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Characterization and identification of pigeonpea [*Cajanus cajan* (L.) Millsp.] genotypes based on quality of seed protein

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ABSTRACT

Tris- and water-soluble seed protein profiling was used to characterize, identify and differentiate 13 genotypes of pigeonpea via SDS–PAGE based electrophoresis of seed protein. Electropherograms, RM-value and UPGMA based cluster (dendrograms) analysis was used to analyze tris- and water-soluble protein banding patterns of these genotypes. It revealed that RM-value of protein bands have differed from all the genotypes for both soluble proteins. Tris-soluble protein banding pattern exhibited 17 bands including 12-15 variate of RM-value (0.083 to 0.98) and two common bands with RM-value 0.083 and 0.78. However, water-soluble protein banding pattern revealed the presence of 16 bands including 8-12 variate of RM-value (0.16 to 0.95) with four common bands of RM-value 0.43, 048, 0.23 and 0.65, respectively. UPGMA cluster analysis was used to group the 13 genotypes into six distinct clusters. The banding pattern of tris-soluble protein was found to be more distinct than water-soluble protein. The result demonstrated that the electrophoretic profile of tris-soluble protein through SDS-PAGE was more effective than the water-soluble protein.

Key words: Pigeonpea genotype, SDS–PAGE, Varietal identification, Tris- and water-soluble protein.

INTRODUCTION

Pigeon pea (*Cajanus cajan* L.) is a multipurpose legume crop grown as a sole or intercrop (Willey *et al*., 1980) with diversified uses (Dasbak and Asiegbu, 2009) which includes as a component of daily diet in the form of daal (dehusked seeds a soup), as feed for livestock and stalks as a raw material in making huts and basket (Joshi *et al*., 2009). In 2017-18, India stands first in area and production of pigeon pea in all over the world and covering an area of about 4.46 million hectares with production of 4.18 million tonnes with an average yield of 937 kg ha^{-1} (DES, 2019). Many varieties of pigeon pea have been developed aimed at increased yield, improved quality, reduced duration, enhanced nutrition and tolerance to biotic and abiotic stresses. Each variety is supposed to possess certain distinct traits, which is reflected in DUS characterization of varieties.

In most of the crops, varietal identification is based on few visual morphological traits. However, on account of limited variation in morphological traits and their expressivity is environmental and age-dependent though it is not a precise tool for varietal identification in present scenario. Further, morphological variations being controlled polygenically, differences within cultivars are too tedious to distinguish between different cultivars. The differences between cultivars should be based on the genic level but direct comparison of genes is too difficult and time consuming. However, the differences can be measured by comparing the product of genes *i.e*. by using protein. Hence, there is need to characterize/ differentiate the varieties/ cultivars with more precision either at phenic level or at protein level.

High diversity in morphological traits of pigeon pea has been reported by Upadhyaya *et al*. (2005) and Singh *et al.* (2014). The differential expression of genes in terms of protein can be studied using SDS-PAGE. It is a routinely used technique due to its validity and simplicity for describing genetic structure of crop germplasm, but its implication has been limited mainly to cereals due to less polymorphism in most of the legumes (Ghafoor *et al*., 2008). PAGE and SDS-PAGE have been utilized in study of genetic relationship in the genus *Cicer* (Ahmad *et al*., 1992 and Asghar *et al*., 2003) and in identification of pigeonpea cultivars (Rani *et al*., 2007). Banding pattern of seed proteins obtained by electrophoresis have been used as genetic markers to resolve the taxonomic and evolutionary problems of several crop plants (Das and Mukharjee, 1995 and Mirali *et al.,* 2007) for analysis of genetic diversity within and between species (Gangwar and Bajpai, 2006) in distinguishing cultivars (Moller *et al*., 1993) in genetic resources conservation and breeding, study of genome relationship and as a tool for crop improvement

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(Das and Mukharjee, 1995 and Murphy *et al*., 1996). The electrophoretic banding patterns of total seed protein are resolved by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS) has been used for identification and differentiation among several crops (Salimi, 2013). The SDS-PAGE is considered to be a practical and reliable method for species identification because seed storage proteins are largely independent of environmental fluctuations (Gepts, 1989).

At present, pigeon pea cultivars/ varieties are being described on the basis of morphological characteristics of plants. The accurate morphological information and rapid reproductive techniques used for varietal identification and characterization which can be conducted in a laboratory is not enough. Although SDS-PAGE has been used in other crops routinely, very few reports are available on pigeon pea (Gangwar and Bajpai, 2006 and Joshi *et al*., 2009). Hence, the present investigation was initiated on seed protein profiling of 13 pigeonpea genotypes/ cultivars for study the genetic variation among them and characterize/ identify unique character to differentiate these genotypes/ cultivars of pigeon pea on the basis of seed tris- and water-soluble protein profiling or banding pattern and its possible relationship with agronomic traits.

MATERIALS AND METHODS

Experimental materials

Genetically pure nucleus seeds of 13 most popular varieties of pigeonpea in plain zone (Table 1) were procured from Pigeonpea Breeder, Economic Botany Section (Legume Section), Department of Genetics and Plant Breeding, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur. These genotypes were selected on the basis their popularity among the farmers community due to its uniqueness, characteristics and performance (Table 1). The thirteen genotypes were characterized and differentiated by tris-soluble and water-soluble protein profiling through SDS –PAGE of seed proteins during 2013-14 in the Biotechnological laboratory.

Preparation of Sample

Varietal characterization of pigeon pea was carried out using two methods *viz*., tris-soluble protein analysis and water-soluble protein analysis. For tris-soluble protein analysis, 0.2 g grinded seed powder (defatted 4 times) of each genotype were taken in 2.0 mL centrifuge tube separately and 0.3 mL of the protein extraction buffer was added. The samples were left for 2 h at room temperature and then kept in a refrigerator overnight. The samples were heated in water-bath for 10 min, then cooled at room temperature and centrifuged at 10000 rpm for 10 min. The clear supernatant was used for electrophoresis. However, for water soluble protein analysis, 0.4 g defatted powder of all genotypes were taken in separate 2.5 mL centrifuge tubes. After addition of 2 mL distilled water in each tube, all the samples were homogenized and centrifuged at 10000 rpm for 5 min. Subsequently, 100 µl of sample buffer for water soluble protein extraction was added in (approximately) 200 µl of supernatant of each variety. The above components were mixed properly and denatured at 100°C in water bath for 10 min and kept the sample in room temperature for cooling. The denatured samples were centrifuged at 10000 rpm for 10 min. Further, the clear supernatant was used for electrophoresis.

Preparation of gel

Seed protein were analysed through SDS-PAGE followed the procedure of Laemmli (1970) using 12% polyacrylamide gel electrophoresis (PAGE). The electrophoresis was conducted in auto-electrophoresis unit using thirteen well for loading the sample (Bio-Rad, USA). Fifty (50) µl of clear supernatant with one drop of tracking dye (Bromophenol blue) was loaded into each well, after well mixing with the help of micro-pipette (Eppendorf, Germany). The electrophoresis was conducted at a constant current of 1.5 mA per well till the tracking dye crossed the stacking gel. Then the current was fixed @ 2 mA per well at 220 V. The electrophoresis was stopped after 3-4 hours or the tracking dye reached the end of the gel.

Table 1: Special features of pigeonpea (13 genotypes) used for identification and characterization.

Name of Variety	Source	Special features
Amar	C.S.A.U.A.&T. Kanpur	Tall in height, spreading type plants, matures in 250-270 days.
KWR2-7	C.S.A.U.A.&T. Kanpur	Tall in height, moderately resistant to wilt and medium maturity.
Shekhar-3	C.S.A.U.A.&T. Kanpur	Medium in height, semi spreading and medium maturing
KA 01-108	C.S.A.U.A.&T. Kanpur	Tall in height, spreading type and medium maturing
KSP-13	C.S.A.U.A.&T. Kanpur	Tall in height, resistant to wilt and medium maturing
UPAS-120	G.B.P.U.A.& T. Pantnagar	Semi spreading, tolerant to pod borer and synchronous maturity.
KAWR-7	C.S.A.U.A.&T. Kanpur	Tall in height, moderately resistant to wilt, matures in 240-250 days.
KSMR-105	C.S.A.U.A.&T. Kanpur	Tall in height, moderately resistant to wilt, matures is 210-248 days.
Pusa 9	R.A.U., Pusa, Bihar	Tall in height, tolerant/ resistant to Alternaria and SMV, matures in 210-248 days, bold seeded
KA 09-01	C.S.A.U.A.&T. Kanpur	Semi spreading, resistant to SMV and medium maturing
T-21	C.S.A.U.A.&T. Kanpur	Spreading type, matures in 140-150 days
KA 01-94	C.S.A.U.A.&T. Kanpur	Tall in height, spreading plant type.
KSMR-88	C.S.A.U.A.&T. Kanpur	Medium in height, spreading plant type, medium seeded

RESULTS AND DISCUSSION

The experiment was conducted to investigate the genetic purity and identify the genotypes of pigeonpea on the basis of SDS -PAGE electrophoresis of tris- and water- soluble proteins. Seed is the basis of vital and cheapest input in realizing the sustained agriculture production. Hence, it becomes mandatory to provide genetic identity of planting material to farmers. To establish genetic identity of crop genotype, a quick, reliable and reproducible technique is of utmost requirement. Thus, majority of pigeonpea genotypes are described on few characters such as yield per plant, height, pod shape, size, pod number, colour etc. at times, these morphological characters are insufficient to clearly distinguish them (Gangwar and Bajpai, 2006 and Singh *et al*., 2014). Contrary to this, biochemical parameters and/ or methods like tris- and water- soluble seed protein have gained reputation as product of gene, since they are formed by amino acids chain and gene based and do not change with the environmental conditions. In this context, seed protein could be used for distinguishing the genotypes, owing to its simplicity, stability, reproducibility and genetically controlled nature by several workers. Therefore, in this study the emphasis is given to evaluate protein based profiling of tris- and water – soluble protein banding pattern of 13 pigeonpea genotypes.

Characterization based on the profile of tris soluble protein through SDS-PAGE

Thirteen genotypes of pigeon pea were distinguished based on presence and absence of protein bands at particular RM value and on total numbers of bands present. In Tris-soluble protein banding profiling the number of bands presents in each genotype ranges from 12-15 with RM value 0.083 - 0.98. The minimum (12) bands were observed in KWR2-7, KSMR-105 and KSMR-88, followed by more number of bands (13) in varieties AMAR, T-21. Higher number of bands (14) were revealed by KA 09-01 and KA 01-94, however, 15 bands were present in genotypes KWR2-7, Shekhar-3, KA O1-108, KSP-13, UPAS-120 and PUSA-9. Based on the RM value, a total no of 17 protein bands at different RM value were identified (Table 2). RM value 0.083 and 0.78 are found common in all of these varieties (Fig 1). Presence and absence of bands were scored by their respective relative mobilites and number in sequence from cathodal region (Table 4). The result obtained after SDS-PAGE analysis indicated that the method provided a powerful tool for reliable varietal identification based on genetic differences in seed storage protein composition of genotypes in pigeonpea crops. This is in confirmation with the reports of Gangwar and Bajpai, (2006).

In UPGMA cluster analysis of tri-soluble seed protein of 13 pigeonpea, a total of six clusters are formed from these genotypes (Fig 3) which showed the genetic diversity among the cultivars. Cluster I, II and III consisted three genotypes (*viz*., Amar, KWR 260 and KSMR 105; Shekhar 3, Pusa 9 and KA0901 and KPS 13 UPAS 120 and KA 0194, respectively) and Cluster IV and V grouped single genotype (KA01108 and KAWR 7) individually, that have wider or distance relationship than other genotypes of pigeonpea. However, cluster VI have two genotypes *viz*., T 21 and KSMR 88.

Table 2: RM value of electrophoretic profiles of Tris-soluble protein.

Genotype	Electrophoretic profiles of Tris soluble protein	No. of bands	Unique RM value variation
Amar	0.083, 0.125, 0.233, 0.291, 0.375, 0.50, 0.56, 0.608, 0.65, 0.66, 0.795, 0.91, 0.98	13	0.208, 0.75, 0.875
KWR2-7	0.083, 0.125, 0.208, 0.233, 0.291, 0.375, 0.50, 0.56, 0.608, 0.65, 0.66, 0.795, 0.875, 0.91, 0.98	15	0.75
Sekhar-3	0.083, 0.125, 0.208, 0.233, 0.291, 0.56, 0.608, 0.65, 0.66, 0.795, 0.875, 0.91, 0.98	13	0.375, 0.50, 0.75
KA01-108	0.083, 0.125, 0.208, 0.233, 0.291, 0.375, 0.50, 0.56, 0.608, 0.65, 0.66, 0.75, 0.875, 0.98	14	0.795, 0.91
KSP-13	0.083, 0.125, 0.208, 0.233, 0.291, 0.50, 0.56, 0.608, 0.65, 0.66, 0.75, 0.795, 0.875, 0.91, 0.98	15	0.375
UPAS-120	0.083, 0.125, 0.208, 0.233, 0.291, 0.375, 0.50, 0.608, 0.65, 0.66, 0.75, 0.795, 0.875, 0.91, 0.98	15	0.56
KAWR-7	0.083, 0.125, 0.208, 0.233, 0.291, 0.375, 0.50, 0.65, 0.66, 0.75, 0.795, 0.875	12	0.508, 0.56, 0.91, 0.98
KSMR-105	0.083, 0.208, 0.233, 0.291, 0.375, 0.50, 0.608, 0.65, 0.75, 0.795, 0.875, 0.98	12	0.125, 0.56, 0.66, 0.91
PUSA-9	0.083, 0.125, 0.208, 0.233, 0.291, 0.375, 0.56, 0.608, 0.65, 0.66, 0.795, 0.875, 0.91, 0.98	14	0.50, 0.75
KA09-01	0.083, 0.125, 0.208, 0.233, 0.291, 0.375, 0.56, 0.608, 0.65, 0.66, 0.875, 0.91, 0.98	13	0.50, 0.75, 0.795
T-21	0.083, 0.125, 0.208, 0.375, 0.56, 0.608, 0.65, 0.75, 0.795, 0.875, 0.91, 0.98	12	0.233, 0.291, 0.50, 0.66
KA01-94	0.083, 0.125, 0.208, 0.233, 0.291, 0.375, 0.608, 0.65, 0.66, 0.75, 0.795, 0.875, 0.91, 0.98	14	0.50, 0.56
KSMR-88	0.083, 0.125, 0.208, 0.56, 0.608, 0.65, 0.66, 0.75, 0.79, 0.875, 0.91, 0.98	12	0.233, 0.291, 0.375, 0.50

Table 3: RM Value of electrophoretic profiles of water-soluble protein.

Genotype	Electrophoretic profiles of Tris soluble protein	No. of bands	Unique RM value variation
Amar	0.24, 0.432, 0.48, 0.528, 0.60, 0.656, 0.84, 0.88	8	0.16, 0.20, 0.384, 0.704, 0.744, 0.76, 0.784, 0.952
KWR2-7	0.20, 0.24, 0.432, 0.48, 0.528, 0.60, 0.656, 0.704, 0.76, 0.784, 0.84, 0.88	12	0.16, 0.384, 0.952
Sekhar-3	0.20, 0.24, 0.432, 0.48, 0.528, 0.60, 0.656, 0.704, 0.744, 0.76, 0.784, 0.84, 0.88, 0.952	14	0.16, 0.384
KA01-108	0.20, 0.384, 0.432, 0.48, 0.60, 0.656, 0.744, 0.76, 0.88, 0.952	10	0.16, 0.24, 0.528, 0.704, 0.784, 0.84
KSP-13	0.20, 0.384, 0.432, 0.48, 0.528, 0.60, 0.656, 0.704, 0.744, 0.76, 0.784, 0.84, 0.88, 0.952	14	0.16, 0.24
UPAS-120	0.24, 0.384, 0.432, 0.48, 0.528, 0.60, 0.656, 0.704, 0.744, 0.76, 0.784, 0.84 , 0.88, 0.952	14	0.16, 0.20
KAWR-7	0.20, 0.24, 0.384, 0.432, 0.48, 0.528, 0.60, 0.656, 0.704, 0.744, 0.76, 0.784, 0.80, 0.88, 0.952	15	0.16
KSMR-105	0.20, 0.24, 0.384, 0.432, 0.48, 0.528, 0.656, 0.704, 0.744, 0.76, 784, 0.84, 0.88, 0.952	14	0.16, 0.60
PUSA-9	0.20, 0.384, 0.432, 0.48, 0.528, 0.60, 0.656, 0.704, 0.744, 0.76, 0.784, 0.84, 0.88, 0.952	14	0.16, 0.24
KA09-01	0.16, 0.24, 0.384, 0.432, 0.48, 0.528, 0.60, 0.656, 0.704, 0.744, 0.76, 0.784, 0.84, 0.88, 0.952	15	0.20
T-21	0.16, 0.20, 0.384, 0.432, 0.48, 0.528 ,0.656, 0.704, 0.744 ,0.76, 0.784, 0.84, 0.88, 0.952	14	0.24, 0.60
KA01-94	0.16, 0.20, 0.384, 0.432, 0.48, 0.528, 0.60, 0.656, 0.744, 0.76, 0.784, 0.84, 0.88, 0.952	14	0.24, 0.704
KSMR-88	0.432, 0.48, 0.528, 0.60, 0.656, 0.744, 0.76, 0.784, 0.84, 0.88, 0.952	11	0.16, 0.20, 0.24, 0.384, 0.704

Characterization based on profiles of water soluble protein through SDS-PAGE

Water soluble protein is also an important technique for identification of genotype (Gepts, 1989, Singh *et al*., 1991 and Gafoor and Arshad, 2008). The SDS-PAGE method revealed different protein banding patterns and distinguished the genotypes under study based on the presence or absence of bands with particular relative mobility (RM) value and were numbered in sequence from cathodal origin (Gangwar and Bajpai, 2006). The number of protein bands present in individual genotype ranged from 8 to 15 with RM value 0.16 to 0.95. The minimum (8) bands were observed in AMAR, 10 bands present in KAO1-108, 11 bands present in KSMR-88, 12 bands present in KWR2-7, 14 bands observed in Shekhar-3, KSP-13, KMR-105, PUSA-9, T-21, and KA01-94. Maximum 15 bands were observed in KAWR-7 and KAO9-01 (Table 3 and Fig 2). Based on the RM value a total number of 16 protein bands at different RM value were identified (Table 5). Bands having RM value of 0.16, 0.48, 0.23 and 0.65 found common in all genotypes.

However, characterization and discrimination of 13 pigeonpea genotypes based on UPGMA cluster analysis of water soluble protein showed in Fig 4. These genotypes of pigeonpea were grouped in 6 distinct clusters. Clusters I, contain AMAR and KWR2-7 which are closely related to each other. The cluster II consisted three genotypes such as Shekhar-3, UPAS-120 and KSMR-105 are grouped which shows UPAS-120 and KSMR-105 are more closer than Shekhar-3. In cluster III, two genotypes are grouped KPS-13 and PUSA- 9 which are closer to each other and cluster VI, genotypes KA09-01, KA01-94 and T-21 are grouped and these genotypes are having close relation rather than T-21. Whereas, cluster VI contains KA01-08 and KSMR-88 that are close to each other on the water soluble protein banding profile.

UPGMA cluster analysis and electrophoregram (both) methods were applied to produce distinct electrophoretic profiles of tris- and water- soluble protein through SDS-PAGE. The protein banding pattern were found in Fig 1 and 2 and electrophoregram of tris-and water- soluble protein bands, had observed more distinctiveness in number of protein bands, Rm value (location in gel) and in their molecular weight. UPGMA cluster analysis of 13 pigeonpea genotypes, fall in six distinct groups for both water- and tris-soluble proteins banding pattern. The tris- soluble protein were found more distinct than water soluble proteins through SDS-PAGE and hence, act as genotypic finger printing as earlier reported by Gangwar and Bajpai, 2006. However, the tris- soluble protein banding pattern was found in SDS-PAGE exhibited more polymorphism and it would be used

Table 4: Score of RM values of Tris-soluble seed protein bands in pigeonpea genotypes.

Band No.	Genotype RM value	Amar	KAWR 2-7	Sekhar -3	KA-01 -108	KSP -13	UPAS -120	KAWR -7	KSMR -105	PUSA -9	KA-09-01	T-21	KAO -1-94	KSMR -88	Genotype RM value
1.	0.083	+	+	+	+	+	+	+	+	+	+	+	+	+	0.083
2.	0.10	-	-	+	+	-	-	-	-	+	+	+	-	-	0.10
3.	0.125	+	+	+	+	+	+	+	-	+	+	+	+	+	0.125
4.	0.208	-	+	+	+	+	+	+	+	+	+	+	+	+	0.208
5.	0.233	+	+	+	+	+	+	+	+	+	+	-	+	-	0.233
6.	0.291	+	+	+	+	+	+	+	+	+	+	-	+	-	0.291
7.	0.375	+	+	-	+	-	+	+	+	+	+	+	+	-	0.375
8.	0.50	+	+	-	+	+	+	+	+	-	-	-	-	-	0.50
9.	0.56	+	+	+	+	+	-	-	-	+	+	+	-	+	0.56
10.	0.608	+	+	+	+	+	+	-	+	+	+	+	+	+	0.608
11.	0.650	+	+	+	+	+	+	+	+	+	+	+	+	+	0.650
12.	0.660	+	+	+	+	+	+	+	-	+	+	-	+	+	0.660
13.	0.750	-	-	-	+	+	+	+	+	-	-	+	+	+	0.750
14.	0.795	+	+	+	-	+	+	+	+	+	-	+	+	+	0.795
15.	0.875	-	+	+	+	+	+	+	+	+	+	+	+	+	0.875
16.	0.91	+	+	+	-	+	+	-	-	+	+	+	+	+	0.91
17	0.98	+	+	+	+	+	+	-	+	+	+	+	+	+	0.98
	Total	13	15	14	15	15	15	12	12	15	14	13	14	12	Total

(+) represents presence and (-) represents absence of band.

Table 5: Score of RM values of water-soluble seed protein bands in pigeonpea genotypes.

Band No.	Genotype RM value	Amar	KAWR 2-7	Sekhar -3	KA-01 -108	KSP -13	UPAS -120	KAWR -7	KSMR -105	PUSA -9	KA-09-01	T-21	KAO -1-94	KSMR -88	Genotype RM value
1.	0.16	-	-	-	-	-	-	-	-	-	+	+	+	-	0.16
2.	0.20	-	+	+	+	+	-	+	+	+	-	+	+	-	0.20
3.	0.24	+	+	+	-	-	+	+	+	-	+	-	-	-	0.24
4.	0.38	-	-	-	+	+	+	+	+	+	+	+	+	-	0.38
5.	0.43	+	+	+	+	+	+	+	+	+	+	+	+	+	0.43
6.	0.48	+	+	+	+	+	+	+	+	+	+	+	+	+	0.48
7.	0.52	+	+	+	-	+	+	+	+	+	+	+	+	+	0.52
8.	0.60	+	+	+	+	+	+	+	-	+	+	-	+	+	0.60
9.	0.65	+	+	+	+	+	+	+	+	+	+	+	+	+	0.65
10.	0.70	-	+	+	-	+	+	+	+	+	+	+	+	-	0.70
11.	0.74	-	-	+	+	+	+	+	+	+	+	+	-	+	0.74
12.	0.76	-	+	+	+	+	+	+	+	+	+	+	+	+	0.76
13.	0.78	-	+	+	-	+	+	+	+	+	+	+	+	+	0.78
14.	0.84	+	+	+	-	+	+	+	+	+	+	+	+	+	0.84
15.	0.88	+	+	+	+	+	+	+	+	+	+	+	+	+	0.88
16.	0.95	-	-	+	+	+	+	+	+	+	+	+	+	+	0.95
	Total	8	12	14	10	14	14	15	14	14	15	14	14	11	

(+) represents presence and (-) represents absence of band

in varietal identification (Gangwar and Bajpai, 2006 and Salimi *et al.,* 2013). The high polymorphic bands were also found in tris-soluble SDS-PAGE in comparison to water soluble SDS-PAGE of these genotypes that was differed in the RM value and molecular weight. The difference observed in varieties in the level of protein bands, means, the varieties having different protein are encoded by different genes or exons. The variation in banding patterns or RM values or molecular weight also generate variation in genomic structure, which resulted variation in protein, which are useful to identification and characterization of varieties. Differences in band mobility, width and intensity were reported in various

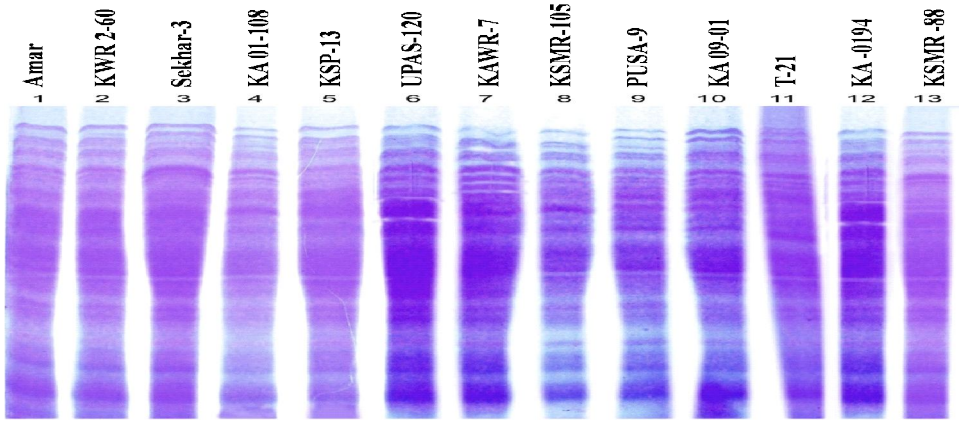

Fig 1: SDS-PAGE electrophoregram of tris-soluble seed proteins in pigeonpea genotypes.

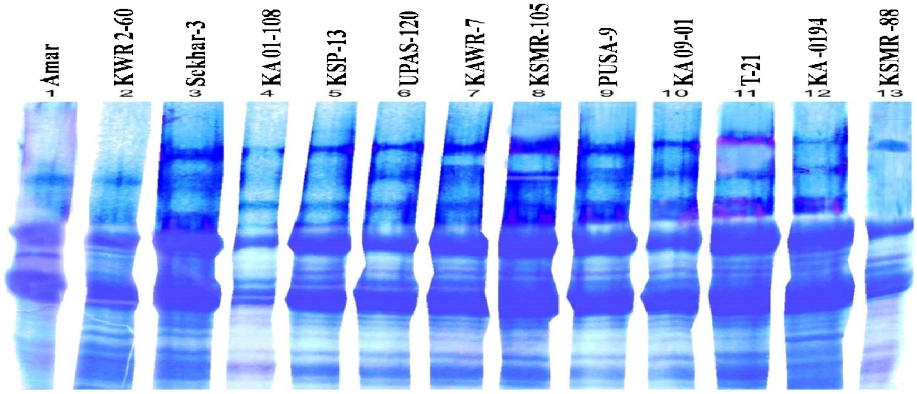

Fig 2: SDS-PAGE electrophoregram of water-soluble seed proteins in pigeonpea genotypes.

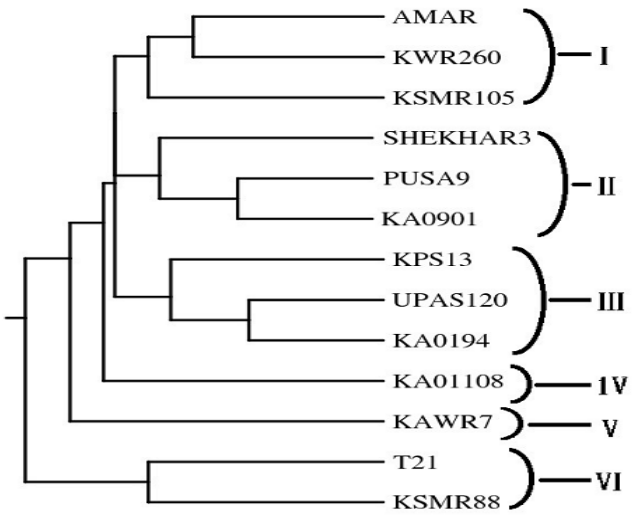

Fig 3: UPGMA cluster analysis showing genetic relationship of tris- soluble protein among thirteen genotypes based on tris-soluble seed protein of pigeonpea genotypes.

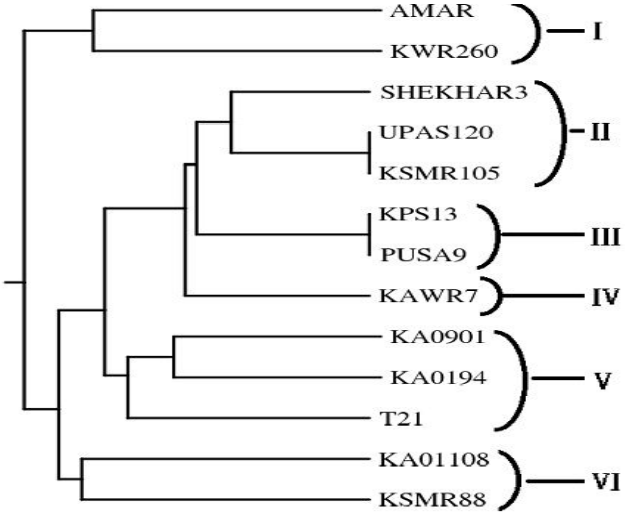

Fig 4: UPGMA cluster analysis showing genetic relationship of water soluble protein among thirteen genotypes based on water soluble seed of pigeonpea genotypes.

legumes like pigeon pea (Singh *et al*., 1991 and Gangwar and Bajpai, 2006), grass pea (Mandal and Das, 2001), pea (Gafoor and Arshad., 2008), soybean (Salimi *et al.,* 2013) and bottle gourd (Srivastava *et al*., 2014).

CONCLUSION

The varieties were characterized and identified using electrophoretic SDS-PAGE based tris- and water- soluble seed protein banding patterns, electrophoregram and RM value. UPGMA cluster analysis of SDS-PAGE banding pattern of both soluble protein showed distinct differences in 13 pigeonpea genotypes, however the electrophoregram results revealed the tris-soluble protein was more distinct than water-soluble proteins. It showed SDS-PAGE is a powerful tool for reliable variety discrimination and identification based on genetic differences in seed storage protein of pigeonpea genotypes. Proteins being the direct gene products reflect the genomic composition of lines accurately to some extent and therefore, are ideal for genotypic distinctness.

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