Electronic Journal of Plant Breeding, 6(1): 74-80 (Mar 2015)
ISSN 0975-928X

Research Article

Genetic diversity study of pigeon pea (*Cajanus cajan* (L.) Millsp.) genotypes using SSR markers

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(Received: 12 Aug 2014; Accepted:05 Feb 2015)

Abstract
Fifty four SSR markers were employed for the genetic diversity study of 40 pigeon pea genotypes, of which 22 markers were polymorphic with polymorphic information content (PIC) of 0.609 to 0.929. The cluster analysis based on Jaccard's similarity coefficient using un weighted pair group method using arithmetic averages grouped the varieties into seven major clusters showing reasonable variability that may be exploited for selecting parents for breeding programme. The similarity coefficient among the genotypes ranged from 0.20 to 0.82 indicating wide genetic diversity. Generally distinct phenotypes identified using SSR markers could be potential sources for genotype identification and crop improvement of pigeon pea.

Key words:
Pigeon pea, Genetic diversity, SSR

Introduction

Pigeon pea [*Cajanus cajan* (L.) Millsp.] is an important protein rich pulse crop of the semi-arid tropics, grown predominantly under rain fed condition. The grain is used as dhal, the green seed as a vegetable and the sticks as fuel wood. India is the largest producer of pulses, of which pigeon pea is the most widely grown legume after chickpea. In crop improvement programmes, estimation of genetic diversity is an essential aspect for breeding highly productive cultivars. The accuracy in estimation of genetic diversity by using morphological and isozyme markers is inadequate and questionable. The DNA based molecular markers are powerful tools for assessment of genetic diversity. Some of the commonly used molecular markers in genetic diversity studies of pigeonpea are random amplified polymorphic DNA (Yadav, 2010, Gunjanjyoti Boora, 2005), amplified fragment length polymorphism (Yan Long Guan, 2007) and simple sequence repeats (SSRs) or microsatellite DNA. Among the DNA based markers, simple sequence repeats (SSR) markers are highly polymorphic, reproducible, co-dominant and occur throughout the genome and have been used in assessment of genetic diversity in pigeonpea (Singh *et al*., 2008) and in many other legumes like chickpea (Sant *et al.*, 1999, Anuradha *et al*., 2011) and soybean (Doldi *et al.*, 1997). With the inevitable narrowing of the genetic base of cultivated plants and the limitations associated with the morphological markers in it becomes necessary to rely on molecular markers for characterization of genotypes and estimation of genetic diversity. Keeping the above aspects in view, characterization and genetic diversity studies were conducted in selected pigeonpea genotypes using SSR markers.

Materials and Methods

Forty genotypes (ICP-2043, ICP-2047, ICP-2048, ICP-2092, ICP-2671, ICP-20108, ICP-85063, ICP-332, ICP-96053, ICP-96061, ICP-99051, ICP-20058, ICP-90048, ICP-20201, ICP-20104, ICPL-20114, ICP-20177, Asha, ICP-20205, ICPL-332WR, ICP-8863, ICPL-13092, ICP-86022, ICP-149, ICP-161, ICP-91024, WC-42, WC-45, WC-13, WC-7, WC-36, WC-25, WC-26, 6364, 20632, LRG-41, LRG-38, Laxmi, PRG-100, and TBB-7) were obtained from International Crops Research Institute for Semi Arid Tropics, Hyderabad and Agricultural Research Station, Tandur, ANGRAU, Hyderabad. The lines were grown in the field during *Kharif*, 2010 with a spacing 90 x 30 cm. The plant characters viz. days to 50% flowering, days to maturity , plant height , pod size , number of seeds per pod, seed size (100 seed weight in grams) were considered for cluster analysis. Fresh young leaves were collected for DNA extraction. Extraction of genomic DNA was carried out by using the CTAB method with little modifications. The purity and concentration of the isolated genomic DNA samples was estimated by UV-absorption spectrophotometer and agarose gel 0.8 % electrophoresis. Fifty four SSR primers all belonging to pigeon pea family were used for PCR amplification, out of which 22 showed polymorphism amounting to 41 per cent. The PCR reactions were

conducted in 10 mixture consisting of 50 nano gram genomic DNA, 0.4 µl Taq DNA polymerase (3U/µl) (Bangalore Genei, Bangalore, India), 0.2 µl SSR primers (5 µM), 1 µl dNTPs (2 mM) and 10X PCR buffer consisting of 10 mM Tris-HCl, 50 mM KCl and 1.5 mM $MgCl_2$. Amplification was performed using Veriti 96 well Thermal Cycler, Applied biosystems, programmed for initial denaturation at 94°C for 5 minutes and 35 cycles of 94°C for 45 seconds, 55°C for 45 seconds and 72°C for 1 minute. The amplification was completed with a 10 minutes final extension at 72°C. The amplification products were separated by electrophoresis in 3% (w/v) agarose gels with 1X TAE buffer, stained by 1 µg/ml of ethidium bromide and documented in gel documentation system (Biorad XR, Biorad, USA).

The SSR data were analyzed using NTSYS-PC (version 2.0) (Numerical Taxonomy and Multivariate Analysis System) computer program (Rohlf, 1998). Each SSR fragment was treated as a unit character and was scored as 1 (present) or 0 (absent). The genetic relatedness among the varieties was estimated using Jaccard's similarity coefficient and clustering was done with UPGMA (Un weighted Pair Group Method using Arithmetic Averages).

Results and Discussion

Genotypes with high molecular diversity could be used in breeding methodologies and development of gene pools with broad genetic base. The genotype specific bands developed by the SSR primers could also be used for genetic diversity studies and cultivar identification. Out of 54 SSR primers 22 were polymorphic, amounting to 41% polymorphism . Sixty alleles were obtained using 22 SSR primers with an average of 2.727 alleles per primer. The number of alleles amplified for each primer ranged from 2 to 4. The polymorphic information content (PIC) for these primers ranged from 0.609 to 0.929 (Table 1). Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (Dewoody *et al*., 1995).

Unweighted pair group method cluster analysis was performed using Jaccard's similarity coefficient matrices calculated from SSR markers to generate a dendrogram for 40 genotypes (Fig.1). The similarity coefficient among the clusters ranged from 0.204 to 0.820 indicated that the genetic diversity among the varieties was quite wide (Fig.2).

The dendrogram showed the grouping pattern of 40 genotypes into 7 clusters. Cluster 1 comprised of seven genotypes (ICP-149, ICP-161, ICP-91024, WC-42, ICP-86022, ICPL-13092 and ICP-8863), which had small sized seeds except ICP-8863. Cluster 2 comprised of four wild collections (WC-36, WC-7, WC-13 and WC-45) with a similarity of 72%. Cluster 3 comprised of five genotypes (ICP-20036, LRG-38, ICP-6364, WC-26 andWC-25) with a similarity of 68%. The genotypes of this cluster were mid-late for maturity. Cluster 4 comprised of three genotypes (Laxmi, PRG-100 and TBB-7) having large sized seed with mid-late duration. Major cluster 5 was divided into two minor sub clusters, sub cluster 1 with three genotypes (ICP-20058, ICP-90048 and ICP-99051) with a similarity of 77 % among them. Sub cluster 2 had two genotypes (ICP-20104 and ICP-20201) with a similarity of 95 % between them. The sub cluster 1 and 2 of major cluster 5 shared a similarity of 70% between them. Cluster 6 comprised of six genotypes (ICP-20177, ICP-20205, ICPL-20114, ICPL-332WR, Asha and LRG-41) and divided into two minor sub clusters, sub-cluster 1 with two genotypes (CP-20177 and ICP-20205) with a similarity of 86 % between them and another sub-cluster 2 with four genotypes (ICPL-20114, ICPL-332WR, Asha and LRG-41) with a similarity of 67 % among them. The sub cluster 1 and sub cluster 2 of cluster 6 shared a similarity of 59 %. Cluster 7 was the largest with ten genotypes (ICP-20108, ICP-85063, ICP-2048, ICP-2092, ICP-2671, ICP-2043, ICP-2047, ICP-96053, ICP-96061 and ICP-332). Major cluster 7 was divided into three minor sub clusters. Sub cluster 1comprised five genotypes (ICP-20108, ICP-85063, ICP-2048, ICP-2092 and ICP-2671), sub cluster 2 comprised of two genotypes (ICP-2043 and ICP-2047) and another sub cluster 3 had three genotypes (ICP-96053, ICP-96061 and ICP-332). These sub clusters 1,2 and 3 of cluster 7 shared a similarity of 57 % (Fig. 1 and Fig. 2)

The cluster analysis showed longest distance 0.820 between clusters 1 and 4 indicating more divergence among the genotypes included. The lowest inter cluster distance 0.49 was recorded between clusters 3 and 4, revealing genetic proximity among the genotypes (Fig. 3). The crosses between genotypes separated by larger statistical distances are likely to yield productive genotypes in the segregating generations (Bhatt,1970). As the genotypes grouped in clusters 1 and 4 comprised of small and large seed size respectively, the crosses among these genotypes may produce transgressive segregates for seed size, which is an important yield attribute in pigeon pea.

The results of the study indicated that SSRs or microsatellite markers provided more definitive separation of clusters indicating a higher level of

efficiency for determining the relationship between closely related varieties. Wang *et al*. (2004) reported that due to the limited number of DNA markers, the legume germplasm is usually described solely by taxonomic and agronomic characters. Hence, the development of more microsatellites is recommended for future genetic studies in pigeon pea (Odey *et al*. 2007).

Acknowledgements:
The authors are highly thankful to the Director , Institution of Biotechnology Acharya N G Ranga Agricultural University and all the supporting staff for extending their co-operation for conducting the laboratory work. Our thanks are also due to pigeon pea breeders at International Crops Research Institute for Semi Arid Tropics and Agricultural Research Station, Tandur, Acharya N G Ranga Agricultural University for seed supply.

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Table 1. List of SSR markers used and their Polymorphic Information Content (PIC)

S. No.	SSR marker	Sequence	No. of alleles	PIC
1	CCttc001	FP.- CGGGCTTCCTTTTCTTCTCT RP.- AAAACCCCGAAAACACCATT	3	0.786
2	CCtta001	FP.- TTCTGGATCCCTTTCATTTTTC RP.- TGACACCCTTCATTTTTC	3	0.811
3	CCat001	FP.- CTTCCCCCAACTAAGATCCA RP.- GTTCGTTCTCTTTAATTGACTTGC	3	0.825
4	CCat002	FP.- TTTCCTGAGCCATCAGTCG RP.- AAGCATCAACGTACCAGCAA	3	0.873
5	CCtta003	FP.- CCAAGAAAAGGTGCTCCAAGT RP.- TTGCTTCTTTTCTCGCTTGC	4	0.929
6	CCtc001	FP.- GACTCTTCACCTCACACTCATCAC RP.- CCTCATACAACAACCCTAAGCAC	2	0.660
7	CCggc001	FP.- CCATTGTGCGTCTTTGTGTT RP.- GCTTTTCCTCTTCCTTTCTCG	3	0.789
8	CCttat001	FP.- TACAGCAGCCACATCAAAGC RP.- TGAACCGTGAAAGTGGGATT	3	0.828
9	CCtta004	FP.- ACCCATTATTGATTTGGGTA FP.- CCAAATTTCACCCAAGAAA	2	0.609
10	CCtta005	FP.- TCTTCCATTGCATGGTGTT RP.- GCATGATATGAGATGATGACGA	2	0.628
11	CCac001	FP.- CTGGGCCTCTAGCATAGCAA RP.- AAACTTCTGGACGCAAAATGA	2	0.744
12	CZ681922	FP.- ACACCACCATGCTAAAGAACAAG RP.- CCAAGCAAGACACGAGTAATCATA	2	0.719
13	CZ681926	FP.- GTAGAGGAGGTTCCAAATGACATA RP.- ATCTGTCTGGTGTTTTAGTGTGCT	2	0.740
14	CZ681946	FP.- TAATCCCATTCCGTTGTCGT RP.- CCCAGGAAGAGATGAGACCA	4	0.918
15	CZ681947	FP.- AGGCTTTCTCCCTTCAATCC RP.- AGGCTTTCTCCCTTCAATCC	3	0.860
16	CZ681973	FP.- ACCTTGCTTGTTTCGCTTTT RP.- AAGGGAGGTGGACTACAAGGA	3	0.868
17	CZ681990	FP.- CAGGTCTGCTACTGCCATCA RP.- CAGGTCTGCTACTGCCATCA	3	0.861
18	CZ681993	FP.- ATCATCAGATTCTTCAGCCGTA RP.- GGTTAGACCAATCCAATCAAGC	3	0.860
19	CZ681995	FP.- CACGATTCCATTGGTGGAG RP.- ACGGTTTCTGGGAGGGTCTA	3	0.837
20	CZ681999	FP.- GACTAGAAAATTCACCTCCGTCTG RP.- TTACAAAGGCTACATTGATGAGAAC	3	0.878
21	CZ682004	FP.- GCCTTTTCAAACTTTTCTCA RP.- CATATGCTTTAAGTGCTTTCCT	2	0.750
22	CZ682005	FP.- TGTATGTTCGTTTAGAGGCTTCC RP.- GCCCCTTTTCACTTTTCTCA	2	0.688

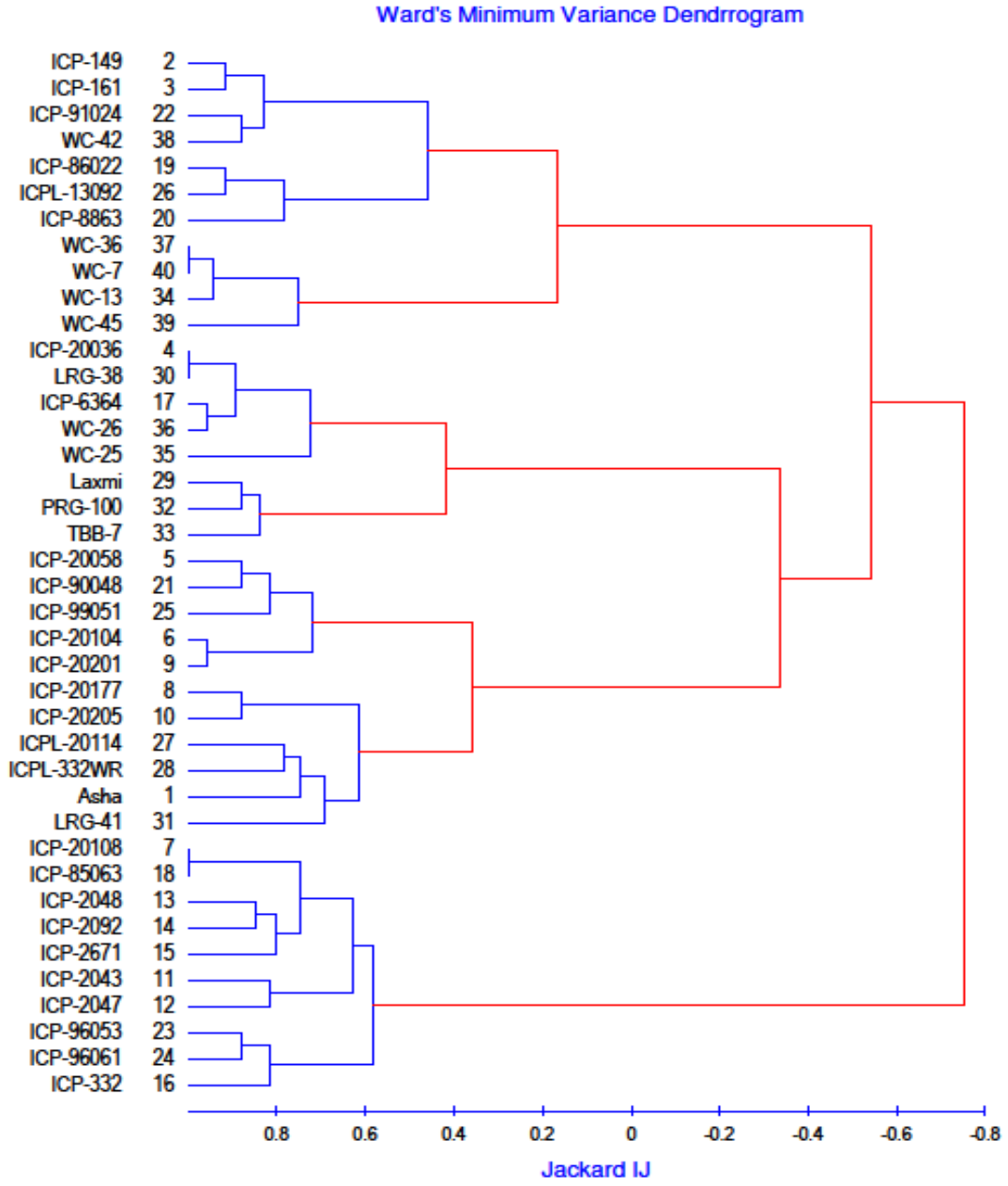

Fig. 1. Dendrogram of 40 pigeon pea genotypes constructed from UPGMA cluster analysis using Jaccard's co-efficient based on data derived from 22 SSR markers

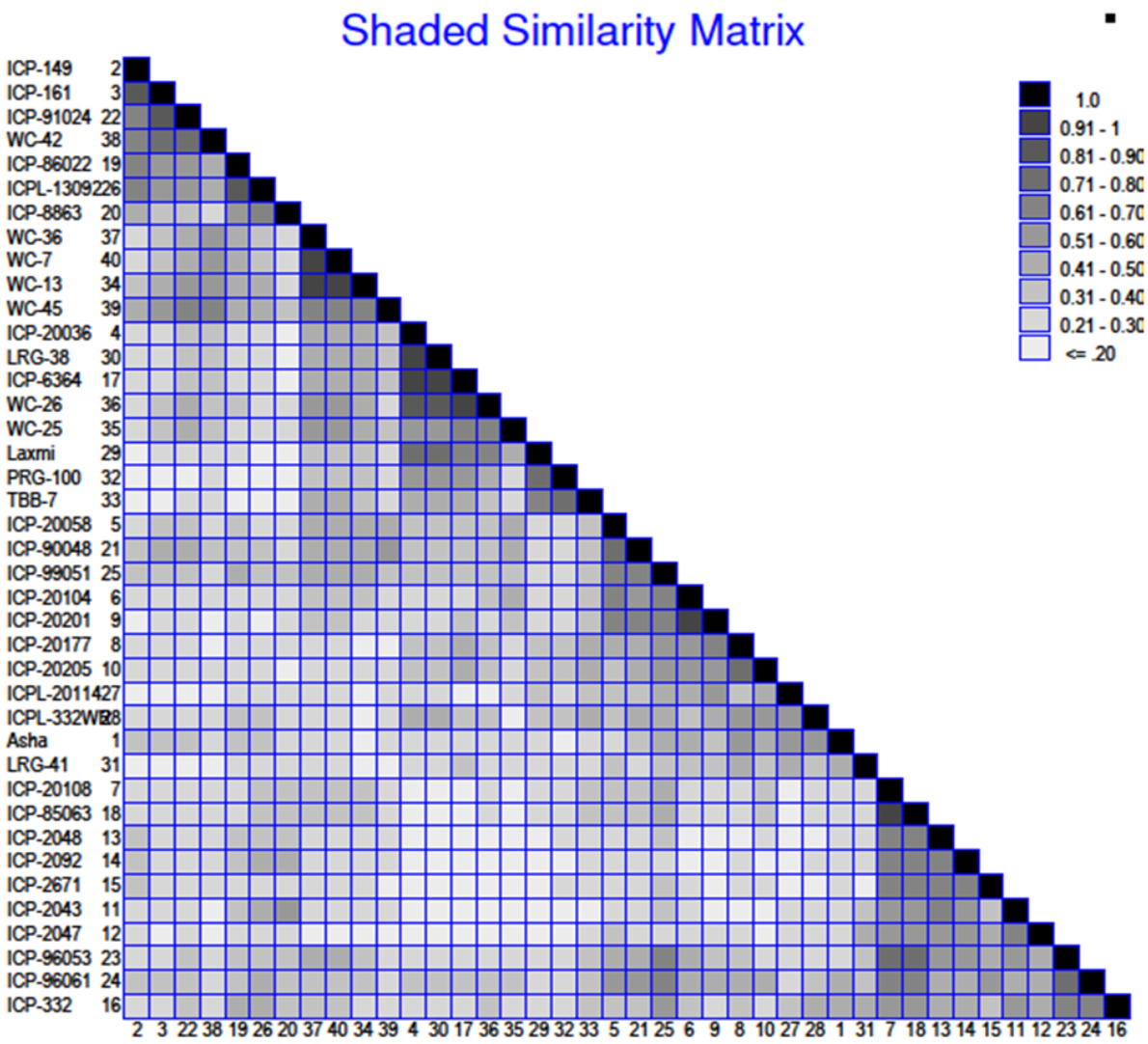

Fig.2 Shaded similarity matrices of 40 pigeonpea genotypes obtained based on data derived from 22 SSR markers

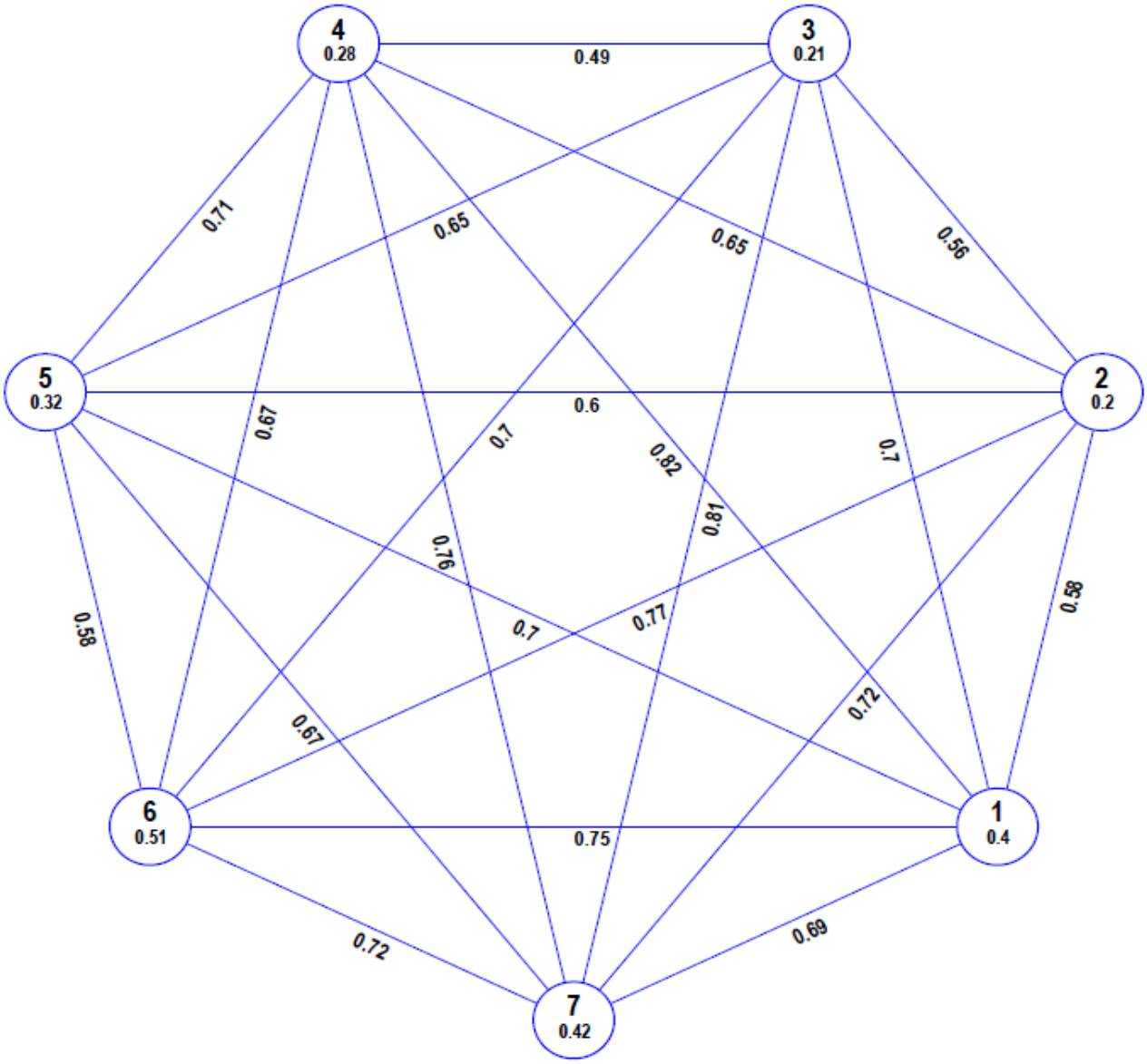

Fig. 3. Statistical distance of seven clusters obtained based on analysis of molecular markers