified fragments per genotype is equivalent to findings of Godwin *et al*., (1997) and Zientkiewicz *et al*., (1994) that reports multiplication of 10 to 60 fragments per genotype during the ISSR. The size of fragments ranged from 210.5 to 1750 bp. Compared to the result of RAPD analysis, there is a shift in the clustering of genotypes in which the relationships of cultivars found in clusters with old landraces can be found. ISSR assays use simple sequence repeat region anchored primers that bind to abundant microsatellite loci of the genome and therefore are useful in detecting genetic polymorphism in large portion of the genome simultaneously (Rajwade, 2010).

Table-3. Jaccard´s distance matrix of 18 *Linum usitatissimum* L. accessions.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
1	.000																	
2	.442	.000																
3	.516	.592	.000															
4	.473	.480	.538	.000														
5	.500	.551	.670	.554	.000													
6	.471	.511	.573	.477	.524	.000												
7	.522	.510	.506	.559	.649	.457	.000											
8	.556	.515	.571	.560	.558	.564	.563	.000										
9	.382	.396	.484	.520	.500	.437	.456	.432	.000									
10	.483	.564	.625	.472	.582	.573	.617	.627	.516	.000								
11	.549	.567	.550	.588	.570	.520	.575	.575	.512	.619	.000							
12	.640	.677	.593	.628	.646	.637	.667	.629	.570	.674	.658	.000						
13	.429	.515	.495	.468	.558	.500	.484	.549	.432	.391	.558	.644	.000					
14	.516	.535	.370	.505	.670	.573	.539	.557	.433	.581	.619	.575	.495	.000				
15	.534	.552	.600	.571	.602	.560	.541	.646	.533	.600	.514	.597	.527	.630	.000			
16	.573	.469	.574	.484	.576	.500	.549	.566	.479	.619	.525	.585	.536	.574	.529	.000		
17	.467	.442	.564	.505	.565	.471	.506	.510	.364	.594	.632	.573	.541	.500	.582	.478	.000	
18	.478	.529	.527	.516	.604	.500	.564	.535	.510	.543	.576	.632	.550	.573	.591	.521	.542	.000

A dendrogram based on the UPGMA algorithm using distance coefficient (Table-3) distributed the accession in six major clusters at various distance coefficient. The genotypes Ilona and Rembrand were the most distinct from other and did not fall in any cluster (Figure-3).

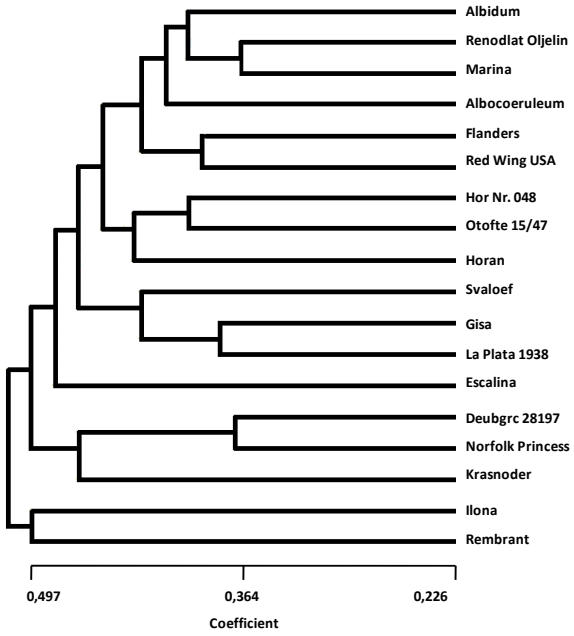

Figure-3. Dendrogram of 18 *Linum usitatissimum* L. accessions based on Jaccard´s similarity coefficient.

IRAP analysis give us no evidential grouping based on straight pedigrees relationships, but the wider genetic backround of old land cultivars with modern ones can be seen, too. In both results for used primers, the old land cultivars Albidum, Albocoeruleum and Deubgrc was grouped with moder cultivars. None of these populations has common characteristics of industrial type or pedigree, but the ties caused by evolutionary stress or old climatic and local effect causes retransposition is refered by retrotransposons (Kalendar *et al*., 2000).

The ability of the flax genome to adapt to environment conditions is confirmed in literature but not for retrotransposon markers. Based on flax genotroph RAPD polymorphism (Cullis *et al*., 1991, 2005) the occurrence of environment induced hereditary changes accompanied by changes of genomic DNA has been confirmed in several genotypes. In contrary the study of Diederichsen and Fu (2006) showed that 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).

As separate clusters were grouped non-European genotypes Flanders, Red Wing, Gisa and Escalina and nested into the European genotypes. The grouping pattern corresponds to the no differentiating between linseed or fiber flax and also no geographical distribution.

Comparing the results of the used techniques, as listed in Table-4, can be seen a high level of polymorphism for all the used markers. Average similarity index calculated for the IRAP was 0.67 (ranged from the 0.4 - 1.00) what is displayed in UPGMA dendrogram, too. The clustrers are joined together in the range of genetic distance coefficients results. There were five groups of the populations indentical withing all the used markers (data not shown). Using the primer Cassandra primers, the IRAP profile was more straightened in the comparison with Tst1 primers. The Nei and Li similarity indexes has ranged from 0.48 to 1.00 wiht the average of 0.81. As populations with the highest values of genetic distance coefficient were recognized the group of american, russian and india populations with some of european provenience population.

Table-4. PCR profile characteristics of the used primers in different techniques.

Primer (primer combination)	**Total fragment levels**	**Percentage polymorphic fragments**	**Polymorphic fragment levels**	**Maximum possible unique fragments per technique**	**PDAI**[a]
P-Tst1-01	13	76,9	10	9	0,176
P-Frodo2-02	16	68,7	11	9	0,215
P-Flax-2	20	65	13	9	0,211
P-Frodo2-02 + P-Flax-2	22	86,4	19	9	0,204

[a] PDAI = 0,25 - (the lowest number of unique fragments up to the maximum possible + the lowest number of unique absent fragments up to the maximum possible) / total sum of fragments

Variability of genetic coefficient values and clusters corresponds to the analyzed intergenic space of old landraces which are mixed among the groups of cultivars and may be the mirror of both environmental and wider pedigree relationships. It corresponds to the previous flax studies where in contradiction to the lack of

diversity indicated by marker studies, the flax genome shows environmentally induced heritable genomic changes (Schneeberger, Cullis, 1991; Cullis, 2005; Chen *et al*., 2005, 2009). Because of this phenomenon we analyzed samples from two seasons growing to ensure the stability of the markers and all the RAPD, ISSR and IRAP fingerprints remained stable (data not shown). The high intergenic space diversity and no grouping patterns when retrotransposon are used as markers is reported by Smýkal *et al*., (2011) as notable because the retrotransposon markers were developed from cultivated accessions.

The advantage of application of retrotransposon-based marker systems consists in the ability to track an insertion event and its subsequent vertical radiation through a pedigree or phylogeny (Shimamura, 1997). Some of the plant retrotransposons are structurally intact and transcriptionaly active (Peterson-Burch *et al*., 2000; Vicien *et al*., 2001 a; 2001 b; Ghany, Zaki, 2002) and presented in many copies throughout plant genomes.

The relative low diversity given by RAPD, ISSR, AFLP and SSR markers in the flax germplasm evaluation (van Treuren *et al*., 2001; Fu 2005; Cloutier *et al*., 2009; Uysal *et al*., 2010) is narrow as a consequence of plant breeding (Tanksley, McCouch; 1997) and Vromans (2006) concluded that further decreases in the genetic variation of especially fiber flax will result if only modern cultivars are used in future breeding. Despite of no clear grouping patterns in the *Linum usitatissimum* L. markers of the intergenic space are able to devide this narrow germplasm and provide an information about the groups of genotypes, where the common molecular marker patterns exists and can be used for specific breeding requirements. Such groupings allow to search for groups with a genetic the same basis, as most ancestral genotypes stored in gene banks is not known. Grouping of european and non-european genotypes in clusters may reflect not only the wider background of the whole process of breeding flax, but also links between the breeding programs of individual countries and the availability of certain genotypes for these programs. The knowledge about diversity and genetic relationships among the primary germplasm and breeding material gives insight into crop improvement strategies and using of molecular genetic distance to select diverse parents is very useful for considerable breeding results.

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