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EXPLORING GENETIC VARIATIONS IN FABA BEAN (*VICIA FABA* L.) ACCESSIONS USING NEWLY DEVELOPED EST-SSR MARKERS

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Abstract

The genetic and phenotypic variability among 22 Jordanian faba bean (*Vicia faba* L.) accessions were explored. Fifteen horticultural traits were evaluated including Biological weight, Days to flowering, Grain yield, Plant height, Highest lowest pod, Number of primary branches, Pod length, Pod width, Pod weight, days to filling, pod number node-^{1}, Pod number plant-^{1}, number of secondary branches, seeds number per pod, 100 seed weight. Statistical analysis indicated significant variability among accessions for days to flowering, pod width, pod weight and grain yield. Genetic relationships among twenty two accessions were constructed using twelve EST-SSR primer pairs and eight AFLP primer combinations. The UPGMA results, based on phenotypical data, were able to divide the accessions into 3 groups. In contrast, the UPGMA results, based on DNA markers data, divided the accessions into five groups with Dice similarity coefficient of 0.62. This obtained information on the genetic and phenotypic diversity within gene pools in breeding programs could help avoid genetic erosion.

Key words: Faba bean, Genetic variation, Phenotypic variation, Accessions, SSR markers, AFLP markers.

Introduction

The breeding of crop species is made possible by introducing novel alleles/genes for agronomic traits. These new alleles/genes are extracted from gene pools. Plant breeders are alarmed at reduced genetic diversity as a consequence of modern farming practices (Lee, 1995). For example, faba bean accessions are becoming extinct and very rapidly replaced by cultivars of better performance.

Breeders have become more alert of the need for sustaining genetic diversity. During the past four decades, several studies have been carried out to compare and estimate genetic diversity based on DNA markers polymorphisms. Among these DNA markers, EST-SSR could show extensive length polymorphisms for different individuals of the same species, a fact that can be utilized for DNA-fingerprinting purposes (Schlotterer & Tautz, 1992).

Expressed sequence tag–simple sequence repeats (EST-SSRs) are gene-derived SSR markers that are developed from complementary DNA (cDNA) clones and any EST databases (Akash & Myers, 2012). The EST-SSR markers consist of highly variable ranges of tandemly repeated sequences that span usually from 2 to 6 base pair (bp). These repeats can be amplified using primers that are complimentary to the flanking regions (Saiki *et al*., 1988).

The AFLP technique combines the high reliability of RFLP and the simplicity of PCR to simultaneously amplify many restriction fragments (Vos *et al*., 1995). This technique was used successfully to evaluate genetic diversity and genetic relationships in most cultivated crops as such DNA marker does not require previous sequencing knowledge (Akash, 2003).

Genetic diversity were observed among 79 inbred lines of recent elite faba bean cultivars of Asian, North African and European origin by AFLP markers (Zeid, *et al*., 2003). In addition, the AFLP markers were utilized to study the relationship between 18 European faba bean lines and to study the performance and heterosis of their hybrid (Zeid, *et al*., 2004). The genotypic variability among 11 faba bean (*Vicia faba* L.) landraces, collected from Jordan, and five imported cultivars were investigated by Abu-Amer *et al*. (2011) using 12 single sequence repeat (SSR) primer pairs and 12 phenotypic traits.

The objectives of this study were to evaluate the phenotypic and genotypic variability within faba bean pool of 22 accessions using EST-SSR and AFLP markers.

Materials and Methods

Seeds of 22 *Vicia faba* L. Jordanian faba bean accessions (Table 1), obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA) were examined. After planting, seeds in pots under field conditions in a randomized complete block design with three replications,young leaves were collected for DNA extraction. Irrigation, fertilization, and other agricultural practices were performed as described by (Abu-Amer *et al*., 2011). Except for days to flowering, days to fruit set, and days to filling, traits were recorded at maturity.

Table 1. Faba bean *(Vicia faba* L.*)* accession numbers collected from different provinces in Jordan.

Sample no.	Accession no.	Province	Longitude	Latitude
1	ICARDA-11195	Zarqa	E36 06	N32 04
2	ICARDA-11197	Amman	E35 56	N31 57
3	ICARDA-11198	Amman	E35 56	N31 57
4	ICARDA-11199	Ma'an	E35 30	N30 29
5	ICARDA-11200	Ma'an	E35 30	N30 29
6	ICARDA-11201	Ma'an	E35 30	N30 29
7	ICARDA-11202	Irbid	E35 54	N32 17
8	ICARDA-11203	Irbid	E35 54	N32 17
9	ICARDA-11204	Irbid	E35 54	N32 17
10	ICARDA-11517	-	-	-
11	ICARDA-11956	Irbid	E35 54	N32 17
12	ICARDA-11957	Irbid	E35 54	N32 17
13	ICARDA-11985	Amman	E35 48	N31 43
14	ICARDA-12752	Amman	E35 56	N31 57
15	ICARDA-12753	Amman	E35 56	N31 57
16	ICARDA-13012	-	-	-
17	ICARDA-13988	-	-	-
18	ICARDA-14124	Amman	E35 56	N31 57
19	ICARDA-74193	Irbid	E35 51	N32 33
20	ICARDA-74194	Zarqa	E35 06	N32 04
21	ICARDA-74195	Al-Tafilah	E35 46	N30 44
22	ICARDA-74196	Amman	E35 55	N31 57

Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method as described by Torres *et al*. (1993) with minor modifications. In addition to the 34 EST_SSR primers developed and validated by (Akash & Myers, 2012), twelve EST-SSR primers were validated in our study (Table 2). Also, eight AFLP primer combinations were included. The EST-SSR PCR reactions were performed according to Pozarkova *et al*. (2002) with minor modifications as explained by (Akash & Myers, 2012) while the AFLP PCR reaction were performed according to Vos, *et al*. (1997) with minor modifications as explained by Akash (2011). GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, Calif.) was used for performing both EST-SSR and AFLP reactions. As melting temperature for the twelve EST-SSR markers spanned from 59.98 to 60.84 C, single annealing temperature of 65 C was used in the amplification step of all EST-SSR primers. The amplified products for EST-SSR were separated in 2% agarose gel and then were visualized using a mini-DOC system (Gel Documentation System, Bio-Rad Hercules, CA, USA). The EST-SSR fragments sizes were determined using DNA PCR marker (100–1500 bp, Promega). The amplified products for AFLP were separated using LiCor DNA Analyzer (LiCor, Lincoln, NE, USA). Amplified bands were scored as 1 for present, 0 for absent, and 9 for missing. Coefficient of similarities among accessions was estimated according to Dice (1945) and cluster analysis was performed using the unweighed pair group method with arithmetic average (UPGMA) and as described by Akash & Kang (2009). Analysis of variance and correlation were performed using SAS software (Anon., 2002) while cluster analysis was performed using NTSYSpc 2.0 software (Rohlf, 1998).

Results

Phenotyping: Significant differences in days to flowering, pod width, pod weight, and grain yield were observed (Table 3). Accession 9, originally collected from Irbid, scored the highest grain yield per plant (2.25 tons ha^{-1}) and at the same time scored the highest pod weight. Concerning days to flowering, accessions 3, 4, 7, and 18 were of the earliest accession in reaching flower stage. Accession 4, collected from Ma'an, scored the highest pod width (1.7 cm) as shown in Table 4.

The highest Pearson correlation coefficient was obtained between pod width and pod weight (r=.94). Both traits have a positive significant correlation with grain yield (r=.73 & .75, respectively; Table 5). The cluster analysis of the phenotypic traits for the 22 vicia faba showed the presences of two major groups; the first group consisted of twelve accessions while the second major group consisted of seven accessions (Fig. 1).

Genotyping: Twelve EST-SSR primers and eight AFLP primer combinations were amplified successfully and found to be polymorphic among at least two of the 22 faba bean accessions. Recorded alleles per EST-SSR marker ranged from 2 to 6 with a mean of 3 alleles per EST-SSR reaction.

A dendrogram based on combined EST-SSR and AFLP data for the 22 faba bean accessions is presented in Fig. 2. A general clustering of accession by collection region was observed as most of the accessions in the first group originated in Amman, while the accessions of the second group originated in Irbid. In the first cluster, consist of 14 accessions, the highest similarity was observed between accession 3 and accession 5 followed by similarity percentage (0.89) between accession 18 and accession 21. The least similarity level (0.69) within the first group was recorded between accessions 18 & 11 and the rest of the other accessions of the same group. The second group, consisted of five accessions, the highest similarity coefficient (0.78) was observed between accession 7 and accession 8. Three accessions did not fall in any group.

Table 2[a]. List of EST-SSR primers including expected product size (bp), annealing temperature °C and sequence (5'→3').

Primer	Primer sequence (5'→ 3')	Expected product size (bp)	Genbank no.	Putative function of the gene in which the SSR resides, together with the E-value for the match
DMA033	GGACAAAGATTAGTGCGAGATAGAG CTTTCACACCTTCACCTTCCAT	199	FL504903.1	dehydration responsive element-binding protein 1 [*Glycine soja*], 9e-43
DMA035	GACGCAGTCACTCTCTGTCTTG AGATGTGTCGGTGACTGACTTG	221	FL506961.1	conserved hypothetical protein [*Ricinus communis*], 9e-59
DMA039	TCCAACACAAAGACAAAGATGC TGCATAACTAACACATTATGCAGGA	164	FL506094.1	defensin-like protein [*Vicia faba*], 2e-28
DMA057	ACACGATCTCATTGACTCTTCATC TCGAGGTTCCTTGGTTGTAGAT	249	FL506437.1	translocon-associated protein, beta subunit precursor, putative [*Ricinus communis*], 5e-53
DMA061	AAAATTCCAGAAATGGCAGCTA TTCGAGGGAAGGAGTATTTGAA	169	FL503455.1	transaldolase [*Gossypium hirsutum*], 6e-22
DMA066	CATTTTCTGAATTTGGGCTCTC TGAATCTAACCAGTCCAAAGCA	197	FL503458.1	Tyrosine protein kinase, active site [*Medicago truncatula*], 2e-63
DMA073	AGGAACAAGAAAGGGTTTGTGA AACCACCCTTTCACCACTTTC	165	FL503298.1	protein F12F1.11 - , putative [*Medicago truncatula*], 9e-49
DMA078	ATGAAACCAGACCCCAAACTTA CCAGTACACAAAAGCCAAAACA	204	FL504892.1	2-Cys peroxiredoxin [*Pisum sativum*], 2e-50
DMA083	GGAGTTTGTTCTCCATTTGAGG ATTTCCCACTTCCCTTTTTCTC	178	FL506741.1	seed albumin PA1 [*Pisum sativum*], 5e-35
DMA095	CACCGTTGATCAGTCATCACAT TGCATATCTGGATACCAAAACTCA	230	FL505651.1	dormancy-associated protein [*Pisum sativum*], 7e-43
DMA106	GGAACCACTGCTCCAACTTATC AAGCTTTAATTTTCTCGCATGG	160	FL505768.1	seed maturation protein [*Glycine tabacina*], 8e-37
DMA111	TTGGGCTACATCTCCTTCAAAT CCACCATCCCTATGTCATTTTT	239	FL507528.1	Like-Sm ribonucleoprotein-related, core [*Medicago truncatula*], 1e-37

Table 2[b]. Adapters and primers used for ligation, pre-amplification and selective amplification steps of the AFLP procedure

Type	Sequence (5'-3')	
	EcoRI	MseI
Adapters	CTCGTAGACTGCGTACC AATTGGTACGCAGTC	GACGATGAGTCCTGAG TACTCAGGACTCAT
Pre amplification primers	GACTGCGTACCAATTCA	GATGAGTCCTGAGTAAC
Selective amplification primers	GACTGCGTACCAATTCAAAG & GATGAGTCCTGAGTAACCAC GACTGCGTACCAATTCAAAG & GATGAGTCCTGAGTAACCCT GACTGCGTACCAATTCAAAC & GATGAGTCCTGAGTAACCCT GACTGCGTACCAATTCAAAC & GATGAGTCCTGAGTAACCAC GACTGCGTACCAATTCAAAT & GATGAGTCCTGAGTAACCCT GACTGCGTACCAATTCAATC & GATGAGTCCTGAGTAACCCT GACTGCGTACCAATTCAAAT & GATGAGTCCTGAGTAACCAC GACTGCGTACCAATTCAATC & GATGAGTCCTGAGTAACCAC	

Table 3. Mean squares for phenotypic traits among 22 faba bean accessions.

Source of variation	df	BWT	DFLR	GY	PHT	HLP	NPB	PL	PW	PWT	DF	PPN	PPP	NSB	SNPP	HSW
Block	2	114.4*	7.6	0.98	191.5	7.2	1.75	0.12	0.02	1.73*	177	0.13	1.13	3.06	2.6	97
Accession	21	29.2	50*	0.85*	56	25.1	1.02	3.15	0.18*	1.3*	59.7	0.12	1.21	0.92	1.91	997
Residual	42	25.3	19.4	0.36	58.5	17.6	.079	1.74	0.07	0.43	98	0.12	0.68	1.56	1.16	520

* Significant at $p \leq 0.05$. BWT: Biological weight; DFLR: Days to flowering; GY: Grain yield; PHT: Plant height; HLP: Highest lowest pod; NPB: Number of primary branches; PL: Pod length; PW: Pod width; PWT: Pod weight; DF: days to filling; PPN: pod number node^{-1}; PPP: Pod number plant^{-1}; NSB: number of secondary branches; NSP: seed number per pod; SNPP: seeds number per pod; HSW: 100 seed weight

Table 4. Mean separation of the four significant phenotypic traitsfor sixteen faba bean genotypes grown in two locations (Krima and Baqa) during 2006/07 growing season.

Accession ID	DFLR	GY	PW	PWT
1	101.0b-d	0.68bc	1.07bc	NE
2	103.7b	0.34bc	1.19a-c	NE
3	95.0cd	0.64bc	1.49 a-c	NE
4	95.0cd	0.98bc	1.70a	NE
5	103.3b	0.14bc	1.06b-d	0.15c-e
6	96.0b-d	0.93bc	1.58ab	1.32b-e
7	95.0cd	0.97bc	1.03c	0.97c-e
8	96.0bd	1.25a-c	1.17bc	1.72a-d
9	96.0bd	2.25a	1.31 a-c	2.61a
11	96.7bd	0.57bc	1.20bc	0.80c-e
12	98.0bd	0.14c	1.03c	0.23e
13	102.2bd	0.99a-c	0.97b-d	1.43a-e
14	94.1d	NE	NE	NE
15	100.3bd	0.20c	1.01c	0.35c-e
18	95.0cd	1.57ab	1.28 a-c	2.28ab
19	94.7bd	NE	NE	NE
20	114.2a	NE	0.36d	0.01de
21	96.0bd	1.56a-c	1.56 a-c	2.03a-c
22	104.7a-c	0.01c	1.56 a-c	0.45c-e

* Means with the same case-letter are not significantly different according to LSD ($p \leq 0.05$). DFLR: Days to flowering; GY: Grain yield; PW: Pod width; PWT: Pod weight. NE: not estimable

Table 5. Pearson correlation coefficientsof the four significant phenotypic traits.

	DFLR	PW	PWT
PW	-0.28		
PWT	-0.18	0.94*	
GY	0.23	0.73*	0.75*

* Significant at $p \leq 0.001$. DFLR: Days to flowering; GY: Grain yield; PW: Pod width; PWT: Pod weight

Discussion

The need for cultivation of adapted vica faba for local environment of Jordan has resulted in better utilization of the available landraces and varieties. However and in order to further increase productivity and quality, efforts should widen the genetic base available vicia faba genotypes. Therefore, this study was performed to evaluate the yield and yield components of 22 landraces collected from different locations from Jordan (Table 1).

Based on the phenotypic analysis of variance, mean squares for days to flowering, pod width, pod weight, and grain yield were significant ($p<0.05$) among the 22 accessions (Table 3). Accession 9 is a promising accession in this study because it scored the highest grain yield per plant (2.25 tons ha^{-1}) and at the same time scored the highest pod weight (Table 4).

The significant correlation between pod width and pod weight with grain yield reflects the importance of the two traits in faba bean breeding for higher grain yield. Furthermore the correlations between traits are of great importance for conducting indirect selections in breeding programs.

The twelve co-dominant EST-SSR and eight dominant AFLP primer combinations produced 36 different EST-SSR markers and 101 different AFLP markers. These 137 markers were able to individualize all the 22 faba bean accessions.

A clear contrast in clustering levels between phenotypical and molecular markers was found. Two groups were identified based on phenotypical data (Fig. 1). Also, two groups were identified based on EST-SSR and AFLP markers. In another genetic diversity analysis of Mediterranean faba bean (*Vicia faba* L.) with ISSR markers, Mediterranean-type faba beans can be subdivided into at least two different germplasm pools (Terzopoulos & Bebeli, 2008).

A general clustering of accession by collection region was observed as most of the accessions in the first group originated in Amman, while the accessions of the second group originated in Irbid. Similarly, the overall results of 802 faba bean landraces varieties from different geographical locations of China and abroad indicated that the genetic relationship of faba bean germplasm was closely associated with their geographical origin and their ecological habit (Wang *et al*., 2012). In another study done by Link *et al*. (1995), three groups of faba bean inbred lines were examined by means of RAPDs (random amplified polymorphic DNAs) assays: 13 European small-seeded lines, 6 European large-seeded lines, and 9 Mediterranean lines. Cluster (UPGMA) and principal coordinate analyses identified European small-seeded lines and Mediterranean lines as distinct groups with European large-seeded lines located in between. In conclusion, genetic clustering of faba bean accessions clearly showed the association of accession with their collection locations. These groups of similar accessions are useful for selecting parents in crossing and in developing inbreds.

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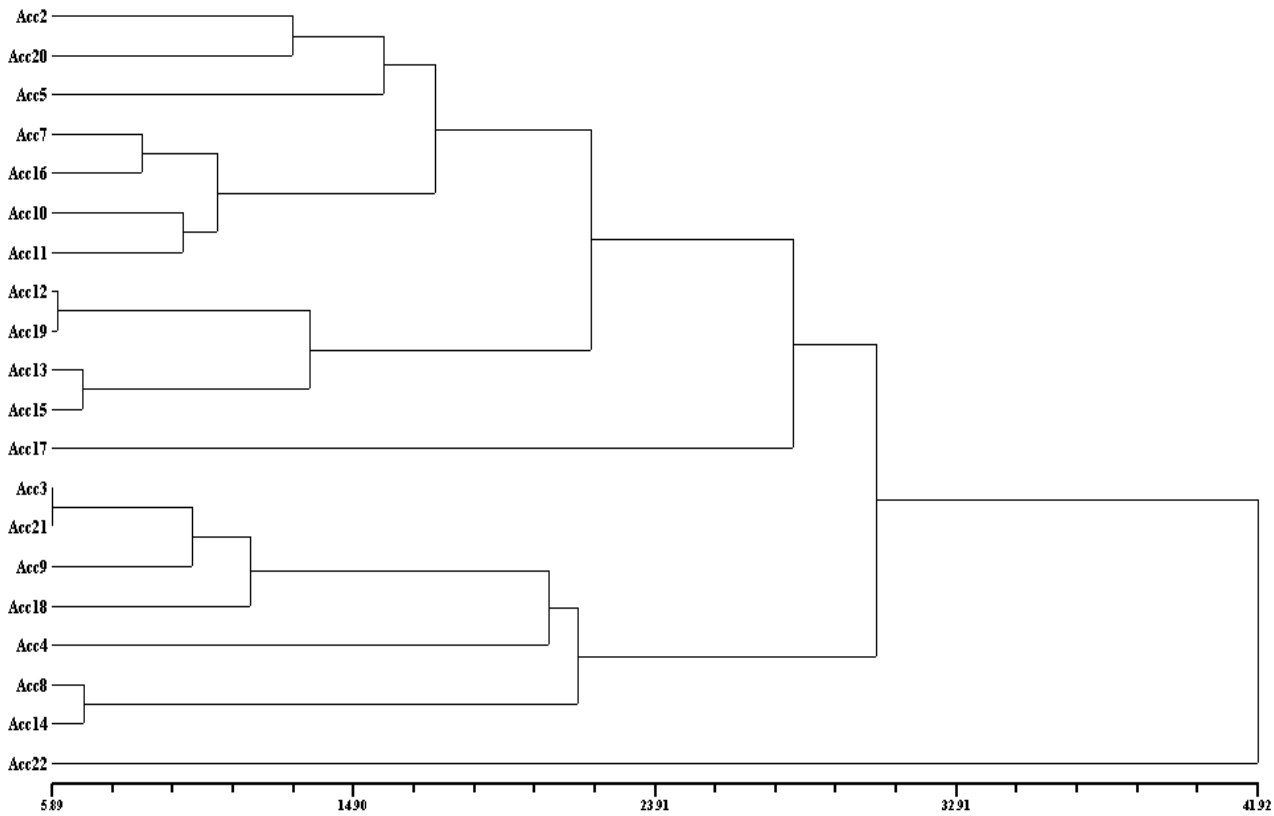

Fig. 1. UPGMA dendrogram for 22 faba bean accessions determined on the basis of phenotypic similarity by means of fifteen horticultural traits.

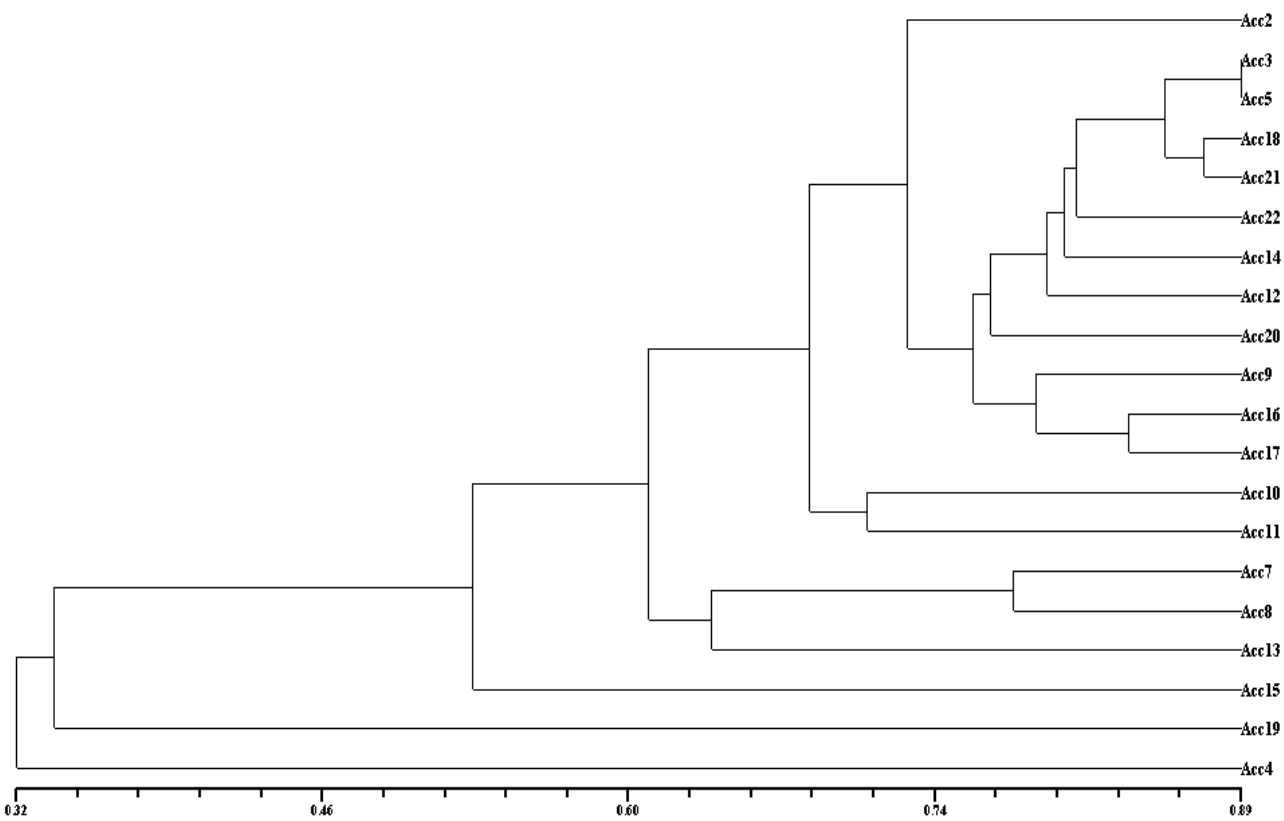

Fig. 2. UPGMA dendrogram for 22 faba bean accessions determined on the basis of genetic similarity by means of twelve EST-SSR primers and eight AFLP primer combinations.

References

Abu-Amer, J.H., H.M. Saoub, M.W. Akash and A.M. Al-Abdallat. 2011. Genetic and phenotypic variation among faba bean landraces and cultivars. *Int. J. Veg. Sci*., 17: 45-59.

Akash, M.W. 2003. Quantitative trait loci mapping for agronomic and fiber quality traits in upland cotton (*Gossypium hirsutum* L.) use molecular markers. Ph.D. dissertation, Louisiana State Univ., Baton Rouge, Louisiana, USA.

Akash, M.W. 2011. Modeling and maximizing AFLP pre-amplification yield using response surface methodology with covariate. *J. Food Agri. & Environ*., 9: 1144-1147.

Akash, M.W. and G.O. Myers. 2012. The development of faba bean expressed sequence tag–simple sequence repeats (EST-SSRs) and their validity in diversity analysis. *Plant Breeding*, 131: 522-530.

Akash, M.W. and M. Kang. 2009. Molecular clustering and interrelationships among agronomic traits of Jordanian barley cultivars. *J. Crop Imp*., 24: 28-40.

Dice, R.L. 1945. Measures of the amount of ecological association between species. *Ecology*, 26: 297-302.

Lee, M. 1995. DNA markers and plant breeding programs. *Adv. Agron*., 35: 265-344.

Link, W., C. Dickens, M. Singh, M. Schwall and A.E. Melchinger. 1995. Genetic diversity in European and Mediterranean faba bean germplasm revealed by RAPD markers. *Theor. Appl. Genet*., 90: 27-32.

Pozarkova, D., A. Koblizkova, B. Roman, A.M. Torres, S. Lucretti, M. Lysak, J. Dolezel and J. Macas. 2002. Development and characterization of microsatellite markers from chromosome 1-specific DNA libraries of *Vicia faba*. *Biol. Plant*., 45: 337-345.

Rohlf, F.J. 1998. NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.02. New York.

Saiki, R.K., D.H. Gelfand, S. Stoffel, S. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis and H.A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239: 487-491.

Anonymous. 2002. SAS/Stat software. Release 9.0. SAS Ins., Cary, N. C.

Schlotterer, C. and D. Tautz. 1992. Slippage synthesis of simple sequence DNA. *Nucl. Acids Res*., 20: 211-215.

Terzopoulos, P.J. and P.J. Bebeli. 2008. Genetic diversity of Mediterranean faba bean (*Vicia faba* L.) swith ISSR markers. *Field Crops Res*., 108: 39-44.

Torres, A.M., N.F. Weeden and A. Martin. 1993. Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. *Theor. App. Gen*., 85: 937-945.

Vos, P.R., M. Hogers, M. Bleeker, T. Reijans, M. Lee, A. Hornes, J. Frijters, J. Pot, M. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.*, 23: 4407-4414.

Wang, H.F., X.X. Zong, J.J. Guan, T. Yang, X.L. Sun, Y. Ma and R. Redden. 2012. Genetic diversity and relationship of global faba bean (*Vicia faba* L.) germplasm revealed by ISSR markers. *Theor App. Gen*., 124: 789-797.

Zeid, M., C.C. Schoen and W. Link. 2003. Genetic diversity in recent elite faba bean lines using AFLP markers. *Theor. App. Gen*., 107: 1304-1314.

Zeid, M., C.C. Schoen and W. Link. 2004. Hybrid performance and AFLP-based genetic similarity in faba bean. *Euphytica*,139: 207-216.

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