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Genetic diversity among *Trigonella foenum-graecum* and *T. carniculata* genotypes for storage seed protein profile

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Abstract

Thirty five fenugreek and 13 Kasuri Methi genotypes were evaluated to determine their seed storage protein subunits profile variation through sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were extracted to represent both water and salt soluble proteins. The protein subunit bands fell in the range of 80 kD to 14 kD of their molecular weight. Both, for fenugreek and Kasuri Methi, the protein bands could be placed in four distinct regions. There were 26 polypeptide bands out of which 19 were polymorphic. The protein subunit banding pattern of fenugreek and Kasuri Methi were different. The polymorphic bands were used to compute Jaccard's similarity coefficient using NTSYS-pc software. The similarity matrix thus prepared was used to construct a dendrogram by UPGMA. The dendrogram distributed the 35 genotypes of fenugreek into 8 clusters whereas 13 genotypes of Kasuri Methi into 4 clusters. In fenugreek the present study revealed genotypic clustering instead of geographical clustering.

Key words: Fenugreek, genetic diversity, kasuri-methi, SDS-PAGE, seed protein

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herbaceous and autogamous crop belonging to family Fabaceae (sub family Papillionaceae) and popularly known by its vernacular name 'Methi'. Its seeds comprise one of the important seed spice crops grown in the country. It has multifarious uses such as vegetable, as condiments in culinary and in a range of medicinal preparations. The seed is bitter in taste due to presence of alkaloids known as "Diosgenin" which is considered as basic material for the synthesis of cellulose, hemicellulose, nutrient and amino acids.

Major fenugreek producing countries are India, Pakistan, Iran, Nepal, Bangladesh, Argentina, Egypt, France, Spain, Turkey and Morocco. The largest producer of fenugreek in the world is India, where during the year 2014-15 it was cultivated in an area of 123 thousand hectares with the production of 131 thousand tones having the productivity of 1065 kg ha^{-1} (Anonymous, 2017). Rajasthan alone accounts for over 77% of the production where, its high market price attracts the farmers to include this crop in their cropping system. However, its productivity has been reported to be stagnant in India and Rajasthan for many years which call for development of suitable high yielding varieties.

For an effective breeding programme, genetic or a heritable variation is a prerequisite. There is substantial variation in the germplasm of fenugreek with respect to different characters. However, it is realized that selection of parents on the basis of molecular typing (besides morphogenetic data) which may include DNA or protein based diversity would be more useful in breeding programs for making choice of parents for hybridization. Keeping these points in view the present study was undertaken to identify diverse genotypes of fenugreek on the basis of their seed storage protein profile. Few genotypes of Kasuri Methi (*Trigonella carniculata*) were also included in the study for comparison purposes.

Material and methods

The present investigation was carried out at S.K.N. College of Agriculture, Jobner. The experimental material was comprised of 35 genotypes of fenugreek (*Trigonella foenum-graecum* L.) and 13 genotypes of Kasuri Methi (*Trigonella carniculata* L.). These genotypes were obtained from the AICRP on Spices, Department of Plant Breeding and Genetics, at S.K.N. college of Agriculture, Jobner. The list of genotypes used and their source of collection are presented in Table 1.

Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was conducted according to the procedure of Laemmli (1970) with minor modifications described by Tripathy *et al.* (2010). For protein extraction, seeds were ground and sieved to get a fine powder. Proteins were

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Table 1. List of fenugreek and Kasuri Methi genotypes used in the study with their source of collection.

Cluster	Genotype numbers	Fenugreek genotypes	Origin	Cluster	Genotype numbers	Kasuri Methi genotypes	Origin
1	1	RMt-365	Jobner, Rajasthan	I	36	JKM-1	Nagaur
	2	RMt-1	Jobner, Rajasthan		38	JKM-3	,,
	8	UM-83	EC-4314		43	JKM-8	,,
	14	UM-64	Tonk, Rajasthan		44	JKM-9	,,
	11	RMt-361	Jobner, Rajasthan		45	JKM-10	,,
	12	UM-38	Tamil Nadu		46	JKM-11	,,
	3	RMt-143	Jobner, Rajasthan		37	JKM-2	,,
	15	UM-142	Australian		42	JKM-7	,,
	16	UM-98	Andhra Pradesh	2	47	JKM-14	,,
	17	UM-90	EC-25913		48	JKM-15	,,
	26	UM-5	Kota, Rajasthan	3	39	JKM-4	,,
2	5	UM-134	Gujrat		40	JKM-5	,,
	6	RMt-351	Jobner, Rajasthan	4	41	JKM-6	,,
	7	RMt-305	Jobner, Rajasthan				
	9	UM-97	Andhra Pradesh				
	24	UM-112	Jaipur Rajasthan				
	13	UM-107	IC-5686				
	21	UM-21	Andhra Pradesh				
	25	UM-261	Chittor, Rajasthan				
	28	UM-181	Tamil Nadu				
	22	UM-178	Tamil Nadu				
	23	UM-113	Banswara, Rajasthan				
3	4	UM-44	Sikar, Rajasthan				
4	10	RMt-303	Jobner, Rajasthan				
5	18	UM-144	Jodhpur, Rajasthan				
	27	UM-50	Sikar, Rajasthan				
	19	UM-54	Sikar, Rajasthan				
	20	UM-82	IC-689				
6	31	UM-68	Pusa Early Bunching				
	35	UM-41	Andhra Pradesh				
	34	UM-152	Tamil Nadu				
7	32	UM-29	Andhra Pradesh				
	33	UM-11	Jhalawar, Rajasthan				
8	29	UM-36	Tamil Nadu				
	30	UM-330	Rajasthan				

extracted using three different procedures described by Hora *et al*., (2013), Erume *et al*., (2011) and Sharma (2012). The procedure of Sharma (2012) revealed relatively more and clear bands, therefore, was used in rest of the study (data not shown). Proteins were extracted by grinding 50 mg of seed powder in 1 ml of distilled water followed by subsequent grinding in 1ml of 1M NaCl. One milliliter of extracted protein samples were transferred into Eppendorf tubes and centrifuged for 5 minutes at 10,000 rpm. One half milliliter (0.5 ml) supernatant was transferred into a fresh Eppendorf tube and denatured with 0.5 ml cracking buffer (0.2M Tris-Hcl buffer pH 6.8, 10% SDS, 20% glycerol, 10 mM β-mercaptoethanol, and 0.05% Bromophenol blue) at 80^{0}C in a water bath for 15 minutes. Bromophenol blue (BPB) was added to the cracking buffer served as tracking dye to monitor the movements of protein bands in the gel. The protein samples were loaded into the wells of the polyacrylamide gel slab prepared for electrophoresis. The electrophoresis was carried out on vertical gel electrophoresis equipment (BioRAD, Model: Protean II Xi Cell) with a power supply maintained at 30 mA for four and half hours. Two separate gels were run under similar electrophoretic conditions in order to check the reproducibility of the results. After electrophoresis gels were stained with Coomassie Brilliant Blue R 250 overnight; followed by distaining overnight and finally washing in distilled water. The molecular weights of the dissociated polypeptides were determined by using standard molecular weight marker. The thoroughly distained gels were put in clear transparent polythene bags for further use in photography.

Data analysis

Gels were scored for the presence ("1") and absence ("0") of every protein subunit bands. These binary data were used to analyze using NTSYS-pc (Numerical Taxonomy System, Version 2.1, Rohlf 2000). The SIMQUAL sub-programme was used to calculate the Jaccard's coefficient using following formula (Jaccard, 1908).

Jaccard's coefficient = $N_{AB}/ (N_{AB}+N_A+N_B)$,

where, N_A and N_B represents number of bands in sample A and sample B, respectively, N_{AB} is the number of bands shared in the samples. Genetic similarity matrices were computed using un-weighted pair group method with arithmetic average (UPGMA) for construction of the dendrograms to elucidate the diversity among the accessions studied (Sneath and Sokal, 1973). Statistical stability of the branches in the cluster was estimated by bootstrap analysis with 1000 replicates, using Winboot software program (Yap and Nelson, 1996).

Results and discussion

The genotypes of fenugreek used in the present study represented a diverse geographical source of collection, and hence, were expected to have differences. It was, therefore, considered justified to substantiate the same on the basis of the protein profile of their seed storage protein. Characterization of genotypes on the basis of seed storage proteins/subunits is well documented in different groups of crops such as, black gram (Ghafoor and Ahmad, 2005; Ghafoor *et al*., 2002), *Capsicum annuum* L. (Anu and Peter, 2003), *Solanum* (Menella *et al*., 1999), *Vigna* spp (Rao *et al*., 1992; Sharma, 2012; Choudhary, 2013), and wheat (Siddiqui and Naz, 2009) including fenugreek (Hora *et al*., 2013).

Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS–PAGE) as originally described by Laemmli (1970) is a powerful and dependable technique for characterization of proteins (or its subunits). In principle, it involves extraction of proteins from the target materials, e.g. cotyledons, leaves etc, followed by its denaturation into polypeptides. The resulting protein subunits are then separated through a gel (polyacrylamide) in an electric field according to their masses.

Fenugreek is a leguminous seed spice crop. Legume seeds in general predominantly contain albumins (20-35%) and globulins (43-55%). The albumins are water soluble whereas globulins are salt soluble. These two proteins together account for 63-90% of the total seed protein (Tchiagam *et al*., 2011). Besides, fenugreek seed is also known to contain alkaloids, known as diosgenin and lectin called trigonellin. For this reason three different protein extraction procedures described by Hora *et al*., (2013), Erum *et al.* (2011) and Sharma (2012) were evaluated on the basis of their ability to generate protein subunit bands in the SDS-PAGE. However, the procedure of Sharma (2012) was followed in rest of the studies as it revealed relatively more number of darkly stained clear protein subunit bands.

The protein bands ranged from slightly less than 80 kD to 14 kD. The observed bands seemed to fall in four distinct regions (Figure 1A & 1B). The first region corresponded to 80-56 kD of molecular weight, whereas the second region spanned over 50-35 kD, third region was around 20-22 kD and last region corresponded to around 14 kD of moleculer weight.

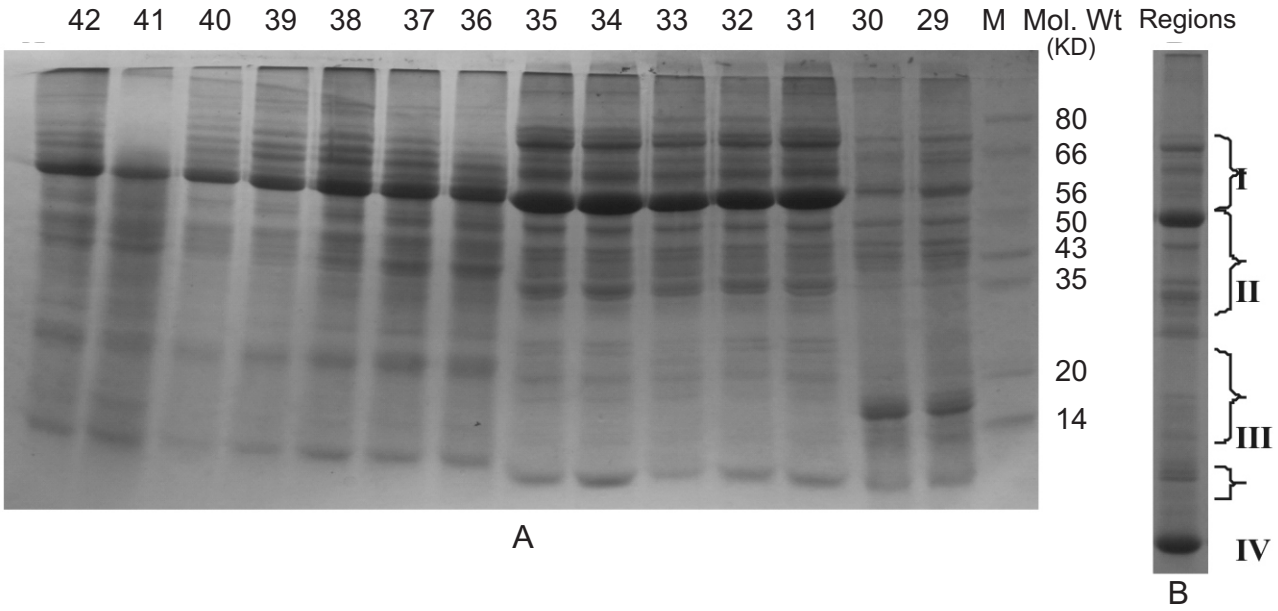

Fig. 1. (A) A view of electrophoregram of fenugreek genotypes 29-35 and Kasuri Methi genotypes 36-42. (B) A view of four regions of protein subunit bands.

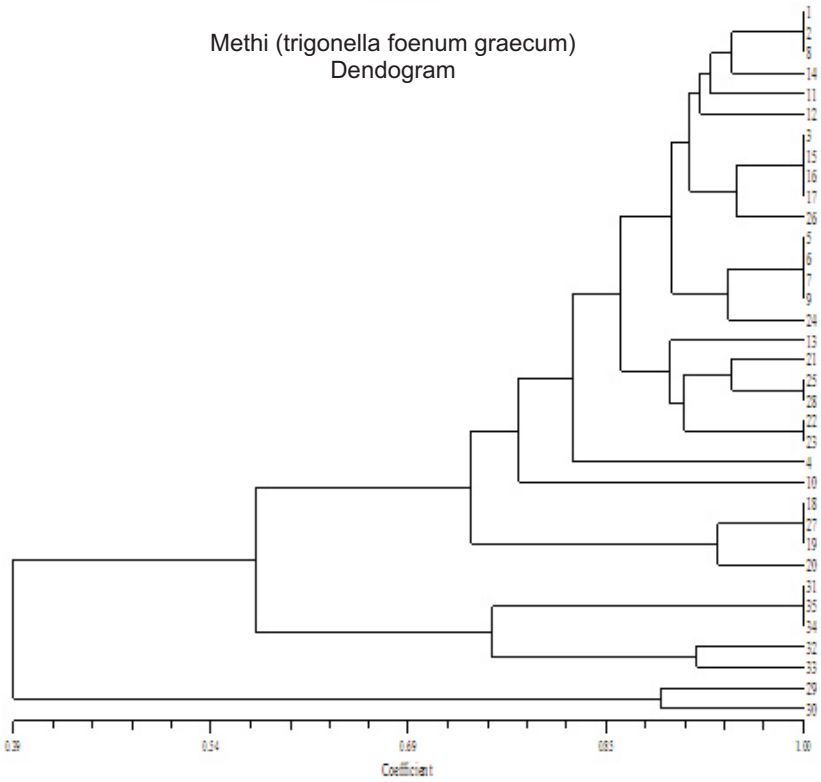

Fig.2. Dendrogram of the 35 fenugreek accession revealed by UPGMA cluster analysis of SDS page based genetic similarity estimates.

In the study performed by Hora *et.al.*, (2013), they reported a different band pattern. They have reported 25 polypeptide bands but only 4 being polymorphic. Where as in the present study we observed 26 polypeptide bands out of which 19 were polymorphic. Such differences can be attributed to the larger number of fenugreek genotypes included in the present study in comparison to only 8 by Hora *et.al.*, (2013). For further description of the results, the electrophoregram of fenugreek and Kasuri Methi are described with separately.

Fenugreek

The 19 polymorphic bands belonged to all the four regions. Using the binary data and NTSYSpc software, the Jaccard's similarity coefficient was obtained. The similarity coefficients ranged between 0.18 to 1.0. Among the 35 genotypes, only one pair showed minimum coefficient of 0.18 where as higher coefficients values such as 1, 0.95, 0.94, 0.89 and 0.88 were associated with 187 cases. It may be inferred that large number of genotypes may have common evolutionary history.

A dendrogram was constructed using Jaccard's similarity coefficients. The cluster analysis of the genotypes revealed 8 clusters at 85% similarity coefficient (Fig.2). The clustering was more apparent after performing the bootstrap analysis using Winboot programme at 1000 cycles. The salient features of clustering includes, (1) The genotypes 29 (UM-36) and 30 (UM-330) were placed in one cluster and found to be most diverse from rest of the clusters. Interestingly, genotype 29 (UM-36) represented Tamilnadu and genotype 30 (UM-330) was a collection from Rajasthan. Further, these two genotypes showed presence of unique bands of ~16 kD and absence of 35 kD bands as compared to rest of the fenugreek genotypes (Fig.1). However, it is highly unlikely that these two genotypes, which are similar and diverse from the rest of the collection studied, would have evolved independently, (2) the released varieties included in the study also showed distinction among them as revealed by clustering at 90%. These results are considerably similar to that observed by Hora *et. al.*, (2013). These authors used 8 varieties of fenugreek in which 6 were similar to our studies. Both RMt-305 (genotype-7) and RMt-303 (genotype-10) were diverse from each other in both the studies but in present study, RMt-351(genotype-6) and RMt-361 (genotype-11) were not as similar as reported by Hora *et al.*, (2013), (3) exotic (Australian) genotype-15 (UM 142) was in the same cluster which included Andhra Pradesh genotypes 16 &17(UM-98 and UM-90). Our data are inadequate to explain how these genotypes of diverse origin resemble each other in this regard and (4) there was no noticeable separation of clusters with geographical diversion which is evident from cluster I and included genotypes from Tamilnadu, Andhra Pradesh, Rajasthan, Gujarat, and Australia. A similar picture was obtained for other clusters as well which included genotypes from different geographical origins. Obviously, clustering revealed genotypic diversity.

Kasuri Methi

All Kasuri Methi genotypes were collected from Nagaur district where this crop is traditionally grown. A perusal of protein bands of 13 genotypes revealed that out of 20 bands obtained 17 were polymorphic and belonged to all the four regions. The presence or absence of data was used to generate binary data for each of 13 genotypes. Using the binary data and NTSYSpc software, the Jaccard's similarity coefficient was obtained. The similarity coefficients ranged between 0.19 to 1.0. Among the 13 genotypes, only two pairs showed minimum coefficient of 0.19 where as higher coefficients values such as 1, 0.94, 0.63 were associated with 45 cases (or 60%). Thus, it may be inferred that large number of genotypes may have shared common development at some point of the evolutionary time scale.

A dendrogram was constructed using Jaccard's similarity coefficient obtained from protein binary data of 13 genotypes of fenugreek employing NTSYS-pc programme (Fig.3). The cluster analysis of the genotypes revealed 4 clusters at 80% similarity. However, only three clusters were apparent after performing the bootstrap analysis using Winboot programme at 1000 cycles. The salient features of the clustering are described as follows: (1) Genotype-41(JKM-6) was most diverse from the rest 12 collections, (2) Genotypes 39 & 40 (JKM 4 & 5) and genotypes 47 & 48 (JKM 14 & 15) were similar among themselves and (3) Genotypes 1,3,8,9,10 and 11 were in subcluster – 1(a) whereas genotypes 37 & 42 (JKM 2 and 7) were in subcluster 1(b).

Conclusion

The results of the present investigation demonstrate that subunit profile of the seed storage proteins can be effectively used to unravel the diversity between fenugreek or even the Kasuri Methi genotypes, however, their distinction based on geographical diversity was absent.

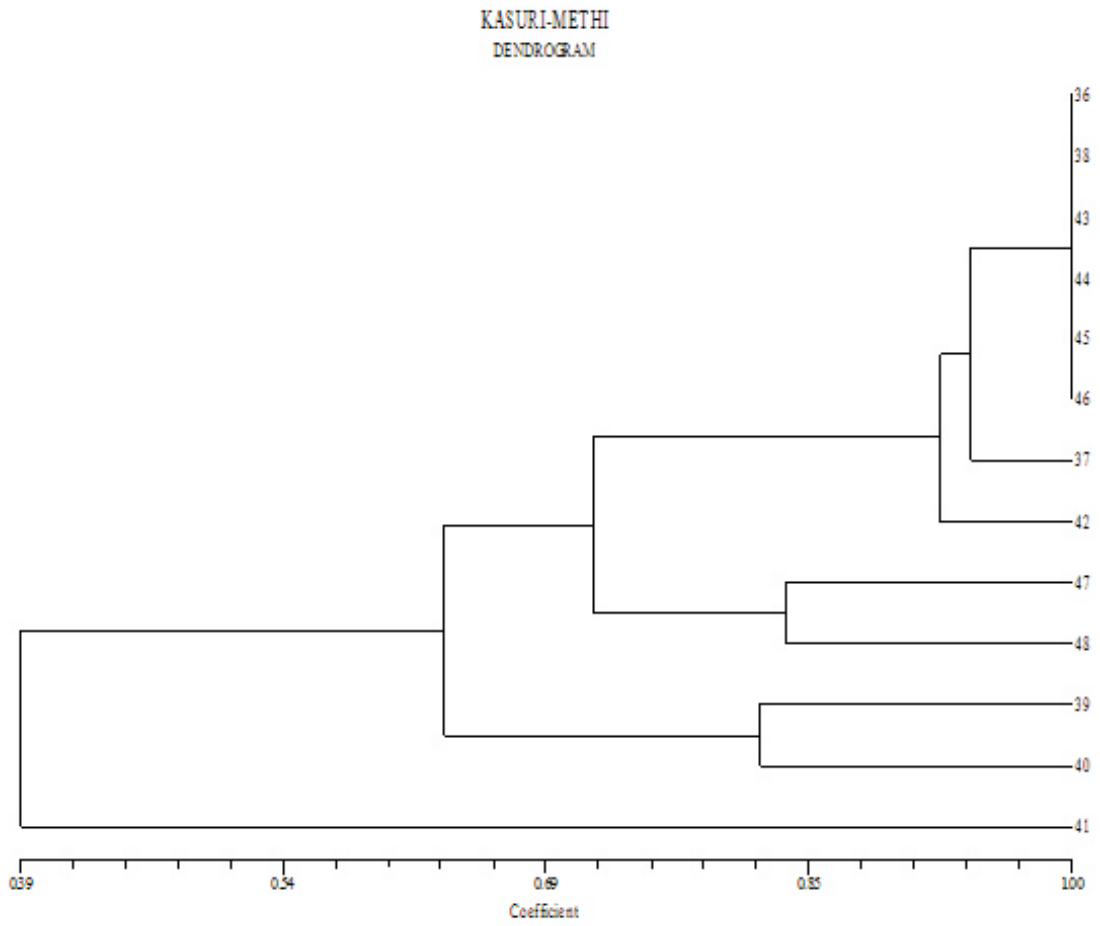

Fig.3. Dendrogram of 13 Kasuri Methi genotypes revealed by UPGMA cluster analysis of SDS page based genetic similarity estimates.

References

Anonymous, 2017. Horticulture Statistics at a glance 2017. Government of India, Ministry of Agriculture & Farmer's Welfare, Department of Agriculture, Cooperation and Farmer's Welfare, Horticulture Statistics Division, New Delhi, pp 144. (www.agricoop.nic.in/statistics/horticulture)

Anu, A. and Peter, K. V., 2003. Analysis of seed protein of 29 lines of *Capsicum annuum* L. by polyacrylamide gel electrophoresis. *Genetic Resources and Crop Evolution*, 50: 239–243.

Choudhary, M. 2013., Electrophoretic characterization of cowpea (*Vigna unguiculata* (L) Walp.) mutants for storage seed protein profile. M.Sc. (Ag.) Thesis submitted to S.K.R.A.U., Bikaner, Campus-Jobner.

Erum, S., Anwar, R. and Masood, S., 2011. Evalution of kasuri methi *Trigonella foenum graecum* L.Var. to establish GI right to Pakistan. *Pakistan J. Agriculture*, 24: 1-4.

Ghafoor, A. and Ahmad, Z., 2005. Diversity of agronomic traits and total seed protein in black gram *Vigna mungo* (L.) Hepper. Acta *Biologica Cracoviensia* Series Botanica, 47(2): 69–75.

Ghafoor, A. and Ahmad, Z., Qureshi, A. S. and Bashir, M., 2002. Genetic relationship of *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R.Wilezek based on morphological traits and SDS-PAGE. *Euphytica*, 123 :367-378.

Hora, A., Chittora, M. and Malik, C. P., 2013. Estimation of genetic diversity based on Seed storage proteins among different varieties of T*rigonella foenum*

graecum. An International J. Life Science, 2 (1) :1-6.

Jaccard, P., 1908. Nouvelles recherches sur la distribution florale. Bulletin de la Société Vaudoise des Sciences Naturelles. 44: 223-270.

Laemmli, U. K., 1970. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature, 227: 680 – 685.

Menella, G., Sanaja, V. O., Tonini, A. and Magnifico, V., 1999. Seed storage protein characterization of *Solanum sps*. and of cultivars and androgenetic lines of S. melogena L. by SDS-PAGE and AE-HPLC. *Seed Science and Technology*, 27: 23-35.

Rao. R., Vagliol, M. D., D'Urzo, M. P. and Month, L., 1992. Identification of *Vigna spp* . through specific seed storage polypeptides. *Euphytica*, 62 : 39-43.

Rohlf, F. J., 2000. NTSYS-pc: Numerical Taxonomy System. Ver. 2.1. Exeter Software, Setauket, NY, USA. 29-34.

Sharma, D. B., 2012. Genetic diversity in cowpea (*Vigna unguiculata* (L) Walp.) using protein profile M.Sc. (Ag.) Thesis submitted to S.K.R.A.U., Bikaner, Campus-Jobner.

Siddiqui, M. F. and Naz, N., 2009. Protein landmarks for diversity assessment in wheat genotypes. *African J. Biotechnology*, 8(9): 1855-1859.

Sneath, P. H. A. and Sokal, R. R., 1973. Numerical Taxonomy: The principles and practice of numerical classification. W.H. Freeman, San Francisco, USA. pp. 573.

Tchiagam, J. B. N., Bell, J. M., Nassourou, A. M., Njintang, N. Y. and Youmbi, E., 2011. Genetic analysis of seed proteins contents in cowpea (*Vigna unguiculata* L. Walp.). *African J. Biotechnology*, 10(16): 3077-3086.

Tripathy, S. K., Sardar, S. S. and Mishra, P. K., 2010. Analysis of seed storage protein pattern: a method for studying genetic variation and diversity among Vigna genotypes. *Indian J. Genetics*, 70 (2): 140-144.

Yap, I. V. and Nelson, R. J., 1996. WinBoot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. IRRI Disc. Ser. No. 14. International Rice Research Institute, Manila, Philippines.

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