Agriculture (Poľnohospodárstvo), 59, 2013 (3): 101−110
DOI: 10.2478/agri-2013-0009

GENETIC DIVERSITY IN DOMESTIC AND INTRODUCED WHEATS

MARTINA HUDCOVICOVÁ[1*], KATARÍNA ONDREIČKOVÁ[1], PAVOL HAUPTVOGEL[1], JÁN KRAIC[1,2]

[1]Plant Production Research Center Piešťany
[2]University of SS. Cyril and Methodius in Trnava

HUDCOVICOVÁ, M. – ONDREIČKOVÁ, K. – HAUPTVOGEL, P. – KRAIC, J.: Genetic diversity in domestic and introduced wheats. Agriculture (Poľnohospodárstvo), vol. *59*, 2013, no. 3, pp. 101–110.

A set of 33 wheat EST–SSR markers was designed and 18 from them were polymorphic and used for assessment of genetic diversity within 36 introduced genotypes of hexaploid bread wheat. Altogether 105 alleles were detected, in average 3.18 alleles per locus. Maximum number of alleles 14 was detected at the locus TDI389708. Five the most polymorphic markers were used for the evaluation and comparison of genetic variation within 46 domestic (Slovak) wheat genotypes and 36 introduced (foreign) wheat genotypes. The number of alleles per used primer pair within domestic genotypes varied from 7 to 19, with an average of 13.2 alleles, an average gene diversity 0.846 and PIC 0.980 per locus. The number of alleles per primer within introduced genotypes varied from 7 to 14, with an average of 10.8 alleles, an average gene diversity 0.780 and PIC 0.958 per locus. The level of polymorphism in EST–SSRs was sufficient for discrimination between genotypes and variation within domestic genotypes was slightly higher than in introduced genotypes. Variation revealed by 5 selected EST–SSR markers clustered genotypes according to origin. Domestic and introduced wheats were grouped distinctly into two separate groups.

Key words: genetic diversity, EST-SSR, microsatellites, polymorphism, bread wheat

The genetic diversity is one of the most important factors for survival and adaptability of all species. Commonly, it is evaluated by pedigree studies, phenotype traits, biochemical and genetic characteristics, and molecular markers. Especially DNA markers are widely used because they are not affected by environmental conditions and they are distributed over whole genome. Microsatellites termed also as simple sequence repeats (SSRs) have been proposed as one of the most valuable molecular markers for assessment of genetic variation. SSRs possess high information content, codominance, locus specificity, simple analysis, multiallelism, and distribution along all chromosomes (Gadaleta *et al*. 2009; Song *et al*. 2012). Large number of SSRs reside in transcribed regions of genomes including protein-coding genes and expressed sequence tags (ESTs), although in general, repeat numbers and total lengths of SSRs in these regions are relatively small (Kantety *et al*. 2002). Expressed Sequence Tags – Simple Sequence Repeats (EST–SSRs), in comparison with microsatellites located in non-coding regions (SSRs), reveal usually only half level of polymorphism but they have much higher information content, provide better description of genetic diversity, higher levels of transferability among related species (Gupta *et al*. 2003), and have high ability for genotypic identification (Song *et al*. 2012). EST–SSR markers have the potential to become markers revealing functional diversity and for this reason the number of subsequent

Mgr. Martina Hudcovicová, PhD. (*Corresponding author), Mgr. Katarína Ondreičková, PhD., Ing. Pavol Hauptvogel, PhD., doc. RNDr. Ján Kraic, PhD., Plant Production Research Center Piešťany, 921 68 Piešťany, Bratislavská 122, Slovak Republic.
E-mail: hudcovicova@vurv.sk
doc. RNDr. Ján Kraic, PhD., University of SS. Cyril and Methodius in Trnava, 917 01 Trnava, Nám. J. Herdu 2, Slovak Republic

analyses should be reduced. Microsatellites located within genes are critical elements for normal gene function, regulation and modulation of gene expression due to their extension or reduction in coding regions directly affecting phenotype manifestation (Li *et al*. 2004). EST–SSR markers have several advantages in comparison to SSRs located within the non-coding regions. They detect variation in coding sequences and represent „perfect markers" indicating status of genes, creation of EST-database is less expensive than genomic SSR-database and can be used for determination of more distant genetic relationships whereas the degree of variation in the coding regions is lower (Gupta *et al*. 2003; Yu *et al*. 2004).

Hexaploid bread wheat (*Triticum aestivum* L.) as one of the world's most important crops shows very low level of intraspecific polymorphism and SSRs have been used as one of the most suitable markers also for the assessment of genetic diversity among bread wheat cultivars and lines (Akkaya & Buyukunal-Bal 2004; Gregáňová *et al*. 2005; Gregáňová *et al*. 2008; Huang *et al*. 2002; Prasad *et al*. 2000). Many ESTs for bread wheat are already available in the public domain (e.g. http://www.ncbEST_summary.html) usable for development of EST–SSR markers and evaluation of genetic diversity (Gupta *et al*. 2003) as well as for identification of bread wheat cultivars (Yang *et al*. 2005; Fujita *et al*. 2009). EST–SSRs were successfully used also for evaluation of genetic diversity in durum wheats (Eujayl *et al*. 2001; Wang *et al*. 2007), hard red spring wheats (Fu *et al*. 2006), tetraploid and diploid wheats (Gadaleta *et al*. 2011), and Persian wheats (Zhuang *et al*. 2011).

Long-time process of breeding and selection decreased genetic variation in modern wheat cultivars due to loss of alleles and relevant traits including resistance against different biotic and abiotic factors (Fu *et al*. 2006; Zhuang *et al*. 2011). Breeding activities in specific climatic conditions practised with limited variation of parental genotypes could lead to reduction of genetic diversity in new-created and cultivated crops. This is very serious reason to evaluate genetic diversity within wheat collections, crucial for effective conservation of the gene pool, and following exploitation of genetic resources.

Therefore the aim of this study was to: i) analyse genetic variation within coding regions in domestic (original Slovak) and introduced (foreign) bread wheat genotypes using the EST–SSR markers, ii) compare content of genetic variation between both subsets of wheats.

MATERIAL AND METHODS

Plant material and DNA isolation

Forty-six domestic (Slovak) cultivars, breeding lines, landraces and 36 introduced (foreign) hexaploid wheat (*Triticum aestivum* L.) genotypes (Table 1) were used for study of genetic diversity. All were obtained from the Genebank of the Slovak Republic (Plant Production Research Center Piešťany). The young fresh leaves were ground to a fine powder using liquid nitrogen, homogenized, and total plant DNA was extracted using the DNeasy Plant Maxi Kit (Qiagen, Germany). Sample of each genotype represented bulk DNA collected from 10–15 individual plants. Concentration and purity of isolated DNA were pre-measured by Nanodrop 1000 Spectrophotometer (Thermo Fischer Scientific Inc.) and samples were diluted to the same final concentration 25 ng/µl.

EST-SSR analysis

Microsatellite sequences of wheat containing di-, tri-, and tetra-nucleotide motifs located in coding regions (Table 2) were obtained from the DNA sequence database GenBank (www.ncbi.nlm.nih.gov). The Primer3 software (http://frodo.wi.mit.edu, Rozen and Skaletsky 2000) was used to design 33 flanking primers according to the following criteria: primer length 18–27 bp with optimum 20 bp, annealing temperature 57–63°C with optimum 60°C, GC content 20–80%, PCR product size 100–200 bp, dimers should be avoided as much as possible. PCR amplification was carried out in 20 µl reaction mixture containing 1 × PCR buffer (Invitrogen™), 1.5 mM $MgCl_2$ (Invitrogen™), 0.2 mM of each forward and reverse primer, 0.2 mM dNTP (Invitrogen™), 0.8 U of *Taq* DNA polymerase (Invitrogen™), and 1 µl template DNA. Amplifications were run in the GeneAmp® PCR System 9700 (Applied Biosystems®) with the following conditions:

T a b l e 1

List of analyzed wheat genotypes

Label	Genotype	Origin
S1	Diosecká 85-6	SVK
S2	Diosecká NR	SVK
S3	Diosecká 1013	SVK
S4	Balada	SVK
S5	Radošinská	SVK
S6	Lontovská	SVK
S7	Bučianska	SVK
S8	Slovenská skorá	SVK
S9	Šamorínska	SVK
S10	Rada	SVK
S11	Blava	SVK
S12	Butin	SVK
S13	Danubia	SVK
S14	Ilona	SVK
S15	Košútka	SVK
S16	Viginta	SVK
S17	Regia	SVK
S18	Solida	SVK
S19	Livia	SVK
S20	Torysa	SVK
S21	Sana	SVK
S22	Agra	SVK
S23	Roxana	SVK
S24	Iris	SVK
S25	Istra	SVK
S26	Solaris	SVK
S27	Barbara	SVK
S28	Kondor (SO-8527)	SVK
S29	Bučianska červenoklasá	SVK
S30	Bučianska V.T.16	SVK
S31	Bučianska 16/438	SVK
S32	Bučianska 106	SVK
S33	Bučianska 202	SVK
S34	Bučianska 316	SVK
S35	Bučianska 316/515	SVK
S36	Čalovská	SVK
S37	Košútská	SVK
S38	Nový Život	SVK
S39	Radošinská Dorada	SVK
S40	Radošinská Karola	SVK
S41	Radošinska Norma	SVK
S42	Radošinská poloraná 562	SVK
S43	Radošinská raná 594	SVK
S44	Slovenská B	SVK
S45	Slovenská intenzívna	SVK
S46	Slovenská 2	SVK
F1	Amazok	FRA
F2	AM 51/59	USA
F3	Arbola	SWI
F4	Boka	CZE
F5	Boundary	USA
F6	Certo	DEU
F7	Clever	GBR
F8	Cortez	DEU
F9	Dekan	AUT
F10	Elpa	DEU
F11	Gene	USA
F12	Charger	GBR
F13	Kraljevica	SRB
F14	Lu Mai	CHI
F15	Mironovskaja 30	UKR
F16	Mobewa	POL
F17	Molera	SWI
F18	MV Koma	HUN
F19	Ouragan	FRA
F20	Ovest	ITA
F21	Perlina Lesostepi	UKR
F22	Pizol	SWI
F23	Semper	NLD
F24	Soraja	POL
F25	Sulamit	CZE
F26	Sultan 95	USA
F27	Svitava	CZE
F28	Titlis	SWI
F29	Toronit	SWI
F30	Tudest	ITA
F31	Zaječarská 75	SRB
F32	Zlatica	YUG
F33	4321.124.05	NZL
F34	4632.44	NZL
F35	4928.14	NZL
F36	5021.16	NZL

T a b l e 2

EST–SSRs and their characteristics

GenBank ID	Gene product	Repetition	Primer sequence [5'-3']	Ta [°C]	size [bp]
TDI389708	γ gliadin	(AAC)29	CAGTGTGCAGCCATCCATAG ATAGTGGCAGCAGGATACGC	58	163
CV782464	anonymous cDNA	(AG)20	GGCGTATTTAAGGTGCGTTC CATCTTGTCGGTCTGAGCTG	58	179
CV782422	anonymous cDNA	(GCCA)6	ACGCGTCCGCTCTCTTTC CCAAGAACCGCAAAATCACT	60	128
AY857761	peroxidase 7	(CG)5	AGCAGAGCAGAATCCGGTAG GCGTAGTACCCGACCCTGAG	62	185
CV782549	anonymous cDNA	(CTT)6	TTAGGCCTATTTAGGTGATCCA CTGACAAGTCAGCGGAATCA	58	153
CV782428	anonymous cDNA	(CCAT)5	GGCCTGAGGGCTATTTAGGT TCATCGCGAGGAACGAC	60	200
CV782531	anonymous cDNA	(TGG)5	AAGTTTGCTGCGGTACCATC CTTTGCCCGGACCTTATCTT	62	124
AY299458	glutenin precursor	(ACA)6	CGCAGCAACCACTATTTTCA ACCTTGCATGGGTTTAGCTG	58	196
AY170867	protein Q	(CCG)4	TTACGCTGCAGCATCATCAG CAACAAGCGTTACCGACGAC	60	192
WHTGLNA	α/β gliadin	(CAA)7	TGTCAACAGCTGTGGCAGAT GTGGGTTTTGCTGAGATGGT	60	184
TSP238590	E2F protein	(CCG)5	AGCCCACCCACCTACCTC CCAGACATAAGCCCGATCTC	62	162
CV782560 I.	anonymous cDNA	(CT)8 N3 (TG)5	TGCTTGTCCTCCCATCTCTT GTGAAATCATGGCCATCTCC	58	137
CV781665	anonymous cDNA	(TG)10	GCAGTGCTGATCATATGTGGA AGATGCAGCAGCTGGCTAGT	56	179
TSP130948 I.	α gliadin	(AAC)5	GGCCATCCACAAAGTTGTTC CCTGTGGGTTTTGCTGAGAT	60	149
CV782529	anonymous cDNA	(CTC)6	GTCCGCCGAGAAGAAGAAG CTAAATAGCGCAGGGAGGTG	60	146
AF519168	Trn T, Trn L, Trn F proteins	(TAT)4	CCCTCTATCCCCAAATCCTC TCTTCGCATTCCTTTGTGAA	62	167
AY162186	extracellular invertase	(GGAT)4	GAGCAGGGCTCAAGCCTATT TGCTGTTCCGAGAAACAATG	62	172
TUR512491	sulphate transporter	(CCA)6	ACCGTCACACAGACAGCAAC GACCTCGGTTGGTCCTTGTA	58	160
TSP130948 II.	α gliadin	(CAG)7 (CAA)13	CACAACCGCAACCACAGTAT TTCCATGCGCTATGTTGTGT	60	183
CV782580	anonymous cDNA	(CGC)5	ATATCCGCACCAGGAATCAA TATTCGCAGAGGGTCTGGAG	58	183
CV782462	anonymous cDNA	(GA)16	TCCAACCTCCACTGAGTGCT TGGAGGAGACAACACAGCAG	62	148
AY650054	ferritin	(CCG)5	GAGATGTTGCCTAGGGTTGC GAGCACCTCCTTCCCCTTC	62	174
AY575717	vacuolar invertase	(GGC)4	AAATCGTCGTCGATGGAGTC CACGACCAGCACCACCAC	60	177
AY748826	LMW glutenin	(ACA)4	TCCTCGTCTTTGCCCTTCTA GGTGGTTGTTGAGGAAATGG	56	175
AY702957	protein Q	(CCG)4 N14 (CCG)4	TTACGCTGCAGCATCATCAG CAACAAGCGTTACCGACGAC	60	192
CV782526	anonymous cDNA	(TAGC)4	CCGGCTTAACCACACTCATC CACAGCCTTGCTGTTGAGAC	58	105
AJ622894	dynamin related protein	(AAG)6	CCTGGGAAGACCATGAAAAA TTCTGGTTGATTTCCTGATCG	62	178
WHTGLNA	α/β gliadin	(ACA)11 N1 (CAA)7	CACAGTATCCGCAACCACAA ATCTTGCATGCGCTATGTTG	56	180
CV782560 II.	anonymous cDNA	(GGC)7	GAGGGAGAAAGGGATGGAAG TTTGTGGAACGTCTGGATCA	62	125
TSP010830	GRAB 2 protein	(GCA)4	CTCATGGACTCTCCGTCTGG CCATAGCCTGGTAGGGTGAG	62	199
TSP010829	GRAB 1 protein	(GC)5	TCGACGGAGAAGAAGAAGTGA CGAACCGGTAGAGGTCGAG	60	200
TSP010829	GRAB 1 protein	(AGG)4	ACCAGTGGGAGAAGATGCAG ACCTCCTCCTTGGGCAGTAT	60	190
AY625682	transcription factor	(CTC)4	GCAGCATTTTTATGCAGTAGC AGGTGGGAACGGAATCAATA	60	122

initial denaturation at 94°C for 4 min, 35 cycles at 94°C for 1 min, annealing temperature (Table 2) for 1 min, 72°C for 1 min, and final extension at 72°C for 10 min. Five microliters of the reaction mixture were loaded into 6% denatured polyacrylamide gels and microsatellites were stained by silver staining method (Bassam *et al*. 1991).

Data analysis

Polymorphic DNA fragments amplified with each EST–SSR primer pair were considered as different alleles and scored as present (1) or absent (0). Based on frequencies of alleles the index of diversity (DI) $1 - \sum P_{ij}^2$ (P_{ij} = frequency of the j^{th} allele of the i^{th} primer), the probability of identity (PI) $\sum p^4_i + \sum\sum(2p_ip_j)^2$, and the polymorphic information context (PIC) $1 - (\sum p^2_i) - \sum\sum(2p^2_ip^2_j)$ were calculated (Paetkau *et al*. 1995; Weber 1990; Weir 1990). The un-weighted pair group method of cluster analysis using arithmetic means (UPGMA) was used for grouping of genotypes. Dendrograms were constructed using the Jaccard's similarity coefficients by the statistic software package SPSS 8.0 (SPSS Inc., USA). Principal component analysis (PCA) was done using the statistical software Statgraphics Centurion XV.II.

RESULTS AND DISCUSSION

Analyses of 36 introduced wheat genotypes by 33 EST–SSRs

Thirty-three EST–SSR primer pairs were tested within group of 36 introduced wheat genotypes and 18 of them revealed polymorphism. Altogeth-

T a b l e 3

Polymorphism characteristics of EST-SSRs of analyses 36 introduced and 46 domestic wheat genotypes

GenBank ID	Introduced genotypes				Domestic (Slovak) genotypes			
	Number of alleles	DI	PI	PIC	Number of alleles	DI	PI	PIC
TDI389708	14	0.831	0.013	0.987	12	0.875	0.033	0.967
CV782464	12	0.839	0.012	0.988	19	0.911	0.009	0.991
CV782422	12	0.781	0.038	0.962	16	0.900	0.014	0.986
AY857761	7	0.682	0.124	0.876	12	0.855	0.017	0.983
CV782549	9	0.769	0.023	0.977	7	0.687	0.030	0.970
Average	10.8	0.780	0.042	0.958	13.2	0.846	0.020	0.980
CV782428	6	0.410	0.371	0.388	–	–	–	–
CV782531	4	0.535	0.333	0.446	–	–	–	–
AY299458	3	0.273	0.576	0.239	–	–	–	–
AY170867	3	0.275	0.534	0.262	–	–	–	–
WHTGLNA	3	0.500	0.382	0.390	–	–	–	–
TSP238590	3	0.488	0.400	0.389	–	–	–	–
CV782560 I.	2	0.392	0.414	0.349	–	–	–	–
CV781665	2	0.162	0.715	0.149	–	–	–	–
TSP130948	2	0.162	0.715	0.149	–	–	–	–
CV782529	2	0.043	0.917	0.042	–	–	–	–
AF519168	2	0.162	0.715	0.149	–	–	–	–
AY162186	2	0.461	0.365	0.393	–	–	–	–
TUR512491	2	0.263	0.577	0.229	–	–	–	–
Average	3.18*	0.446	0.401	0.465	–	–	–	–

*average number of alleles per locus was calculated including 15 monomorphic loci

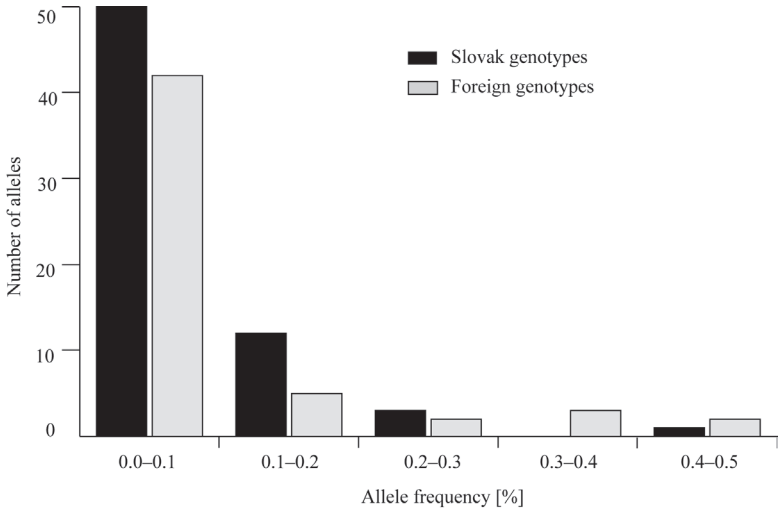

Figure 1. The frequency distribution of analysed EST–SSR alleles

er 105 alleles (including null alleles) were detected, with an average of 3.18 alleles per locus. Maximum number of alleles (14) was detected at the locus TDI389708. Diversity index varied from 0.043 for locus CV782529 (with PIC 0.042) to 0.839 for locus CV782464 (PIC = 0.988), with an average DI 0.446 and PIC = 0.465 per locus (Table 3). These results are comparable to others who revealed wheat genetic diversity by EST–SSRs (Eujayl *et al*. 2001; Fu *et al*. 2006; Fujita *et al*. 2009; Wang *et al*. 2007; Zhuang *et al*. 2011). Gregáňová *at el*. (2005) evaluated genetic diversity of 44 bread wheat genotypes of the Slovak and Czech origin by 15 genomic SSRs. An average number of alleles was 6.33 per locus and average diversity index was 0.68 per locus. Lower EST–SSR diversity within group of introduced wheat genotypes was found in our study. Lower numbers of alleles (1–5 per locus) and lower values of gene diversity (0.105–0.780 per locus) at the EST–SSR loci detected Gupta *et al*. (2003). Results of their analyses within 52 bread wheat genotypes showed lower polymorphism detected by EST–SSRs than by SSRs, which was found also by Eujayl *et al*. (2001), Fu *et al*. (2006), Gadaleta *et al*. (2009, 2011).

Comparison of EST–SSR polymorphism in domestic (Slovak) and introduced (foreign) wheats

Five the most polymorphic loci (TDI389708, CV782422, CV782464, CV782549, and AY857761) were used for the evaluation and comparison of genetic variation of 46 domestic wheats and 36 introduced genotypes. Numbers of alleles per each primer pair were relatively high (Table 3), overall 66 alleles for domestic and 54 for introduced genotypes (including the null alleles) were detected. Null alleles were detected in all loci besides the locus CV 782549 within domestic genotypes and besides loci AY857761 and TDI389708 within introduced genotypes. According to Cordeiro *et al*. (2001) high frequency of null alleles in the EST–SSRs may be due to deletion or substitution at the 5′-end of the primer binding site (Gadaleta *et al*. 2009).

The number of alleles per locus within the domestic genotypes varied from 7 (locus CV782549 with gene diversity 0.687 and PIC = 0.970) to 19 (locus CV782464 with gene diversity 0.911 and PIC = 0.991) with average of 13.2 alleles, average gene diversity 0.846, and PIC = 0.980 per locus. The number of alleles per primer within introduced genotypes varied from 7 (locus AY857761 with gene diversity 0.682 and PIC = 0.876) to 14 (locus TDI389708 with gene diversity 0.831 and PIC = 0.987), with an average of 10.8 alleles, average gene diversity 0.780,

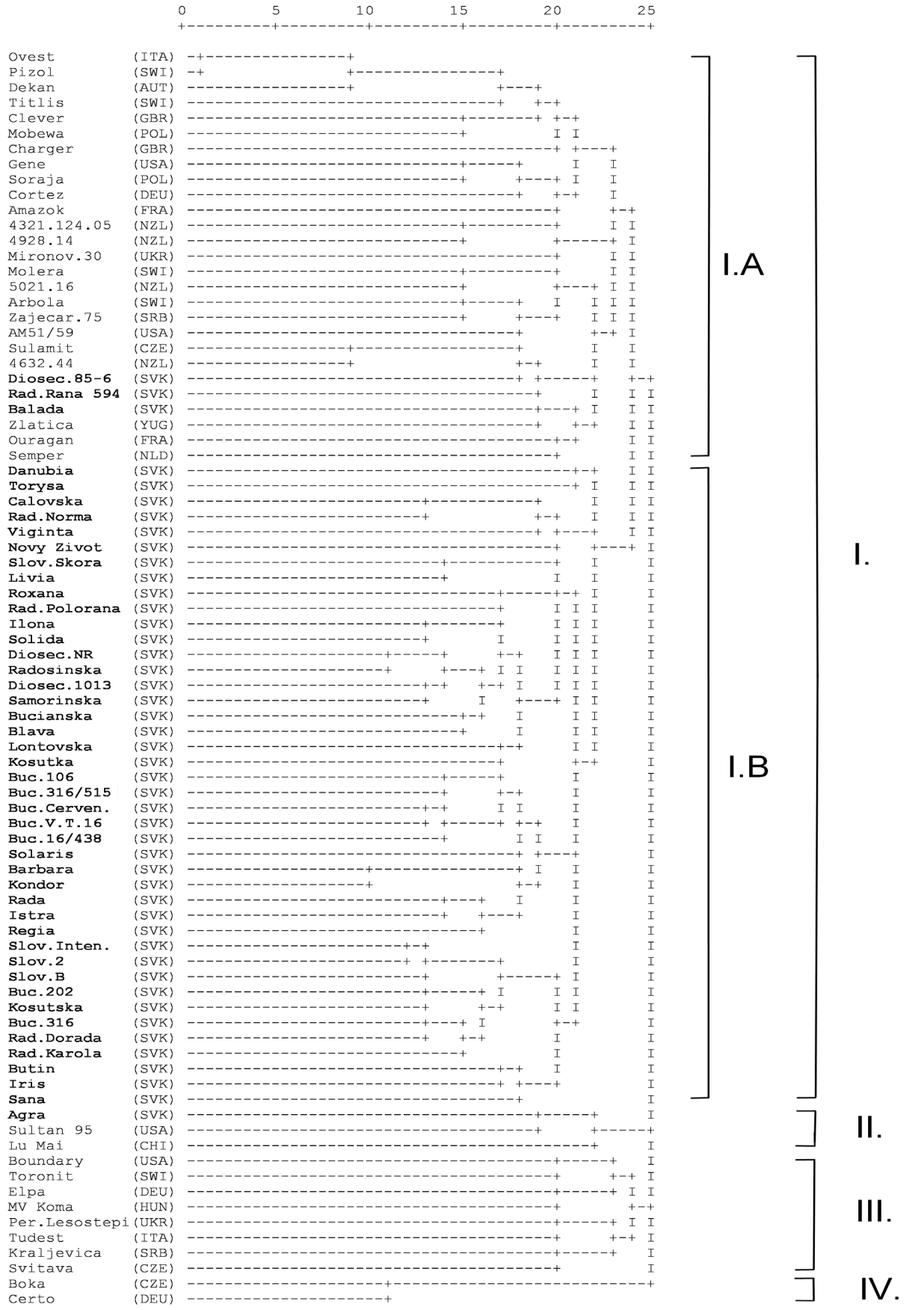

Figure 2. The dendrogram of 46 domestic and 36 introduced wheat genotypes differentiated by 5 EST-SSR markers (domestic genotypes highlighted by bold letters)

and PIC = 0.958 per locus. In comparison with results of other authors these values are rather high as we used 5 selected the most polymorphic EST–SSR loci. Similar to this study Gupta *et al*. (2003) found that each primer pair gave multiple bands, and they suggested conservation of EST–SSRs in 2–3 related wheat genomes. This is in agreement with findings that one-quarter of all genes motifs within the wheat genome are represented by two or more duplicate loci (Akhunov *et al*. 2003). Eujayl *et al*. (2001) also showed that many durum wheat genotypes had 2 or 3 alleles due to their heterogeneity or heterozygosity as expected because genotypes were not pure inbred lines.

Due to relatively high variation at five selected EST–SSR loci and high PIC values, the indices of probability had relatively low values (Table 3). DI and PIC values were slightly higher for domestic genotypes than for introduced ones, what shows rather high diversity within domestic group and can be connected to presence of not only cultivars, but also landraces and breeding lines within this group. The frequency distribution of all alleles within domestic and introduced genotypes shows Figure 1. The observed allelic frequencies ranged from 0.006 to 0.484 with an average 0.076 within domestic genotypes and from 0.028 to 0.500 with an average 0.093 within introduced genotypes. Fifty alleles (75.76%) within domestic genotypes and 42 alleles (77.78%) within introduced genotypes appeared with the frequencies of 0.10 or lower suggesting the evidence of mutation or introduction of new gene resource in a germplasm pool (Wang *et al*. 2007)

Five of selected EST–SSRs were sufficient for definite distinguishing of all compared wheats. The cluster analysis based on the calculated Jaccard's coefficients (Figure 2) presents four main clusters (I.–IV.). Out of the 46 domestic wheats analyzed, forty-five were grouped into the clusters I., three of them into the cluster I.A and 42 genotypes formed separate the cluster I.B. Within

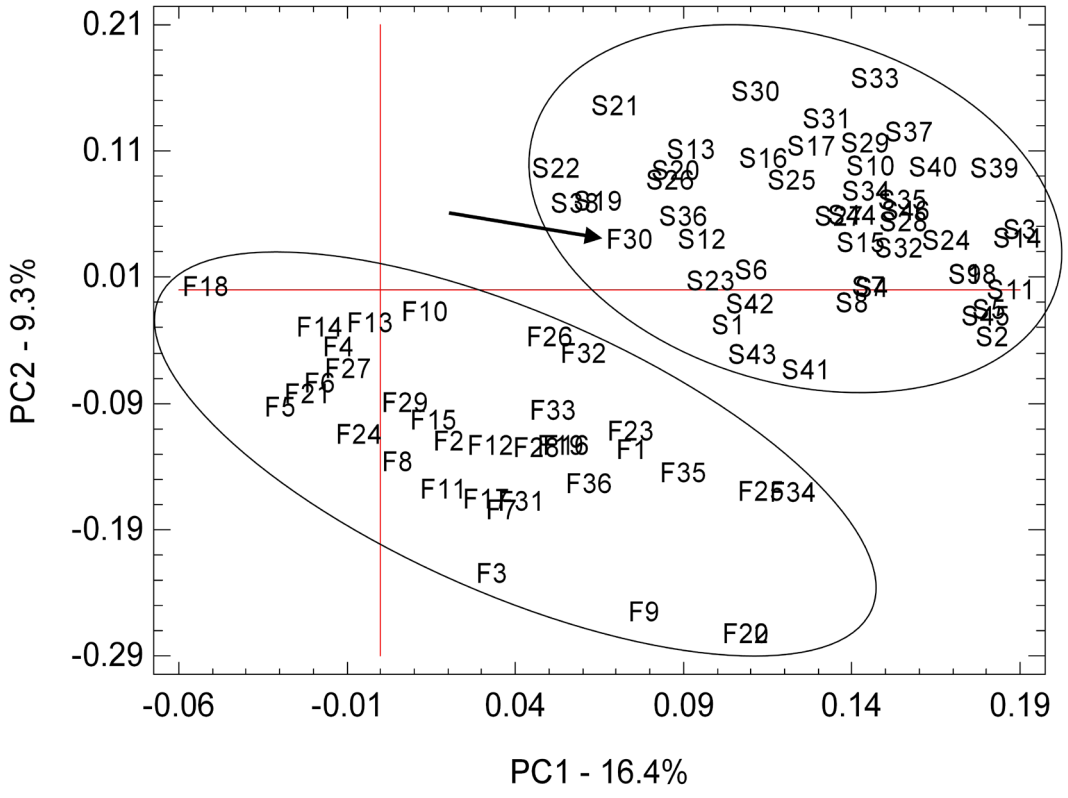

Figure 3. PCA analysis was done using binary data of 46 domestic (Slovak = S) and 36 introduced (foreign = F) wheat genotypes using 5 the most polymorphic EST-SSRs (labelling of samples is according to Table 1)

the cluster I.B several domestic genotypes with similar pedigree grouped to smaller subclusters (different lines of Bučianska and Slovenská). This classification partially shows grouping of genotypes according to their country of origin as well as to pedigree, similar as found by Eujayl *et al*. (2001) within durum wheats, or by Zhuang *et al*. (2011) within the Persian wheat accessions. The most similar wheat genotypes from all analyzed were Ovest (from Italy) and Pizol (Switzerland) in the cluster I.A. They differ by only one allele. Only a single domestic cultivar (Agra) was included into the cluster II., although placed close to other domestic wheats in dendrogram. This domestic cultivar is grouped together with wheat cultivars originated from the USA and China. The second largest is the cluster III. including 8 introduced wheats originating from different European countries and one cultivar from the USA. The cluster IV. consisted of only two wheats originating from the Czech Republic and Germany.

PCA analysis better indicates differences between groups of domestic and introduced wheats (Figure 3). Introduced wheats showed slightly higher degree of diversity among themselves. In the group of introduced wheat genotypes separate smaller subgroups composed from one or two wheat genotypes were created. Simultaneously, cultivars Ovest and Pizol (F20 and F22), as in the dendrogram, showed great similarity by using the PCA analysis. Introduced cultivar Tudest from Italy (F30) is located in a group of domestic wheat cultivars, while the domestic cultivars form a distinct group separated from introduced. Group of domestic cultivars, contrary to introduced, has higher cohesion.

CONCLUSION

Results showed that EST–SSR markers are suitable for evaluation of genetic differences within and between groups of domestic and introduced wheat genotypes and for genetic comparison between them. Genetic diversity of introduced wheats was evaluated by 18 EST–SSRs and comparison of domestic (Slovak) and introduced (foreign) wheats by 5 the most polymorphic EST–SSRs. The level of polymorphism in EST–SSRs was sufficient for discrimination between genotypes. Variation of domestic genotypes was slightly higher than those of introduced genotypes. Analysis based on used 5 EST–SSR markers showed clustering of genotypes according to origin, domestic and introduced wheats were grouped distinctly into two separate groups.

Acknowledgements. This work originated thanks to the support within Operational Programme Research and Development for the project: "*Transfer, use and dissemination of research results of plant genetic resources for food and agriculture*" (ITMS: 26220220058), cofinanced from the resources of the European Union Fund for Regional Development.

REFERENCES

AKHUNOV, E.D. – AKHUNOV, E.D. – GOODYEAR, A.V. – GENG, S. – QI, L.-L. – ECHALIER, B. – GILL, B.S. – MIFTAHUDIN – GUSTAFSON, J.P. – LAZO, G. – CHAO, S. – ANDERSON, O.D. – LINKIEWICZ, A.M. – DUBCOVSKY, J. – LA ROTA, M. – SORRELLS, M.E. – ZHANG, D. – NGUYEN, H.T. – KALAVACHARLA, V. – HOSSAIN, V. – KIANIAN, S.F. – PENG, J. – LAPITAN, N.L.V. – GONZALEZ-HERNANDEZ, J.L. – ANDERSON, J.A. – CHOI, D.-W. – CLOSE, T.J. – DILBIRLIGI, M. – GILL, K.S. – WALKER-SIMMONS, M.K. – STEBER, C. – MCGUIRE, P.E. – QUALSET, C.O. – DVORAK, J. 2003. The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. In *Genome Res*earch, vol. *13*, pp.753–763. DOI:10.1101/gr.808603.

AKKAYA, M.S. – BUYUKUNAL-BAL, E.B. 2004. Assessment of genetic variation of bread wheat varieties using microsatellite markers. In *Euphytica*, vol, *135*, pp. 179–185. DOI: 10.1023/B:EUPH.0000014908.02499.41.

BASSAM, B.J. – CAETANO-ANOLLES, G. – GRESSHOFF, P.M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. In *Analytical Biochemistry*, vol. *196*, no. 1, pp. 80–83. DOI: 10.1016/0003-2697(91)90120-I.

CORDEIRO, G.M. – CASU, R. – McINTYRE, C.L. – MANNERS, J.M. – HENRY, R.J. 2001. Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to *Erianthus* and sorghum. In *Plant Science*, vol. *160*, pp. 1115–1123. DOI: 10.1016/S0168-9452(01)00365-X.

EUJAYL, I. – SORRELLS, M.E. – BAUM, M. – WOLTERS, P. – POWELL, W. 2001. Assessment of genotypic variation among cultivated durum wheat based on EST–SSRs and genomic-SSRs. In *Euphytica*, vol. *119*, pp. 39–43.

FU, Y.B. – PETERSON, G.W. – GAO, J.K.Y.L. – JIA, J. – RICHARDS, K.W. 2006. Impact of plant breeding on genetic diversity of the Canadian hard red spring wheat germplasm as revealed by EST-derived SSr markers. In *Theoretical and Applied Genetics*, vol. *112*, pp. 1239–1247. DOI: 10.1007/s00122-006-0225-2.

FUJITA, Y. – FUKUOKA, H. – YANO, H. 2009. Identification of wheat cultivars using EST–SSR markers. In *Breeding Science*, vol. *59*, pp. 159–167. DOI: 10.1270/jsbbs.59.159.

GADALETA, A. – GIANCASPRO, A. – GIOVE, S.L. – ZACHEO, S. – MANGINI, G. – SIMEONE, R. – SIGNORILE, A. – BLANCO, A. 2009. Genetic and physical mapping of new EST-derived SSRs on the A and B genome chromosomes of wheat. In *Theoretical and applied genetics*, vol. *118*, pp. 1015–1025. DOI: 10.1007/s00122-008-0958-1.

GADALETA, A. – GIANCASPRO, A. – ZACHEO, S. – NIGRO, D. – GIOVE, S.L. – COLASUONNO, P. – BLANCO, A. 2011. Comparison of genomic and EST-derived SSR markers in phylogenetic analysis of wheat. In *Plant Genetic Resources*, vol. *9*, pp. 243–246. DOI:10.1017/S147926211100030X.

GREGÁŇOVÁ, Ž. – KRAIC, J. – GÁLOVÁ, Z. 2005. Effectiveness of microsatellites in differentiation of elite wheat cultivars. In *Biologia*, vol. *60*, pp. 665–670.

GREGÁŇOVÁ, Ž. – VIVODÍK, M. – GÁLOVÁ, Z. 2008. Analysis of diversity of wheat by microsatellites. In *Biotechnology 2008* [CD-ROM]. České Budějovice : University of South Bohemia České Budějovice, pp. 109–111. ISBN 80-85645-58-0.

GUPTA, P.K. – RUSTGI, S. – SHARMA, S. – SINGH, R. – KUMAR, N. – BALYAN, H. S. 2003. Transferable EST–SSR markers for the study of polymorphism and genetic diversity in bread wheat. In *Molecular Genetics and Genomics*, vol. *270*, pp. 315–323. DOI: 10.1007/s00438-003-0921-4.

HUANG, X.Q. – BORNER, A. – RODER, M.S. – GANAL, M. 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. In *Theoretical and Applied Genetics*, vol. *105*, pp. 699–707. DOI: 10.1007/s00122-002-0959-4.

KANTETY, R.V. – LA ROTA, M. – MATTHEWS, D.E. – SORRELLS, M.E. 2002. Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. In *Plant Molecular Biology*, vol. *48*, pp. 501–510. DOI: 10.1023/A:1014875206165.

LI, Y.CH. – KOROL, A.B. – FAHIMA, T. – NEVO, E. 2004. Microsatellites within genes: structure, function and evolution. In *Molecular Biology and Evolution*, vol. *21*, pp. 991–1007. DOI: 10.1093/molbev/msh073.

PAETKAU, D. – CALVERT, W. – STIRLING, I. – STROBECK, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. In: *Molecular Ecology*, vol. *4*, pp. 347–354. DOI: 10.1111/j.1365-294X.1995.tb00227.x.

PRASAD, M. – VARSHNEY, R.K. – ROY, J.K. – BALYAN, H.S. – GUPTA, P.K. 2000. The use of microsatelites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. In *Theoretical and Applied Genetics*, vol. *100*, pp. 584–592. DOI: 10.1007/s001220050077.

ROZEN, S. – SKALETSKY, H. 2000. Primer3 on the www for general users and for biologist programmers. In MISENER, S. – CRAWETZ S.A. (eds.) *Bioinformatics Methods and Protocols. Methods in Molecular Biology*, vol. *132*, pp. 365–386.

SONG, Z.P. – JIANG, X.B. – ZHANG, M. – WANG, Z.L. – BO, W.H. – AN, X.M. – ZHANG, D.Q. – ZHANG, Z.Y. 2012. Differences of EST-SSR and genomic-SSR markers in assessing genetic diversity in poplar. In *Forestry Studies in China*, vol. *14*, pp. 1–7. DOI: 10.1007/s11632-012-0106-5.

WANG, H.Y. – WEI, Y.M. – YAN, Z.H. – ZHENG, Y.L. 2007. EST-SSR DNA polymorphism in durum wheat (*Triticum durum* L.) collections. *In Journal of Applied Genetics*, vol. *48*, no. 1, pp. 35–42. DOI: 10.1007/BF03194655.

WEBER, J.L. 1990. Informativeness of human $(dC\text{-}dA)_n$. $(dG\text{-}dT)_n$ polymorphism. In *Genomics*, vol. *7*, pp. 524–530. DOI: 10.1016/0888-7543(90)90195-Z.

WEIR, B.S. 1990. *Genetic data analysis. II. Methods for discrete population genetic data*. 2nd ed. Sinauer Associates : Sunderland, Mass., p. 370.

YANG, X.Q. – LIU, P. – HAN, Z.F. – NI, Z.F. – LIU, W.Q. – SUN, Q.X. 2005. Comparative analysis of genetic diversity revealed by genomic-SSR, EST–SSR and pedigree in wheat (*Triticum asetivum* L.). In *Acta Genetica Sinica*, vol. *32*, pp. 406–416.

YU, J.-K. – DAKE, T.M. – SINGH, S. – BENSCHER, D. – LI, W.L. – GILL, B. – SORRELLS, M.E. 2004. Development and mapping of EST-derived simplesequence repeat markers for hexaploid wheat. In *Genome*, vol. *47*, pp. 805–818. DOI: 10.1139/g04-057.

ZHUANG, P.P. – REN, Q. – LI, W. – CHEN, G.Y. 2011. Genetic diversity of Persian wheat (*Triticum turgidum* ssp. *carthlicum*) accessions by EST–SSR markers. In *American Journal of Biochemistry and Molecular Biology*, vol. *1*, pp. 223–230. DOI: 10.3923/ajbmb.2011.223.230.

Received: August 1th, 2013