Indian J. Hort. 66(2), June 2009: 147-153

Genetic variability studies among apricot populations from cold arid desert of Ladakh using DNA markers

Meetul Kumar*, G.P. Mishra, Raghwendra Singh, P.K. Naik, Sanjai Dwivedi***, Zakwan Ahmad**** and S.B. Singh**

Defence Institute of High Altitude Research (formerly FRL), Defence Research and Development Organization, C/o 56 APO, Jammu & Kashmir 194 101

ABSTRACT

Twelve apricot (*Prunus armeniaca* L.) genotypes were collected from two valleys *viz.*, Nubra (9,600 ft) and Leh (11,500 ft) of trans-Himalayan region of Ladakh (Jammu & Kashmir). These genotypes were characterized using random decamer RAPD primers. Initially, 20 decamer primers were used to screen the 12 accessions of which 16 produced the reproducible amplicons. These primers generated a total of 740 scorable bands with an average of 46.25 bands per primer of which 88.1% were polymorphic. The size of amplicon ranged from 250 to 2,100 bps. The resolving power of primer ranged from 3.5 to 15, whereas the polymorphic bands ranged from 4 to 10. The mean coefficient of gene differentiation (Gst) was 0.1072, indicating 89.28 % of the total genomic diversity within populations. Estimated value of gene flow for RAPD (Nm = 4.164) indicated that there was very high gene flow among the populations. Similarity index value ranged from 0.49 to 0.84 indicating the high level of genetic diversity within the 12 apricot accessions. Genotypes from two valleys clustered into three non-specific groups with 4, 7 and 1 genotypes, respectively. Cluster I represented four genotypes of which three were from Leh and one was from Nubra valley. While, cluster II had seven genotypes of which five were from Leh while two are from Nubra valley. The third cluster consisted of only one genotype from Leh valley. The result showed high genetic diversity in apricot grown in Ladakh region of Jammu Kashmir.

Keywords: Apricot, genetic diversity, *Prunus armeniaca*, RAPD.

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is the most important fruit crop of cold arid regions of India which cover 3,200 ha with a total production of 5,200 MT (Dwivedi and Attrey, 2). Apricot is believed to have originated in Sogdiana, western China. Wild apricot popularly called as *Zardalu* appears to be originated in Indian Himalayas. In apricot, three regions have been identified as the centre for origin, *i.e.* (1) Chinese Centre (China and Tibet), (2) Central Asia (from Tien-Shan to Kashmir, India) and (3) Near-Eastern (Iran, the Caucasus and Turkey) (Vavilov, 18). The Central Asian group is the oldest and richest in biodiversity includes local apricots from central Asia, Xinjiang, Afghanistan, Baluchistan, Pakistan and northern India (Kashmir). It is believed to have been introduced in cold arid Ladakh *via* Baltistan though some experts opined that it was introduced directly from China *via* Tibet.

Classical approaches for identification and analysis of genetic variability in fruit crops are based on morphological, physiological and agronomic traits (UPOV, 17). However, these traits have limitations as they are influenced by environmental factors. Also, the morphological and agronomic traits are limited and do not cover the entire genome, which limits their use in assessing genetic diversity. With the advent of newer techniques based on biochemical methods like isozyme genetic variability in different *Prunus armeniaca* fruit crops have been distinguished (Badenes *et al.*,1), but due to low polymorphic levels, has led to search for alternatives like DNA markers, which are independent of environmental conditions and unaffected by developmental stages of the plant. Different molecular markers such as RFLP, RAPD, AFLP and microsatellite markers are commonly employed to identify different apricot cultivars. A large number of polymorphic markers are required to measure the genetic relationships and genetic diversity in a reliable manner (Santalla *et al.*, 13). DNA marker such as RAPD (Williams *et al.*, 19) is quite stable and highly polymorphic in nature. DNA markers simply detect differences in genetic information. In other words, they are based on polymorphism in DNA sequences carried by two or more individuals. The use of DNA based

*Corresponding author's E-mail: meetulkumar@gmail.com
**JUIT, Waknaghat, Solan, HP.
***PEACE Metcalf House, New Delhi.
****DARL, DRDO, Pithoragarh, Uttarakhand .

genetic markers is efficient and reliable also in the case of species having low genetic diversity, such as peaches, nectarines and almonds. In present study, RAPD marker technique was used to determine the genetic variability in apricot accessions from two different valleys, i.e. Leh and Nubra of Ladakh region in India. To our knowledge, no report has been reported on the genetic diversity, population structure and gene flow among the populations of *Prunus armeniaca* in the trans-Himalayan region with molecular markers like RAPD for diversity analysis. In this study, we investigated the genomic relationship among the different populations of *Prunus armeniaca* and their relationship in Ladakh region, with the aim to provide insight to facilitate conservation management of natural populations.

MATERIALS AND METHODS

The plant materials used in the study were obtained from two valleys (Leh and Nubra) with altitude ranging from 9,600 (Nubra) to 11,500 m (Leh) from the cold arid desert of Ladakh (Table 1). There are 12 genotypes collected in previous years and maintained at the Defence Institute of High Altitude Research (DIHAR), Leh were selected for the present investigation. About 5 g of young leaves from each representative plant (termed as clones) were collected in aluminium foil and dipped in liquid nitrogen for few minutes and then stored at -80 °C until use.

Total genomic DNA was extracted from frozen leaves (5 g) by the CTAB method (Saghai-Maroof *et al.*, 12) with minor modifications, which included the use of 200 mg per sample polyvinyl pyrollidone (PVP, MW: 40,000).

Twenty random decamer primers (IDT Tech, USA) were used individually as primers for RAPD analysis of which 16 primers produced reproducible amplicons (Table 2) . DNA was amplified following the protocol of Williams *et al.* (19). Amplification reactions were performed in volumes of 25 µl containing 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM of each dNTPs, 0.4 µM primer, 20 ng template DNA and 0.5 unit of *Taq* polymerase (Sigma). DNA amplification was performed using a Biometra Gradient (Germany), thermal cycler. The first cycle consisted of denaturation of template DNA at 94 °C for 5 min, primer annealing at 37 °C for 1 min and primer extension at 72 °C for 2 min. In the next 40 cycles, the period of denaturation was reduced to 1 min at 92 °C, while the primer annealing and primer extension time remained the same as in the first cycle. The last cycle consisted of only primer extension (72 °C) for 7 min. The PCR products were stored at 4 °C before analysis.

Amplification products were electrophoresed on 1.5% agarose gel (Life Science Technologies, USA) run at constant voltage (50 V) in 1 × TBE for approximately 2 h visualized by staining with ethidium bromide (@0.5 µ g ml^{-1}) and a total of 2.5 µl loading buffer (1.0 × TBE, 50% glycerol, 0.25% xylene cyanol) was added to each reaction before electrophoresis. After electrophoresis, the gels were observed under an UV-transilluminator, documented on a gel documentation

Table 1. Details of 12 apricot genotypes used in the investigation.

S. No.	Genotype	Valley	Year of collection
1	Afghani	Nubra	1999
2	Special R Karpo	Nubra	2002
3	Khantay	Nubra	2005
4	Koban	Nubra	2006
5	Kaisi	Nubra	2006
6	Halman	Leh	1999
7	Nari	Leh	1999
8	Khantay	Leh	2000
9	Nari Special	Leh	2001
10	Afghani	Leh	2001
11	Safeda 1	Leh	2003
12	Khantay	Leh	2004

Table 2. List of 20 RAPD primers used for analysis in different apricot accessions.

S. No.	Primer	Primer sequence (5'-3')	Tm (°C)
1	S 21	CAGGCCCTTC	36.4
2	S 22	TGCCGAGCTG	40.7
3	S 23	AGTCAGCCAC	34.3
4	S 24	AATCAGCCAC	30.1
5	S 25	AGGGGTCTTG	32.6
6	S 26	GGTCCCTGAC	35.2
7	S 27*	GAAACGGGTG	33.2
8	S 28	GTGACGTAGG	31.1
9	S 29	GGGTAACGCC	37.4
10	S 30	GTGATCGCAG	33.1
11	S 31	CAATCGCCGT	36.7
12	S 32	TCGGCGATAG	34.0
13	S 33	CAGCACCCAC	37.7
14	S 34	TCTGTGCTGG	34.3
15	S 35	TTCCGAACCC	34.2
16	S 36	AGCCAGCGAA	38.3
17	S 37*	GACCGCTTGT	35.7
18	S 38*	AGGTGACCGT	36.2
19	S 39	CAAACGTCGG	34.2
20	S 40*	GTTGCGATCC	33.5

Note: * Primers which did not get amplified.

system. Molecular size of amplicons was estimated using a 100 bp DNA ladder (Bangalore Genei, Bangalore). The amplification for each primer was performed twice independently with same procedure in order to ensure the fidelity of RAPD.

Data obtained with RAPD was subjected to similarity matrix and cluster analyses using the Numerical Taxonomy and Multivariant Analysis System program package for PC (NTSYS-pc, version 2.11U; Rohlf, 11). The bands were scored as 1 (present) or 0 (absent) for all markers and for all individuals in the study, and a binary matrix was constructed from the data. A conservative criterion for the selection of bands was used. Only reproducible and well-defined bands for each of the tree replications were considered as potential polymorphic markers. Using the raw data, similarity matrix values were calculated using simple matching coefficient. Percentage of polymorphic bands was defined as the percentage of the number of polymorphic bands amplified by a single primer to that of the total number of bands produced by the same primer. The dendrogram was constructed employing an unweighted pair group method with arithmetic averages (UPGMA) using sequential agglomerative, hierarchical and nested cluster (SAHN) (Sneath and Sokal, 16). The data matrix of RAPD were also used for assessment of genetic structure, genetic differentiation, gene flow and diversity. Measurement of diversity including gene diversity (H), gene flow and Shannon's information index (I) were estimated by POPGEN 1.32 software. The RAPD data was subjected to a hierarchical analysis of molecular variance (AMOVA) as described by Excoffier *et al.* (4) using three hierarchical levels; individual, population and their regions. The analysis was performed using GenALEx software (Peakall and Smouse, 9). Principal component analysis (PCA) was also estimated.

The resolving power of the primers used in the present study was calculated using the formula: Resolving power (Rp) = ΣIb; where Ib is the band Informativeness. Band Informativeness was calculated for each band scored by the primer individually; Ib = 1[2(0.5-p)]; p is the proportion of occurrence of band in the species out of total number of species screened (Prevost and Wilkenson, 10).

RESULTS AND DISCUSSION

Considering the high genetic differentiation among the different apricot populations, conservation of only a few genotypes may not adequately protect the genetic variation within the species in this fragile trans-Himalayan region. At present, the rate of propagation of *Prunus armeniaca* is far less than the rate of its exploitation. This species or at least a large part of its genetic diversity may be lost in the near future owing to its importance and consequent exploitations as a fruit plant, if appropriate conservation measures are not adopted. In the present study, an attempt has been made to examine the level of genetic variation within apricot genotypes and probably it is the first report on fingerprinting of existing apricot genotypes from Ladakh.

Twelve genotypes from two valleys were amplified using 16 RAPD makers which generated a total of 126 bands (an average of 7.88 bands per primer) out of which 111 (88.10 %) were polymorphic and only 15 (11.90 %) were monomorphic bands. Number of bands was maximum for primer S21 (90 bands) and minimum for primer S36 (21 bands). The size of amplified fragments produced ranged from 250 to 2,100 bp. The percent polymorphism ranged from 77.78 (S34) to 100 (S 23, 25 and 36). The resolving power (Rp) of the 16 RAPD primers ranged from 3.5 for primer S36 to 15.0 for S21. Three RAPD primers (S21, 31 and 34) possess the higher Rp values (15.0, 11.16 and 12.16 respectively) and were able to distinguish all 12 genotypes (Table 3).

Data for observed number of alleles, effective number of alleles, Nei's genetic diversity, Shannon's information index, for both the populations were analyzed using sixteen RAPD markers and their respective values were found as 1.992, 1.545, 0.328 and 0.499. Value for total genotype diversity among population (Ht) was 0.328 while within population diversity (Hs) was found to be 0.293. The mean coefficient of gene differentiation (Gst) was 0.1072, indicating 89.28% of the total genetic diversity within the populations. Based on the Gst value, the mean estimated number of gene flow (Nm) between populations was found to be 4.164 (Table 4). Nei's gene diversity (H) values calculated for RAPD, 0.328±0.131 which showed overall 25.14 ± 2.43 % to 25.14 ± 4.32% heterozygosity. Studying the sampling variance of heterozygosity and genetic distance estimates, Nei (8) reported that a relatively reliable estimate of average heterozygosity can also be obtained from a small number of individuals if a large number of loci are examined. Similarly, the Shannon's information indices (I) was 0.499 ± 0.157 for RAPD, which shows gene diversity measurement among the different valleys with an average of 3% among the groups (Table 4). Three percent molecular variance was found among population, while within the population, this value was found to be 97% (Table 5) indicating that almost all the variations are present within the population.

Polymorphism in a given population is often due to the existence of genetic variants represented by the number of alleles at a locus and their frequency of distribution in a population. Heterozygosity corresponds to a probability that two alleles taken at random from a population can be distinguished using the marker in question.

Table 3. Amplified band details of 16 primers and their resolution power (Rp) values of all the DNA samples obtained from 12 apricot genotypes.

Primer No.	No. of total bands	Monomorphic bands	Polymorphic bands	Polymor-phism(%)	Total No. of bands	Resolution power
S21	12	02	10	83.33	90	15.00
S22	08	01	07	87.5	32	05.33
S23	07	0	07	100	33	05.50
S24	07	01	06	85.7	48	08.00
S25	04	00	04	100	29	04.83
S26	07	01	06	85.7	28	04.66
S28	06	01	05	83.3	38	06.33
S29	08	01	07	87.5	50	08.33
S30	06	01	05	83.3	31	05.16
S31	10	01	09	90.0	67	11.16
S32	08	01	07	87.5	41	06.83
S33	10	01	09	90	59	09.83
S34	09	02	07	77.78	73	12.16
S35	09	01	08	88.89	49	08.16
S36	04	0	04	100	21	03.50
S39	11	01	10	90.10	51	08.50
Total	126	15	111	88.10	740	7.705

Table 4. Details of genetic variability across 12 apricot genotypes using 16 RAPD primers.

Observed No. of alleles	Effective No. of alleles	H	I	Ht	Hs	Gst	Nm
1.992 (0.089)	1.545 (0.298)	0.328 (0.131)	0.499 (0.157)	0.328 (0.017)	0.293 (0.015)	0.107	4.163

(where, H = Nei's Gene Diversity; I = Shannon's Information Index; Ht = Total genotype diversity among population; Hs = Within population diversity; Gst = Mean coefficient of gene differentiation; Nm = Mean estimated number of gene flow).

Table 5. Summary of analysis of molecular variance (AMOVA) based on sixteen RAPD data of twelve apricot genotypes (levels of significance are based on 1000 iteration steps).

S. No.	Source of variance	df	SSD	Variance component	Percentage	P-value
1	Among groups	1.00	26.60	0.75	03	Not significant
2	Among population within groups	10.00	222.22	22.22	97	0.169
3	Total	11.00	248.83			

(where df = degree of freedom; SSD = sum of square deviation; P-value = Probability of null distribution).

Twelve accession of apricot were fingerprinted by using 16 RAPD markers. The genotypes were selected on the basis of year of collection and the valleys from where they were taken. These RAPD markers produced 740 bands with an average 46.25 bands per primers. A high percentage of polymorphism was detected in both the apricot populations. The observed high proportion of polymorphic loci suggests that there is a high degree of genetic variation existing among the two apricot populations. The genetic variation estimated by RAPD markers are PPB (% Polymorphic bands) = 88.10 %, I = 0.499±0.157 at the populations level of apricot. The

RAPD primers in the present study yielded 111 polymorphic markers that unambiguously discriminated 12 genotypes into 3 clusters. Geographically isolated population accumulates genetic differences as they adapt to different environments. Variations between the populations of different valleys are not significant. This might be due to the fact that apricot is clonally propagated. In population genetics, a value of gene flow (Nm) < 1.0 (less than one migrant per generation into a population) or equivalently, a value of gene differentiation (Gst) > 0.25 is generally regarded as the threshold quantity beyond which significant population differentiation occurs (Slatin, 15). In the present study, Nm and Gst was found as 4.163 and 0.107 respectively. The average resolving power (Rp) of RAPD primers was 7.705. There is seemingly linear relationship between the Rp of each primer and the number of recorded marker. Prevost and Wilkinson (10) have studied the nature of this relationship using a total of 371 hypothetical primers producing 8, 10 or 12 band position (r = 0.98). The evolution of varieties in distinct agro-climatic zones demonstrates significant levels of variation in response to the selection pressure in the zones (Singh *et al.*, 14).

Genetic similarity was calculated from the Jaccard similarity index value for all the 12 apricot genotypes. Based on RAPD marker, the similarity index values ranged from 0.49 to 0.80. These values were used to construct a dendrogram using UPGMA. Genotypes from two valleys clustered into three non-specific groups. The genotypes were distributed into three main clusters with 4, 7 and 1 genotypes respectively (Fig. 1). Cluster I represented four genotypes of which three (Sp_R_Karpo_2002, Kaisi_2006 and Afghani_1999) are from Leh valley while one (Khantay_2000) was from Nubra valley. Cluster II had seven genotypes of which five (Nari_1999, Nari_sp_2001, Safeda1_2000, Afghani_2001, Khantay_2004) were from Leh valley while two (Khantay_2005 and Koban_2006) from Nubra valley. The third cluster consisted of only one genotype i.e. Halman_1999 from Leh valley. The result indicated high genetic diversity in apricot grown in Ladakh region, of Jammu & Kashmir. Bootstrapping was done using the Treeview (http//www.treeview.net) programme to estimate the relative support at clades. The robustness of the cluster was not very strong in selected genotypes. The results of PCA analysis were comparable to the cluster analysis. The first three most informative PC components explained 50.99 % total variation. Genotype Halman_1999 appear to be distinct from other genotypes in the PCA too (Fig. 2). There were three Khantay genotypes collected from both the valleys of which Khantay_2000 (from Leh) was in cluster I, while Khantay_2004 (from Leh) and Khantay_2005 (from Nubra) grouped in the cluster II. Similarly, Afghani_1999 (from Nubra) and Afghani_2001 (from Leh) clustered in clusters I and II, respectively. These results indicate that the genetic purity of the genotypes was not maintained both within and between the valleys. Nari and Nari Special were together in cluster II. It means that both the genotypes are not much different at the genotypic level too. There is no significant difference with respect to altitude for the genetic variability and genotypes were scattered randomly in different clusters representing the two valleys.

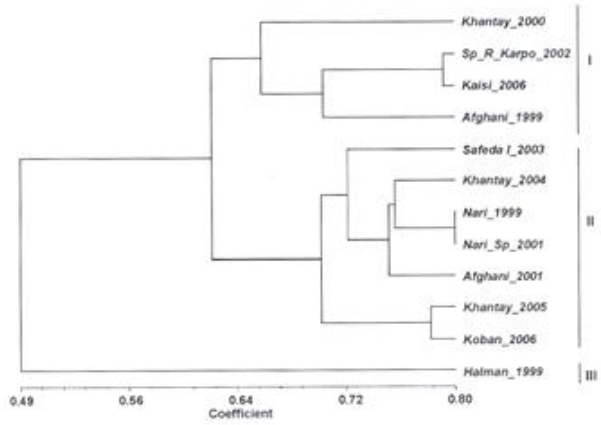

Fig. 1. Dendrogram illustrating genetic relationships among 12 apricot genotypes generated by UPGMA cluster analysis calculated from 740 RAPD bands produced by 16 primers. The numbers at the forks indicate the confidence limits for the grouping of those species in a branch occurred, based on 1,000 cycles in bootstraps analysis.

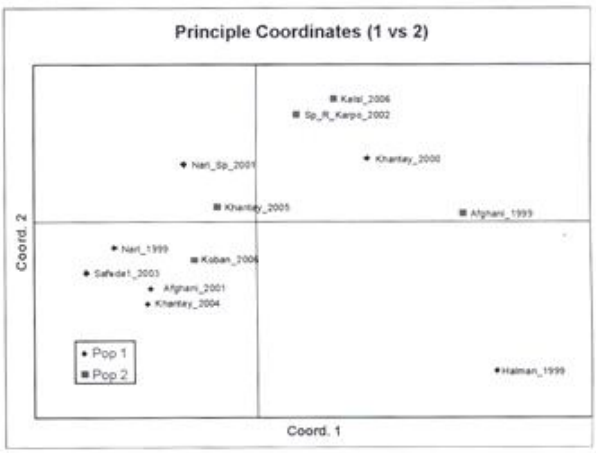

Fig. 2. Two-dimensional plot of principle component analysis of twelve elite apricot genotypes using RAPD analysis. The numbers plotted represents individual genotypes. (where, Pop 1 and Pop 2 represents Leh and Nubra valleys, respectively).

Cluster analysis using RAPD markers have revealed independent distribution of different genotypes with respect to their geographical locations. This indicated that gene flow in the apricot genotypes occurred within and between the valleys. Moreover, RAPD markers used here were able to differentiate the apricot populations collected from the valleys. The study also indicates that *Prunus armeniaca* genotypes in trans-Himalayan region are highly genetically diverse. The high genetic variations in apricot may be attributed partially to the cross-pollinated nature of *P. hexandrum*. Although clonal propagation contributes towards genetic uniformity within each population, Hangelbroek *et al.* (6) reported that clonal plant species can have high levels of genetic variation in some cases. The high gene flow among genotypes detected in this study point towards the possibility of instances of several isolated populations possessing unique genotypes not found in other populations. Based on polymorphic feature, genetic diversity, genetic similarity, gene flow among the populations of apricot based on RAPD study we recommend that any future conservation plans for this species should be specifically designed to include representative populations with the highest genetic variation for both *in situ* conservation and germplasm collection expeditions.

Dendrogram in the present study did not indicate very clear pattern of clustering according to the altitude or valleys in which they were collected. Similar results were obtained in Azukibean (Yee *et al.*, 20) and groundnut (Dwivedi *et al.*, 3). Cluster III is unique as it contains only one genotype from Leh valley, i.e. Halman_1999. Similar results were observed in blackgram (Gaffor *et al.*, 5) and mungbean (Lakhanpaul *et al.*, 7).

ACKNOWLEDGEMENTS

The authors are thankful to DRDO HQ for its support and funding the project. The encouragement and guidance received from Dr W. Selvamurthy, DS & CC R&D (LS & HR), Dr Narendra Kumar, Sc H, DOP and Dr RC Sawhney, Sc G, DLS, DRDO HQ, New Delhi, are also gratefully acknowledged.

REFERENCES

1. Badenes, M.L., Asíns, M.J., Carbonell, E.A. and Llácer, G. 1996. Genetic diversity in apricot (*Prunus armeniaca*) aimed at improving resistance to plum pox virus. *Plant Breed.* **115**: 133-39.

2. Dwivedi, S.K. and Atrey, D.P. 2002. Possibilities of apricot production in high altitude regions of Uttaranchal. **In**: *National Seminar on Development of Horticulture in Uttaranchal.* Directorate of Horticulture, Govt. of Uttaranchal, Dehradun. 3-4 September, 2001, pp.17-20.

3. Dwivedi, S.L., Gurtu, S., Chandra, S., Yuejin, W. and Nigam, S.N. 2001. Assessment of genetic diversity among selected groundnut germplasm using RAPD analysis. *Plant Breed.* **120**: 345-49.

4. Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analyses of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics,* **131**: 479-91.

5. Gaffor, A., Sharif, A., Ahmad, Z., Zahid, M.A. and Rabbani, M.A. 2001. Genetic diversity in blackgram (*Vigna mungo* L. Hepper). *Field Crop Res.* **69**: 183-90.

6. Hangelbroek, H.H., Ouborg, N.J., Santamaria, L. and Schwenk, K. 2002. Clonal diversity and structure within a population of pondweed *Potamogeton pectinatus* foraged by Bewick's Swans. *Mol. Ecol.* **11**: 2137-50.

7. Lakhanpaul, S., Chadha, S. and Bhat, K.V. 2000. Random amplified polymorphic DNA (RAPD) analysis in Indian mungbean [*Vigna radiata* (L.) Wilczek] cultivars. *Genetica,* **109**: 227-34.

8. Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individual. *Genetics,* **89**: 583-90.

9. Peakall, R. and Smouse, P.E. 2001. GenAlEx V5: Genetic Analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia, (http://www.anu.edu.au/BoZo/GenAlEx/)

10. Prevost, A. and Wilkinson, M.J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* **98**:107-12.

11. Rohlf, F.J. 1998. NTSYSpc. **In**: Numerical Taxonomy and Multivariate Analysis System. Version 2.0. Exeter Softwater, Setauket, New York.

12. Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. 1984. Ribosomal spacer length in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci.* **81**: 8104-18.

13. Santalla, M., Power, J.B. and Davey, M.R. 1998. Genetic diversity in mungbean germplasm revealed by RAPD markers. *Plant Breed.* **117**: 473-78.

14. Singh, A.K., Smart, J., Simpson, C.E. and Raina, S.N. 1998. Genetic variation *vis-a-vis* molecular polymorphism in groundnut (*Arachis hypogaea* L.). *Genet. Resour. Crop Evol.* **45**: 119-26.

15. Slatin, M. 1987. Gene flow and geographic structure of natural populations. *Science,* **236**: 787-92.

16. Sneath, P.H.A. and Sokal, K. 1973. **In**: *Numerical Taxonomy*. WHF, San Francisco. pp. 100-308.

17. UPOV. 1976. Guidelines for the conduct of test for distinctness, homogeinity and stability of the cherry. UPOV, TG/35/3.

18. Vavilov, N.I. 1951. Phytogeographic basis of plant breeding. The origin, variation, immunity and breeding of cultivated plants. K.S. Chester [Translated]. *Chronol. Bot.,* **13**: 13-54.

19. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers and useful as genetic markers. *Nucleic Acids Res.* **18**: 6531-35.

20. Yee, E., Kidwell, K.K., Sills, G.R. and Lumpkin, T.A. 1999. Diversity among selected *Vigna angularis* (Azuki) accessions on the basis of RAPD and AFLP markers. *Crop Sci.* **39**: 268-75.

Received: June 2008; Revised: March 2009;
Accepted : April 2009