GENETIC DIVERSITY AMONG ASPARAGUS SPECIES USING MORPHOLOGICAL CHARACTERISTICS AND RAPD MARKERS IN PAKISTAN

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Abstract

The aim of present research was to study the genetic diversity among *Asparagus* species and its cultivars using morphological characteristics and RAPD markers. *In-vitro* and field experiments of 14 germplasm sources of *Asparagus* species and its cultivars were conducted at the Kohat University of Science and Technology to estimate the comparative performance. Highest genotypic variance, phenotypic variance, genotypic and phenotypic coefficient of variance were observed in shoot height in shoot related traits were 318.40, 320.30, 939.34 and 944.96, respectively, whereas highest values for root related traits were observed in root length that were 21.84, 22.35, 141.60 and 144.91 respectively. Among the shoot related traits maximum heritability, genetic advance and genetic gain were calculated for shoot length, while in root related traits these characters were highest for root dry weight. RAPD markers were used to evaluate genetic diversity analysis of 14 germplasm sources of *Asparagus* species and its cultivars. RAPD markers generated a total of 247 bands, of these 239 bands were polymorphic with average of 34.1 bands per primer. Cluster analysis based on Neighbor Joining methods showed that wild species (*A. adsendens, A. densiflorus, A. capitatus, A. gracelus, A. plumosus, A. racemosus* and *A. setaceus*) were genetically distant from *A. officinalis* and its cultivars (Abril, Apollo, Gersengum, Huchel, Para seletion and Taranga). The results of the present investigations could be particularly used for authentic identification and would be useful for evaluation of genetic improvement of *Asparagus* species and its cultivars.

***Key words*:** Seed sources; *Asparagus officinalis*; Genetic advance; Heritability; RAPD; Genetic diversity.

INTRODUCTION

Asparagus Linnaeus (1753) belongs to the family Aspargaceae, which are extensively distributed all over the world. The natural habitats of *Asparagus* are tropical and sub-tropical regions. It is a perennial, herbaceous plant having a height of 100-150 cm. The roots of *Asparagus* are moist tuberous extending up to 30-100 cm and up to 2 cm in thickness. The roots are arranged in cluster form, attached at the base of the stem. The stem is fat having many soft branches. The leaves are needle like having a length of 6-12 mm and width of 1 mm, arrange in clusters. Flowers are greenish-white to yellowish, and round in shape. The fruits are small red berry, 6-10 mm in diameter and are poiso*nou*s to human (Chen *et al.* 2000).

Asparagus prefers different types of soil for their growth including wobbly soil which is suitable for its profound growth. Naturally *Asparagus* is grown in slightly alkaline soils (Thompson and Kelly 1957). Many species of *Asparagus* are valued vegetable crops; whereas *A. adscendens*, *A. capitatus* and *A. racemosus* are widely used for medicinal purposes. *A. densiflorus, A. setaceus* and *A. plumosus* have economic importance for both horticultural and ornamental purposes. *A. adscendens* are specifically confined to Kashmir regions of Pakistan and considered as important medicinal plants that can be used for various medical purposes including antifungal, anti-inflammatory, anti-mutagenic, diuretic and as anti-cancer drug. Roots of *Asparagus* are found to possess antioxidant, anti-ADH activity, anticancer

activity, anti-ulcerogenic activity, anti-inflammatory activity and antimicrobial activity (Mandal *et al*. 2000). It has been also used for cleaning, strengthening and nourishing female reproductive system. It is also used for premenstrual syndrome (PMS) and also for sexual weakness (Frawley 1989). The main active components of the roots of *Asparagus* are saponins and steroidal, while Asparagine, Arginine, Tyrosine, Flavonoids (kaempferol, quercetin and rutin), Tannin and Resin are other primary chemical constituents (Negi *et al*. 2010).

Conventionally, evaluation and characterization of genetic diversity are based on the variation in quantitative and qualitative traits. The evaluation of agro-morphological traits does not usually require complex experiments and advance instruments. Therefore, experiments for these traits are relatively inexpensive, rapid and simple to analyze. For this reason, to evaluate genetic variation morphological parameters are important (van Beuningen and Busch 1997).

RAPD is based on the amplification of genomic DNA with single primers of arbitrary nucleotide sequence (Williams *et al*. 1990). RAPD primers identify polymorphism in the absence of definite nucleotide sequence information. It is not possible to differentiate the amplified DNA fragment whether it is homozygous (two identical copies) or heterozygous (two different copies) at exact locus because RAPD markers are mostly dominant. In recent years, fingerprinting systems based on RAPD study have been progressively more utilized for detecting genetic polymorphism in several plant genera. Due to technical simplicity and speed, RAPD methodology has been used for diversity analysis in many medicinal plant species (Li *et al*. 2002). PCR based RAPD markers have been widely used in assessing genetic variation within a species by measuring genetic diversity in many species, including medicinal plants.

MATERIAL AND METHODS

Germplasm and leaf samples of *Asparagus* species were collected from different regions of Pakistan including Islamabad, Lahore, Kohat and Swat as indicated in *flora of Pakistan*. The samples were collected and immediately stored. Sampling areas and geographical description are presented in Table 1.

Table 1. Collection sites and environmental parameters for *Asparagus* species and cultivars.

Species & Cultivars	Type	Collecting sites	Altitude	Latitude	Longitude
Asparagus officinalus	Vegetative	NARC, Islamabad	490 m	33° 43'N	73° 04' E
A. officinalus C.V. Abril	CV	ARI, Mingora	984 m	34°78' N	72°36' E
A. officinalus C.V. Appollo	CV	ARI, Mingora	984 m	34°78' N	72°36' E
A. officinalus C.V. Gersengum	CV	ARI, Mingora	984 m	34°78' N	72°36' E
A. officinalus C.V. Huchel	CV	ARI, Mingora	984 m	34°78' N	72°36' E
A. officinalus C.V. Para selection	CV	ARI, Mingora	984 m	34°78' N	72°36' E
A. officinalus C.V. Taranga	CV	ARI, Mingora	984 m	34°78' N	72°36' E
A. adsendens	Medicinal	Jerma, (Kohat)	489 m	33° 58'N	71° 43' E
A. capitatus	Medicinal	Ghalegay (Swat)	950 m	34°69' N	72° 26' E
A. gracelus	Medicinal	Shamozu (Swat)	974 m	34°51' N	72°25' E
A. densiflorus	Arnamental	More green Nursery (Lahore)	217 m	31° 34'N	74° 22' E
A. plumosus	Medicinal	Mingora (Swat)	950 m	34° 69′N	72°26′ E
A. racemosus	Medicinal	Charbhage (Swat)	1032 m	34° 83'N	72° 44'E
A. setaceus	Arnamental	Bhage Jinnah (Lahore)	209 m	31° 54′N	74° 33′E

Germination of Asparagus

Germplasm of *Asparagus* species was sterilized by treating with 3% sodium hypochlorite and then washed three times with sterile water. One percent Agar medium was prepared by adding one gm Agar into 100 ml distilled water and sterilized for 20 minutes at 120°C. Agar medium was cooled up to 50^{o}C and poured around 20 ml medium into Petri dishes. After solidification of the medium, healthy seeds that sank in the water were selected and inoculated for germination under sterilized condition by placing

them at 25°C ± 2°C in a growth chamber. The swollen seeds with green embryos were observed after 12 days of incubation and were considered as germinated (Hailes and Seaton 1989).

Morphological traits

The field trial was established each with ten replications and ten seeds in a plot for evaluation of the *Asparagus* growth performance. The optimum seed to seed distance was 5 cm and row to row distance was 10 cm in order to achieve the full coverage of the soil which minimized the competition with weeds and assured best performance. The germination of germplasm was started after twenty days. Plants were harvested at the age of two and half months, and growth performance data for nine different morphological traits of the seedlings were recorded that included number of shoot, shoot height, shoot weight (wet and dry), number of roots, root length, root diameter and root weight (wet and dry) for each plant.

RAPD Primers and RAPD PCR amplification

A total of seven RAPD primers (Operon Technologies, Alameda, CA, USA) was used for PCR amplification of DNA template of *Asparagus* species and *A. officinalis* cultivars. 7 RAPD primers including (RPI 4, RPI 5, TIBM BA-04, TIBM BA-14, TIBM BB-12, TIBM BB-13, and TIBM BD-19) are illustrated in Table 2.

The PCR reaction mixture with a total volume of 25 µl containing 0.5 µl of assay buffer, 0.2 mM dNTPs, , 1U of Taq DNA polymerase, 1.5 mM of $MgCl_2$, 0.2 pico moles of primers, 50 ng of template DNA. DNA amplification was performed with arbitrary Polymerase Chain Reaction (PCR) in an ABI thermal cycler (Applied Biosystem Inc, USA). Each of the 35 PCR thermal Cycles standardized for this study, consisted of denaturation of DNA at 94 ºC for 45 seconds, primer annealing at 37 ^{0}C for 1 minute and primer extension at 72 ^{0}C. All PCR samples were subjected to an initial denaturation at 94 ºC for 5 minutes and final extension at 72 ºC for 10 minutes followed by hold temperature at 4 ^{0}C. Amplified PCR products were size-separated by carrying out electrophoresis on 1.5 % agarose gels, stained with ethidium bromide in 1×TBE buffer for 1 hour at 125 V. Gels with amplified products were visualized and photographed using Gel documentation system.

Data analysis

The genotypic and phenotypic components and variances for the various traits among *Asparagus* species were calculated by following the procedure described by Burton (1952). By using the following formula $h^2 = \sigma^2g/\sigma^2p$, broad sense heritability (h^2) was estimated where σ^2g is genotypic variance and σ^2p is phenotypic variance (Lush 1949). The genetic advance was calculated as described by Johnson *et al*. (1955) for all traits studied. Genetic advance (Gs) was calculated as $Gs = K \cdot h^2 \cdot \sqrt{\sigma^2p}$, where K is the selection differential [2.06 at 5% selection intensity (Cotterill and Dean 1990)], and $\sqrt{\sigma^2p}$ is Phenotypic standard deviation. The expected genetic gain, percent of mean was calculated following the procedure of Burton and Devane (1953). Genetic gain was calculated as Genetic gain = (*Gs* / mean) × 100, where *Gs* = Genetic Advance. The correlation among the traits was analyzed to find out the relationship of one trait and its influence on other traits using Mstat C and statistic 9 software. Unequivocally, scorable and consistently reproducible amplified DNA fragments were transformed into binary matrix (1 = presence, 0 = absence). Data analyses were conducted using only the polymorphic bands. Genetic similarity matrix was generated using Nei genetic similarity coefficient (Nei and Li 1979), the cluster analysis was performed based on Neighbor joining algorithm and dendrogram was constructed by help of NTSYSpc 2.02 software (Rohlf 2000).

Analysis of the amplified DNA fragments for RAPD bands were scored manually as presence (1) or absence (0) of data. The results were analyzed on the principle that a band is considered to be 'polymorphic' if it is present in some individuals and absent in others and 'monomorphic' if present in all individuals. Numerical Taxonomy System (NT-SYS), version 2.11 from applied biostatis Inc. (2002), was used to analyze the result obtained from scoring. For measuring similarity among *Asparagus* species and *A. officinalis* cultivars, Nei and Lie genetic similarity coefficient was used (Nei and Li 1979).

RESULTS AND DISCUSSION

In-vitro seed germination

Asparagus species and cultivars were germinated on Agar medium, After nine days of sowing, germination was started in *Asparagus* species (Fig. 1). After 12 days germination, highest percent germination (100 %) was observed in *A. officinilis*, *A. officinilis* Cv. Abril and *A. officinilis* Cv. Taranga, while lowest percent germination (33.33 %) was observed in *A. capitatus*, *A. racemosus* and *A. setaceus* (Fig. 2).

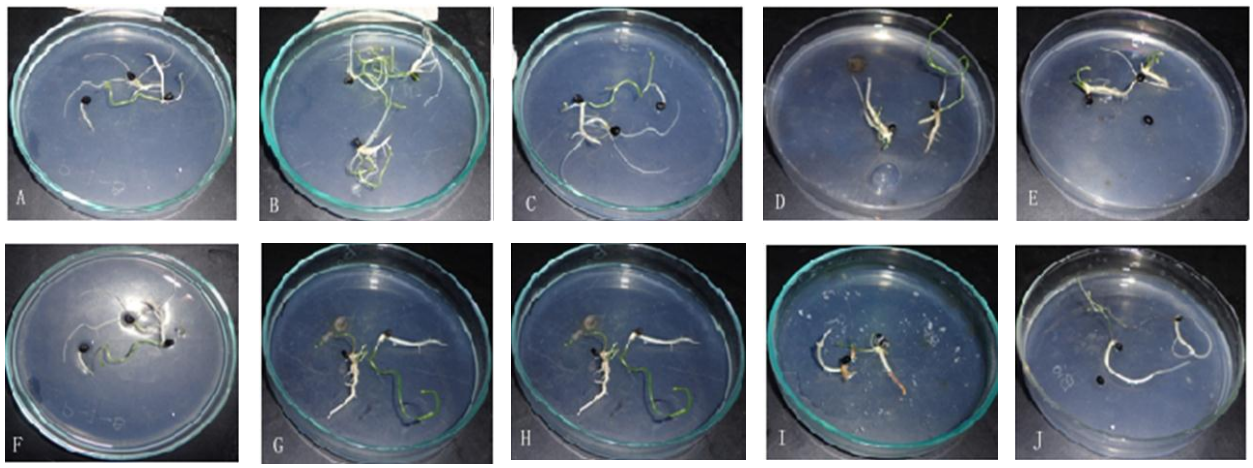

Fig. 1. *Asparagus* growth in Agar medium: **A.** *A. officinalis*; **B.** *A. officinalis* cv. *Taranga*; **C.** *A. officinalis* cv. Huchel; **D.** *A. officinalis* cv. Gersengum; **E.** *A. officinalis* cv. Para; **F.** *A. officinalis* cv. Apollo; **G.** *A. officinalis* Abril; **H.** *A. racemosus;* **I.** *A. adscendens*; and **J.** *A. gracilus.*

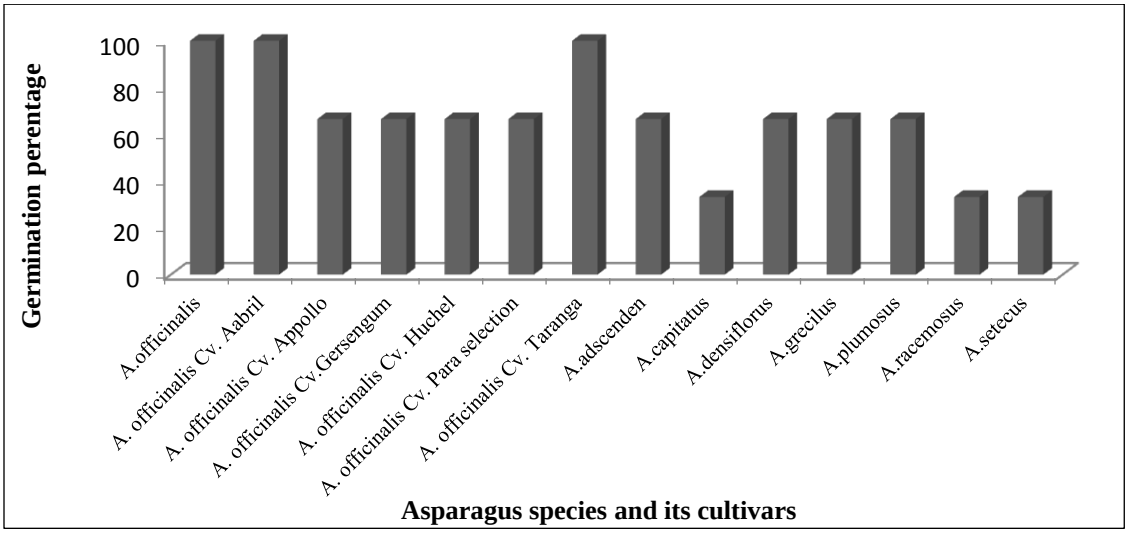

Fig. 2. Percent germination of different *Asparagus* species.

Morphological parameters

For the evaluation of morphological parameters, growth data were collected for *Asparagus* species and were statistically analyzed using MstatC (Table 2). The maximum coefficient of variation (CV) was observed in root diameter (74.50 %), whereas minimum coefficient of variation was observed in shoot height (19.95 %).

Table 2. Morphological parameters studied for different *Asparagus* species.

Static	Shoot				Root				
	Number	Height	Fresh weight	Dry weight	Number	Length	Diameter	Fresh weight	Dry weight
Average	2.49	16.94	0.29	0.09	3.13	7.71	2.01	0.41	0.13
Maximum	6	39	0.89	0.28	8	15	4	1.71	0.48
Minimum	1	3.3	0.04	0.01	1	2.7	0.5	0.03	0.01
SD	1.06	7.87	0.21	0.07	1.39	2.51	1.02	0.35	0.11
cv	35.25%	19.95%	41.77%	49.63%	23.62%	22.69%	74.50%	46.95%	45.15%

The analysis of variance (ANOVA) showed that most of the traits observed for *Asparagus* species were found significant at the significance level of 0.005 (Table 3).

Table 3. Analysis of variance for morphological traits of *Asparagus* species.

Traits	Degree of freedom	Sum of squares	Mean sum of squares	Frequency	P value
No. of shoot	5	6.857	1.371	1.766	0.006
Shoot height	5	227.808	45.562	3.9862	0.003
Shoot fresh weight	5	0.643	0.129	8.6587	0.002
Shoot dry weight	5	0.035	0.007	4.0954	0.003
No. of Root	5	15.821	3.164	5.8745	0.001
Root length	5	33.799	6.76	2.2069	0.004
Root diameter	5	14.238	2.848	1.105	0.006
Root fresh weight	5	1.167	0.233	6.2946	0.001
Root dry weight	5	0.104	0.021	6.69	0.003

Components of variance including genotypic variance, phenotypic variance, genotypic coefficient of variance and genotypic coefficient of variance for the different morphological traits of *Asparagus* species are shown in Table 4.

Table 4. Components of variance for the nine morphological traits of *Asparagus* species.

Character	Genotypic variance	Phenotypic variance	Genotypic coefficient variance	Phenotypic coefficient of variance
No. of shoots	2.4605	2.69	49.21	51.8
Shoot height	318.4	320.305	939.344	944.964
Shoot fresh weight	0.1485	0.151	25.428	25.856
Shoot dry weight	0.02	0.021	12.45	12.65
No. of root	3.085	29.679	49.276	473.954
Root length	21.846	22.357	141.6	144.91
Root diameter	5.165	5.595	119.849	129.814
Root fresh weight	0.485	0.492	59.25	60
Root dry weight	0.052	0.053	21.17	21.37

The maximum genotypic variance was observed for shoot height (318.4), while the minimum genotypic variance was observed for shoot dry weight (0.02). The maximum phenotypic variance, genotypic coefficient of variance and phenotypic coefficient of variance were observed in shoot height (320.305, 939.344 and 944.964), whereas the minimum values were found in shoot dry weight (0.021, 12.45 and 12.65, respectively). The heritability value ranged from 0.10 to 0.99 (Table 5).

Table 5. Genetic parameters for the nine morphological traits of *Asparagus* species.

Characters	Heritability (broad sense)	Genetic advance	Genetic gain
No. of shoots	0.91	2.96	118.22
Shoot height	0.99	36.54	215.59
Shoot fresh weight	0.98	0.78	267.36
Shoot dry weight	0.95	0.28	334.28
No. of roots	0.1	0.38	12.01
Root length	0.98	9.41	121.96
Root diameter	0.92	4.32	200.55
Root fresh weight	0.98	1.41	344.93
Root dry weight	0.98	0.46	371.68

The maximum heritability was found for shoot height (0.99) whereas the minimum value was observed in the number of roots (0.10). The genetic advance value ranged from 0.28 to 36.54. The maximum value was observed for shoot height (36.540) whereas the minimum value was found for shoot dry weight (0.28). In case of genetic gain the value ranged from 12.01 to 317.68 (Table 5). The correlation among nine morphological traits of *Asparagus* species growth ranged from 0.10 to 0.931 (Table 6). The maximum significant correlation was observed for shoot fresh weight (0.931) and shoot dry weight (0.894) whereas the minimum correlation was found between number of shoots and shoots length (0.10).

Table 6. Correlations among nine morphological traits of *Asparagus* species.

Traits	No. of shoots	Shoots length	Shoots fresh weight	Shoots dry weight	No. of roots	Roots diameter	Root length	Root fresh weight
No. of shoots	-							
Shoots length	0.100	-						
Shoots fresh weight	0.590	0.526	-					
Shoots dry weight	0.631	0.545	0.894	-				
No. of roots	0.514	0.583	0.633	0.629	-			
Roots diameter	0.288	0.584	0.479	0.485	0.653	-		
Root length	0.348	0.653	0.617	0.635	0.764	0.614	-	
Root fresh weight	0.325	0.711	0.645	0.666	0.768	0.656	0.852	-
Root dry weight	0.344	0.656	0.661	0.700	0.745	0.620	0.839	0.931

Significant at level of 0.05.

RAPD analysis

RAPD primers were used for genetic diversity among *Asparagus* species and its cultivars. The amplification of DNA generated from 7 random oligonucleotide primers with each of the 14 *Asparagus* species produced a total of 239 polymorphic bands out of 247 produced (Table 7 and Fig. 3).

Table 7. Polymorphism of RAPD markers for *Asparagus* species and its cultivars.

Primers	Sequences	No of bands	Polymorphic bands	Polymorphism
RPl 4	AATCGCGCTG	23	19	82.60 %
RPl 5	AATCGGGCTG	51	47	92.15 %
TIM BA-04	TCCTAGGCTC	26	26	100 %
TIM BA-14	TTCGGCCGAC	24	24	100 %
TIM BB-12	CTTCGGTGTG	61	61	100 %
TIM BB-13	TCGGGAGTGG	41	41	100 %
TIM BD-19	GGTTCCTCTC	21	21	100 %
Total		247	239	-
Average		35.3	34.1	96.4

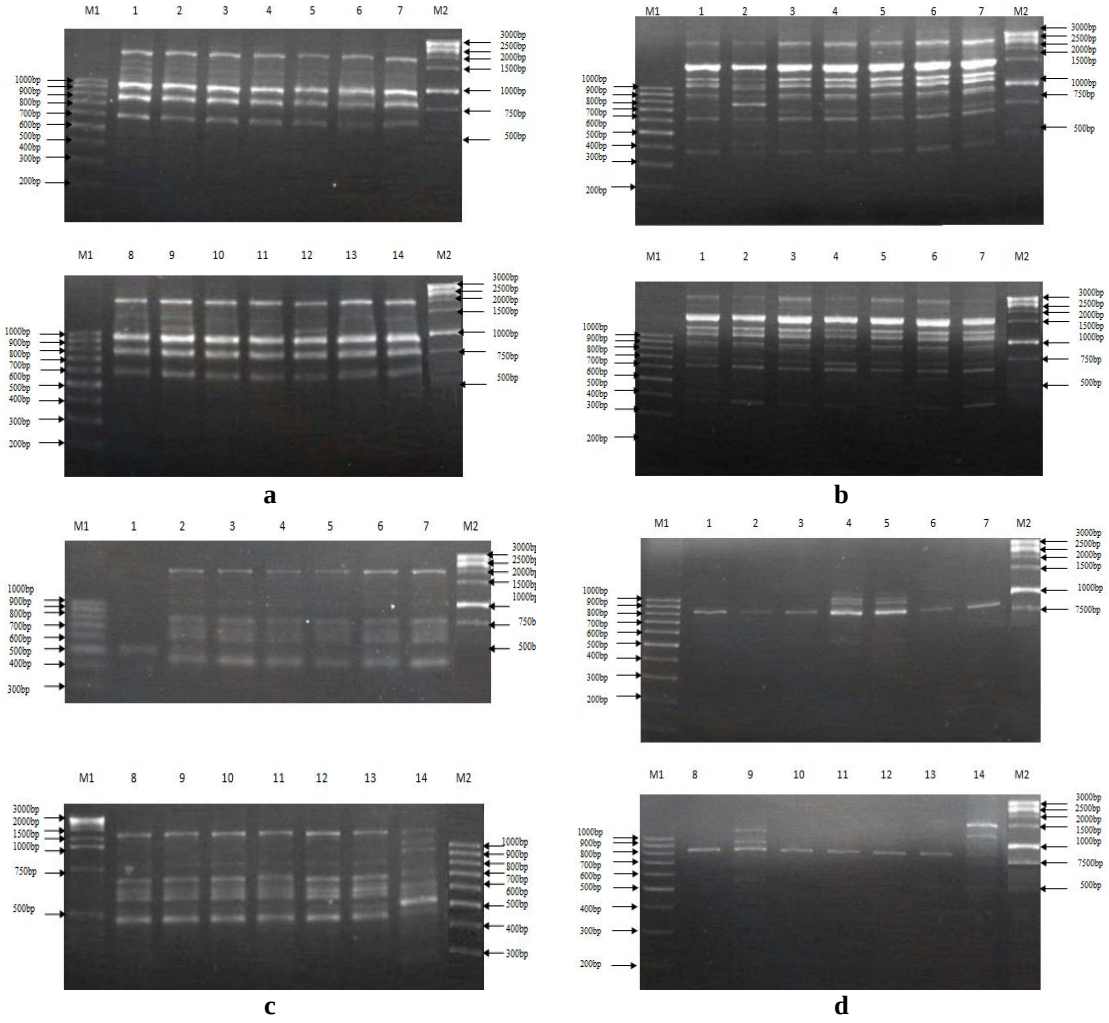

Fig. 3. RAPD pattern generated using primer: **a**. Rpl4; **b**. Rpl5; **c**. TIMBB-13; and **d**. TIMBB-19: Lane M1 = 100bp marker, Lane M2 = 1kb marker, Lane (1) *A. officinalis*, (2) *A.officinilis* Cv. Abril, (3) *A. officinilis* Cv. Apollo, (4) *A. officinilis* Cv. Gersengum, (5) *A. officinilis* Cv. Huchel, (6) *A. officinilis* Cv. Para, (7) *A. officinilis* Cv. Taranga, (8) *A. adsendens*, (9) *A. capitatus*, (10) *A. densiflorus*, (11) *A. plumosus*, (12) *A. racemosus*, (13) *A. setecus*, (14) *A. gracelis.*

The calculation of genetic similarity coefficient was based on 239 polymorphic bands. The number of bands for each primer ranged from 21 to 61 with an average of 34.1 bands per primer. The similarity matrix obtained from RAPD analysis showed similarity coefficient ranged from 0.54 to 0.97 (Table 8).

Table 8. Genetic similarity indexes of *Asparagus* species and cultivars using RAPD analysis.

	A. officinalis	*A. officinalis* Cv. Abril	*A. officinalis* Cv. Apollo	*A. officinalis* Cv. Gersengum	*A. officinalis* Cv. Huchel	*A. officinalis* Cv. Para	*A. officinalis* Cv. Taranga	*A. adscenden*	*A. capitatus*	*A. densiflorus*	*A. plumosus*	*A. racemsus*	*A. setaceus*	*A. gracelis*
A. officinalis	-													
A. officinalis Cv. Abril	0.69	-												
A. officinalis Cv. Apollo	0.85	0.76	-											
A. officinalis Cv. Gersengum	0.71	0.71	0.84	-										
A. officinalis Cv. Huchel	0.74	0.74	0.85	0.85	-									
A. officinalis Cv. Para	0.71	0.71	0.85	0.92	0.82	-								
A. officinalis Cv. Taranga	0.79	0.74	0.94	0.86	0.86	0.90	-							
A. adscenden	0.66	0.71	0.75	0.69	0.76	0.73	0.81	-						
A. capitatus	0.68	0.67	0.75	0.70	0.76	0.73	0.76	0.78	-					
A. densiflorus	0.72	0.72	0.89	0.77	0.77	0.82	0.91	0.85	0.75	-				
A. plumosus	0.72	0.72	0.84	0.77	0.76	0.81	0.89	0.88	0.78	0.93	-			
A. racemsus	0.75	0.75	0.91	0.80	0.76	0.80	0.89	0.82	0.77	0.97	0.91	-		
A. setaceus	0.69	0.69	0.78	0.67	0.75	0.72	0.80	0.87	0.80	0.87	0.86	0.85	-	
A. gracelis	0.54	0.48	0.59	0.55	0.57	0.62	0.64	0.59	0.72	0.61	0.65	0.60	0.54	-

The highest similarity 0.97 was observed between *A. racemosus* and *A. densiflorus*, while lowest similarity (0.54) was observed between *A. officinalis* and *A. gracelis*, and also between *A. gracelis* and *A. setaceus*. In cluster analysis using neighbour joining algorithm, *Asparagus* species were divided into two main clusters (Cluster A and Cluster B) (Fig. 4). Cluster A is further divided into two sub cluster including Cluster I and Cluster II. Cluster I consists of *A. officinalis*, *A. officinalis* cultivars, namely Apollo, Abril, Gersengum, Para selection, Huchel and Taranga, while Cluster II comprised of *A. adscenden*, *A. setaceus*, *A. plumosus*, *A. densiflorus*, and *A. racemosus*. Cluster B comprised of *A. capitatus* and *A.gracelis* (Fig. 4).

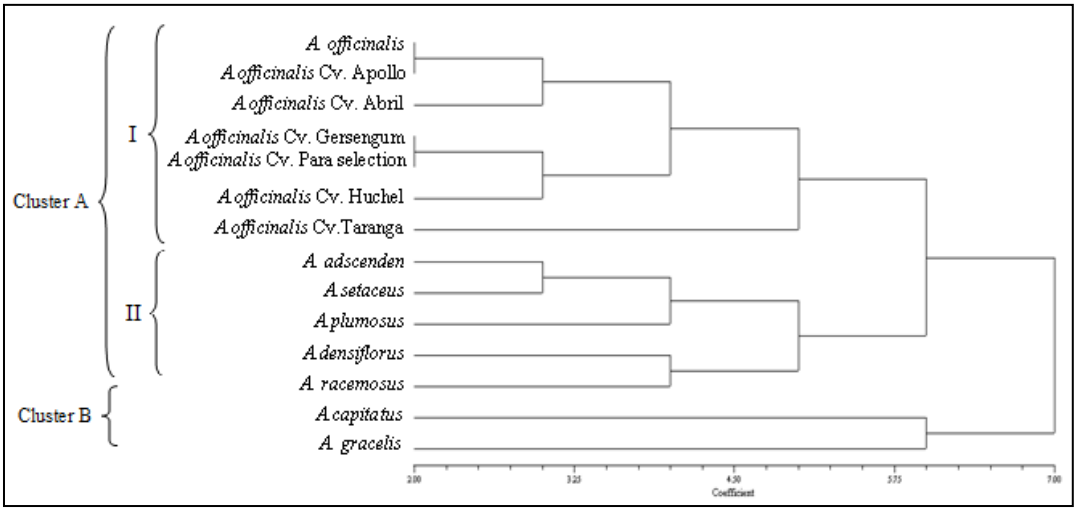

Fig. 4. Phenogram of *Asparagus* species and its cultivars based on Neighbor joining method using RAPD markers.

In the present investigation, *in vitro* germination of *Asparagus* species and cultivars was found successful by forming healthy seedlings as shown in Fig. 1. Germination test on fresh germplasm showed high germination power. Germination percentage among *Asparagus* species and its cultivars ranged from 33.33 to 100 %. Our results are consistent with the previous works suggested that, *Asparagus* species show no deep dormancy (Baskin and Baskin 2004, Mario *et al.* 2005).

The genetic parameters are tremendously significant in predicting the amount of genetic gain to be expected from an improvement program (Kumar *et al.* 2010). To obtain a considerable amount of genetic gain at a realistic cost while maintaining sufficient genetic variability in the breeding population to ensure future gain is the important objective of plant improvement programs (Zobel and Talbert 1984). The present work analysis showed that morphological parameters could be helpful for biodiversity among *Asparagus* species. The root related characters showed moderate to high coefficient of variance (CV). In case of heritability, both shoot related and root related characters influenced the vigor of the species which showed high value. In this investigation nine traits including number of shoots, shoot height, fresh shoot weight, dry shoot weight, number of roots, root length, roots diameter, roots fresh weight and dry roots weight were observed. The growth data for the *Asparagus* species and cultivars showed that mostly cultivars had high values for morphological traits. The most important character was observed in shoot length ranged from 6.0 cm to 28.67 cm, whereas in *A. officinalis* cultivars, the values of shoot length were above 20 cm. Another important character observed in *A. officinalis* cultivars was the root length ranged from 3 cm to 10.58 cm, whereas the shoot length ranged above 7 cm. These investigations showed that cultivars are well adapted to the environment.

Heritability percentage was calculated as a ratio between the genotypic variance and the total phenotypic variance. The value of heritability in broad sense was high for all studied characters ranging from 91 to 99 %. High heritability estimates were useful while making selection on the basis of phenotype. For all *Asparagus* species which showed that assortment process for these characters would definitely bring development in the genotypes. The phenotypic co-efficient of variance was a little higher than genotypic co-efficient of variance for all the traits.

The identification of *Asparagus* species is more difficult through vegetative characters, although phenotypic expression showed less variation. Besides, morphological and chemical characters cannot determine genetic differentiation and plasticity in population adaptation (Gepts 1993). So they lack the resolving power for individual genotype identification. In the early stages of *Asparagus*, identification is more difficult. Thus, for the identification of variation among *Asparagus* species, different molecular techniques have been exploited. For *Asparagus* species due to the erratic flowering and lack of morphological differences, the recognition of genetic relationship is extremely difficult. Reliable identification of taxa is not only necessary for breeders but also necessary for consumers. Nowadays traditional method of species identification by morphological parameters is gradually being replaced by protein or DNA profiling which is more reliable.

The RAPD techniques are quite sensitive because different DNA profiles were generated by each primer for each of the cultivar and species. Using RAPD techniques, unique DNA profiles were obtained for *Asparagus* species and cultivars. In present study, 7 random oligonucleotide primers (RPI 4, RPI 5, TIBM BA-04, TIBM BA-14, TIBM BB-12, TIBM BB-13, and TIBM BD-19) were used for genetic characterization of 14 *Asparagus* species and its cultivars. All these RAPD primers showed good amplifications that were satisfactory and reproducible. The results of the RAPD analysis revealed a total of 239 polymorphic bands with an average of 34.1 bands per primer as shown in Table 7. These results contradict with those of those of Sarabi *et al.* (2010) and indicate a broad genetic basis of this species in Pakistan; however, consistent with Lal *et al.* (2011) using 20 RAPD primers, of which 6 primers were reproducible and polymorphic which produced 258 polymorphic bands with an average of 43 bands per

primer. TIBM BA-04, TIBM BA-14, TIBM BB-12, TIBM BB-13, and TIBM BD-19 primers showed highest number of polymorphism (100 %), while RPI 5 primer showed 92.14 % of polymorphism whereas the lowest number of polymorphism was showed by RPI 4 primer (82.60 %). The RAPD primers used in the present work produced fragments of different size ranging from 150bp to 3000bp. The minimum (1500 bp) sized fragment was amplified by TIMBB-19 while maximum (3000bp) sized fragment was amplified by primer Rpl 5. Dendrogram constructed on the basis of Neighbor joining algorithm, clustered *Asparagus* species into two main clusters (Cluster A and Cluster B). Cluster A is subclustered into Cluster I and Cluster II, Cluster I included only *A. officinalis* and its cultivars while Cluster II comprised of 5 wild *Asparagus* species. Cluster B made of *A. capitatus* and *A. gracelis* were clustered far from the other, suggesting that these may be due to mutation and genetic change or in their genetic background. The clarity of the differentiation of wild species by RAPD in the present work was in consistent with work of Lal *et al*. (2011), where they clustered *Asparagus* species on the basis of their geographical isolation.

It was concluded that comprehensive morphological study is advantageous in order to understand all aspects of this variation. RAPD markers for evaluated diversity analysis was highly reproducible, revealed sufficient genetic diversity and a high level of genetic polymorphism. These would be further useful for evaluation of genetic improvement of *Asparagus* species and its cultivars. Furthermore, we suggest that, RAPD method is convenient for a better understanding of the distribution of genetic variation at intra-specific level as well as for effective conservation.

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