Volume 1, Issue 3. December, 2021. www.jbbt.org

MOLECULAR CHARACTERIZATION OF PEPPER (*CAPSICUM ANNUM* L.) GENOTYPES FROM DIVISION MUZAFFARABAD.

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ABSTRACT

Thirty pepper (*Capsicum annum* L.) genotypes were characterized using Twelve PCR-DNA based ISSR markersand Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis(SDS-PAGE). Healthy seedswere collected from different regions of Division Muzaffarabad Azad Jammu & Kashmir Muzaffarabad.A field experiment was carried out in the NIGAB (National Institute of Genomics and Advanced Biotechnology), NARC, and Islamabad.Fresh leaves were used to extract DNA using CTAB method.Bivariate 1-0 data matrix and dendrogram was obtained by using UPGMAfor cluster analysis of genotypes.Overall 84 bands were produced the highest polymorphism observed was 89%. SDS-PAGE was used for seed protein profiling of 30 chili genotypes.The total 112 bands were identified. In present investigation the genotypes showed considerable variation in protein banding pattern.

Key words: Polymorphism, *Capsicum annum* L., ISSR, SDS-PAGE, cluster, genetic diversity.

INTRODUCTION

Hot pepper (*Capsicum annum* L.) belongs to the genus Capsicum and family Solanaceae, widely consider as first spice to have been used by the human. Among vegetables, after tomatoes and peas it ranks third in the world (Ali, 2006). After tomatoes the capsicum is considered as the second most important vegetable of family Solanaceae (Yoon *et al*., 1989). Chili (Capsicum annum L.) is cultivated mostly in temperate and tropical zones of the world because it is an important commercial crop worldwide. The genus Capsicum contains about 31 species (Moscone *et al*., 2007) of which five species are domesticated, namely C. pubescens R., C. frutescens L., C. annuum L. C. chinenseJacq. and C. baccatum L. Out of five domesticated species of pepper, chili is the most widely cultivated and is used as vegetable and spice. Pepper consists of 12 chromosome pairs with a variable genome size from 3,200 to 5,600 Mb (Pakozdi *et al*. 2002).

The genetic diversity arises due to genetic barriers to cross ability or geographical differences. Different molecular, biochemical and morphological markers are used to carry out the genetic diversity analysis of capsicum species. The taxonomy of genus capsicum is complex. Its limitation into some species can shift extensively dependent upon the variations in characteristics of fruits, leaves and flowers and the variation in climatic and geographic conditions of the plant habitat (Petters *et al*., 2002). In plant breeding programs the basic requirement is the information on phylogenetic relationship and genetic diversity between the lines of a germplasm of a crop. Molecular markers or morphological characters could be used to obtain pedigree analysis and genetic relationship (Mohammadi and Prasanna, 2003). DNA markers are highly polymorphic, stable and can be obtained easily. The markers data can be used to establish various core collections recently (Zhao *et al*., 2016). The presence of microsatellites throughout the genome is a source to study polymorphism by using ISSR (inter simple sequence repeat) (Zietkiewicz *et al*., 1994).

Biochemical categorization has some important parameters like enzymes and proteins that are considered as principal source of gene products. Because of the nonessential nature of the storage proteins that are characterized by electrophoresis are considered to experience the procedure of advancement (Margoliash and Fitch, 1968) and enzymes are believed to be incredibly sensitive for development and necessary for existence of the living being (McDaniel, 1970). The seed proteins are primary products of structural genes that were used as genetic markers and have the greater precision to measure genetic diversity conveyed by these markers (Srivalli *et al.*, 1999). The characterization of Capsicum annuum L. was done by seed protein electrophoresis technique (Panda *et al*., 1986). The phylogenetic relationship and genetic diversity among chili varieties is studied by seed protein profiling (Srivalli *et al*., 1999) has initiated the diploids and tetraploid hybrids of Capsicum. Species identification by electrophoresis of seed protein is a useful technique for back-tracking the diversity of various groups of plants and frequently employed as an additional approach (Ladizinski and Hymowitz, 1979).

Genetic variability among twenty three chili genotypes observed through morphological and SDS-PAGE analysis during the cropping seasons of 2011 and 2012 at National Agricultural Research Centre (NARC), Islamabad, Pakistan, low variation in chili accessions was observed (Mushtaq *et al.,* 2018).Six hybrids of C. frutescens and C. annuum were characterized with 10 ISSR primers which produce polymorphism average of 60%. This study was considered a significant approach to detect genetic diversity among capsicum (Ahmed, 2013).

MATERIALS AND METHODS

Plant material

Seeds of thirty capsicum genotypes were collected from Division Muzaffarabad Azad Jammu and Kashmir. Thirty Capsicum genotypes were selected which were sown in a greenhouse at National Institute of Genetics and Biotechnology Islamabad, Pakistan. Fourteen genotypes were collected from Muzaffarabad, ten from Jhelum valley and six from Neelum valley.

DNA extraction and PCR amplification

DNA was extracted from young leaves of 3-week-old seedlings by using a standard protocol (Doyle and Doyle, 1990). Bulk genomic DNA samples were made from 30 individual seedlings of each genotype for studying ISSR analysis. Total 12 microsatellite markers (ISSR) (table 1) were used for detecting genetic divergence in pepper genotypes. All primers produced polymorphic bands, therefore they were included in further study. PCR amplification was done at 94ºC for three (3) minutes. It consists of 3 steps; first step was of de naturation which was at 94ºC for three (3) minutes followed by 40 cycles for 40 seconds and second step primer annealing was 40 seconds and extension for 1 minute at 72ºC. Final extension period was at 72ºC for 10 minutes.

Electrophoresis to visualize the amplified PCR products

For the visualization of PCR products 2% agarose gel was prepared. 4µl of ethidium bromide was used as dying material to visualize the bands at gel documentation system. 100bp ladder was also loaded on gel with PCR product so to check the presence or absence of bands by comparing the bands with DNA ladder of known base pair size the scoring was done.

Characterization (*Capsicum annum* l.) genotypes using sodium dodecyl sulphate polyacrylamide gel

Seed protein extraction:

Total seed protein was extracted using 25 mg of seed in 1 ml of protein extraction buffer and incubated for 1 hour at 4°C. The extraction buffer contained 50mM Tris-HCl, 0.2% SDS, 5.0 M urea and 0.1% mercaptoethanol. After 1 hour the tubes were vortexed and then centrifuged for 10 minute at 12000rpm. . Supernatant was taken and transferred to new labeled eppendorf tubes and stored at 4°C.

Seed protein profiling

Proteins profiling of samples was performed using SDS-polyacrylamide gels as described by Laemmli (1970).Total proteins were resolved on 4.5% stacking (pH 6.8) and 10% separating (pH 8.8) of 90 × 80 × 1mm SDS-PAGE (ATTO AE-6530m PAGE system). The protein samples were mixed with 3ul loading dye.

Gel documentation and analysis:

. The protein bands were assessed by making comparison of bands with protein ladder of known band size in Gel documentation system.

Data Analysis

The recorded protein and molecular data was evaluated through bivariate data matrix in which all bands were counted on the basis of their presence (1) or absence (0). Construction of dendrogram was done by using UPGMA (Unweighted Pair Group Method and Arithmetic Mean') method, to assess genetic associations by using NT SYS software. The polymorphic information content of ISSR markers was calculated using following formula by (Smith and Smith, 1992).

RESULTS AND DISCUSSION

Molecular tools ISSR markers are considered to be very useful for explaining population structure and genetic diversity among them. In present study one set of molecular marker viz., ISSR markers. Overall 84 bands were produced by pepper genotypes under investigation, out of which 71 were polymorphic bands and 13 were monomorphic bands representing 51.5% of entire amplified loci with an average of 5.9 polymorphic bands for each primer. The greatest number of alleles produced by ISSR UBC 827 (9), whereas UBC826 and UBC813 generated lest number of alleles (5). The largest polymorphism was given away by ISSR UBC827 (89%) and lowest polymorphism (71.4%) was depicted by UBC810. Average polymorphism percentage observed for all ISSR primers was 84%. The PIC value calculated assorted from 0.88473 for UBC825 to 0.68903 for UBC809. Average PIC value observed for all primers was 0.8091 (Table 1).

It has been noticed that the present study is useful in different ways. Polymorphism and genetic diversity in genotypes was detected through cluster analysis. The grouping of pepper genotypes through molecular and protein markers revealed significant data to considerate more on the genetic diversity among pepper genotypes of district Jehlum valley and district Muzaffarabad Azad Jammu and Kashmir. It provides beneficial direction in pepper breeding programs, to select particular genetic diverse background germplasm.

Similarly, four capsicum accession were characterized using 41 ISSR primers, high variability was observed among the accessions (Dias *et al*., 2012).

Similar findings were provided by (Patel *et al*., 2011), who used five ISSR primers for the estimation of genetic diversity among thirteen genotypes. Five ISSR primers amplified 204 reproducible bands of which 139 were polymorphic. The highest polymorphic bands obtained by the use of primers UBC-809 (34) and UBC-66.

The gel pictures of primers which shown highest polymorphism are given below.

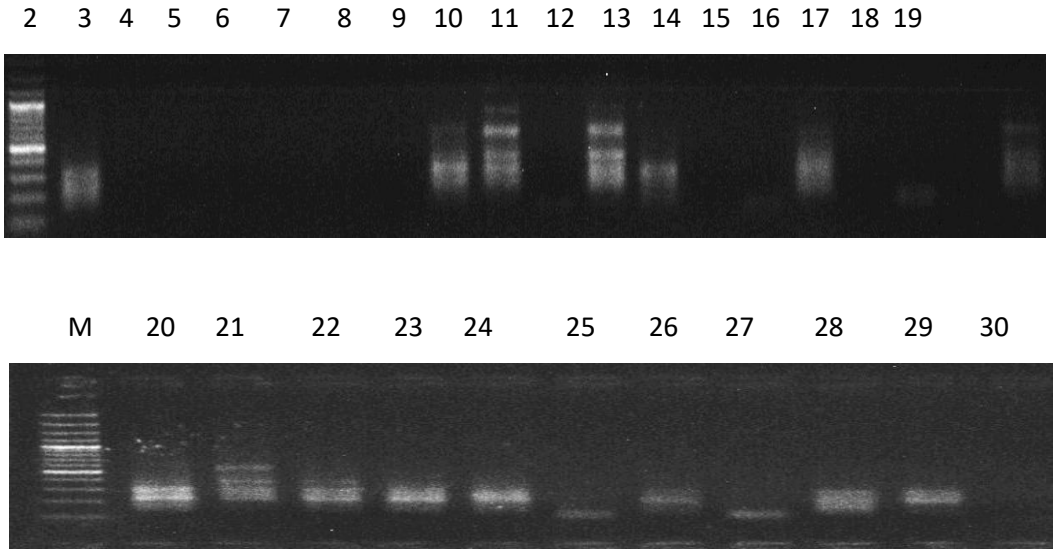


Figure 1. UBC 809 ISSR marker, DNA ladder 100bp

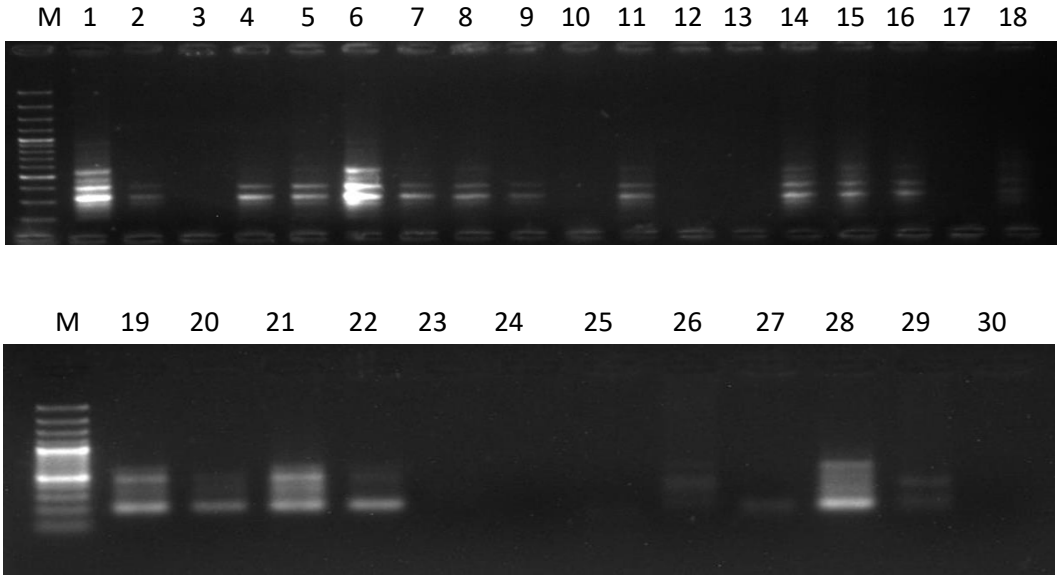
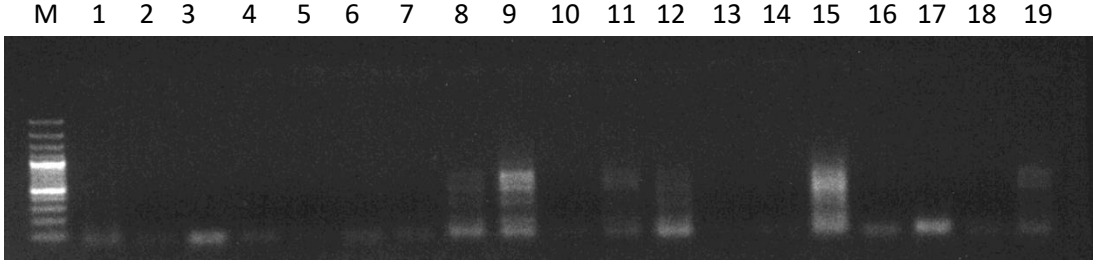


Figure 2. ISSR UBC807, DNA ladder 100bp



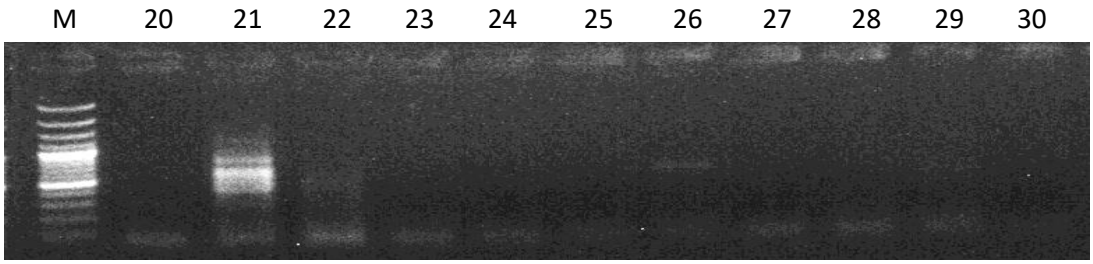
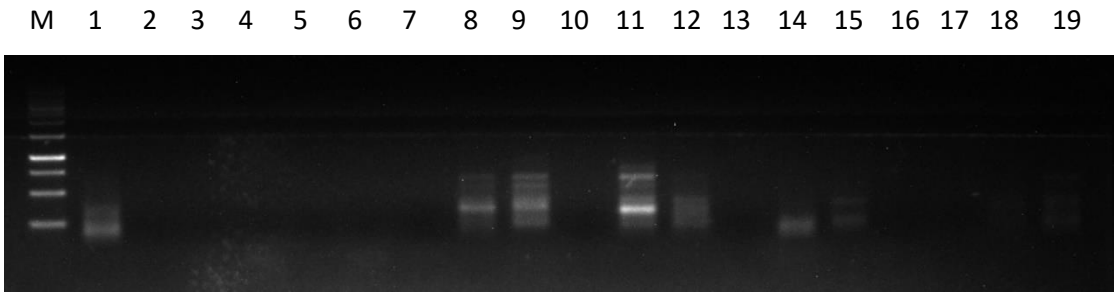
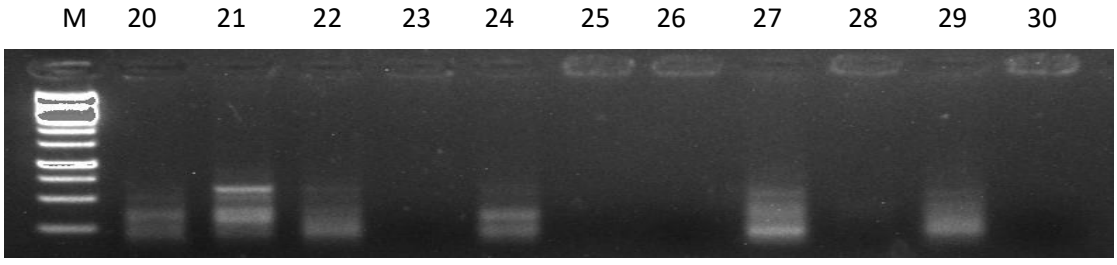
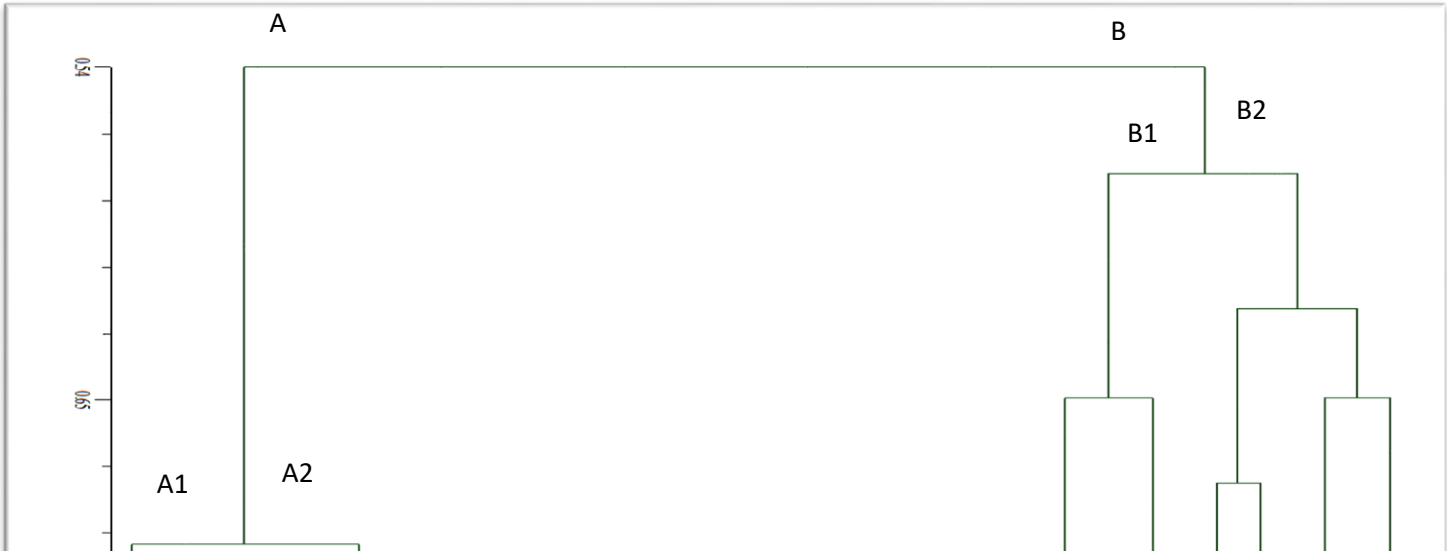


Figure 3. ISSR UBC825, DNA ladder 100bp







Out of 30 genotypes, total percentage of genotypes present in clusters A2-1 and A2-2 was 16.66% and 50% respectively, while the percentage of genotypes in sub-group A1 was 3.33% is not further divided into clusters and has only one genotype. The sub-groups B1 and B2 hold percentage of genotypes 13.33% and 16.66% respectively.

Figure 4. ISSR UBC827, DNA ladder 100bp

Group's distribution dendrogram for cluster analysis of pepper genotypes on the basis of ISSR markers

Table 1. List of ISSR primers, number of amplified product and number of polymorphic bands, percentage of polymorphism and PIC value obtained by thirty genotypes:

Primer	Total number of bands	Number of polymorphic bands	%age of polymorphism	No of loci amplified	Number of genotype amplified	PIC value
UBC 826	5	4	80%	35	13	0.78695
UBC 809	7	6	86%	38	18	0.68903
UBC 807	8	7	87.5 %	67	18	0.83227
UBC 810	7	5	71.4%	48	19	0.82985
UBC 815	8	7	87.5%	36	11	0.84722
UBC 825	8	7	87.5%	68	24	0.88473
UBC 823	7	6	86%	53	17	0.78586
UBC 827	9	8	89%	66	15	0.83148
UBC 844	6	5	83.3%	80	14	0.849376

UBC 891	6	5	83.3%	19	10	0.720239
DBD	8	7	87.5%	54	16	0.790814
UBC813	5	4	80%	54	16	0.86147
TOTAL	84	71	1009	618	191	9.709
MEAN	7	5.9	84.0%	51.5	15.9	0.8091

Characterization of protein in 30 genotypes of *Capsicum annum* L. using (SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis)

Out of 30 genotypes, total percentage of genotypes present in sub-group A1 and clusters A2-1 and A2-2 is 10% , 3.33% and 40% respectively (Fig. 6).. While in subgroup B1 comprising 2 genotypes having total percentage of genotypes is 6.66% and cluster B2-1 and B2-2 have total percentage of genotypes 23.33% and 16.66% respectively. CM1 and CM14 comprise the protein with 10kDa molecular weight. While CM11 and CM13 contain (15kDa and 40kDa), CJ8 and CJ9 contain (10kDa and 15kDa), CM2, CM3, CM4, CM5, CM7, CM8, CJ3, CJ4, CJ6, CJ10, CN1, CN3, and CN6 comprise different proteins of different molecular weight (10 kDa, 15 kDa, 25 kDa, 26 kDa, 38kDa, 40kDa, 70kDa). CM6, CM10 and CJ2 contain higher proportion of proteins of

different molecular weight (10kDa, 15kDa, 25kDa, 26kDa, 27kDa, 38kDa, 70kDa) respectively, while CM12, CJ1, CJ5, CJ7, CN2, CN4, and CN5 contain no protein content. May be the proteins of these genotypes degraded after extraction (Cseke *et al*., 2004) or some other factors involved.The total 112 bands were identified.

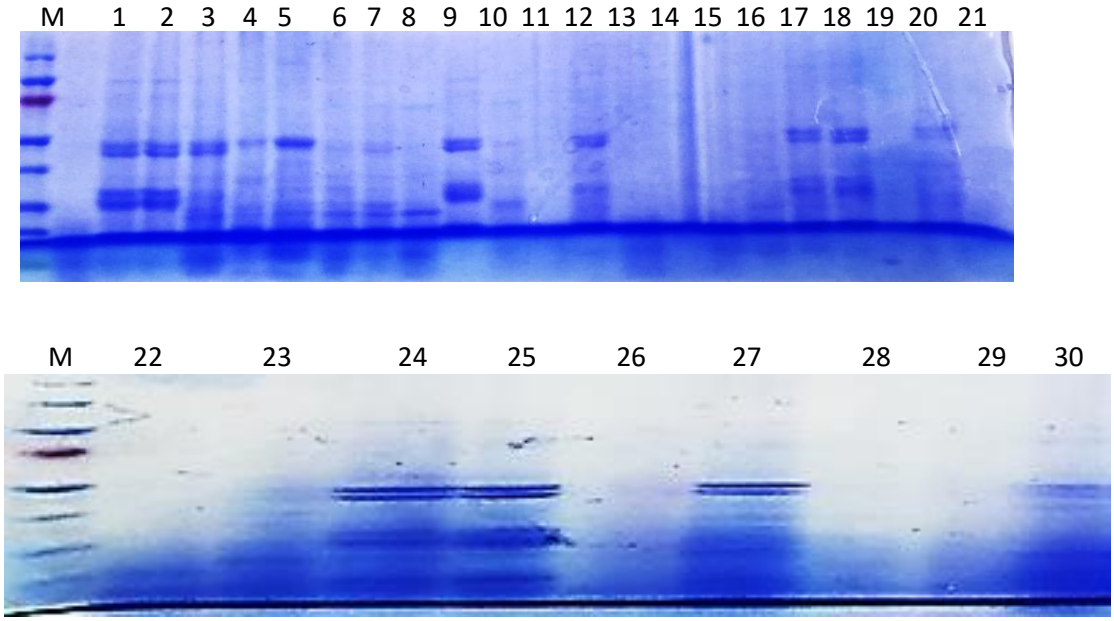


Figure 5. Gel picture showing the pattern of protein distribution in seeds of 30 genotypes of *capsicum annum* L.

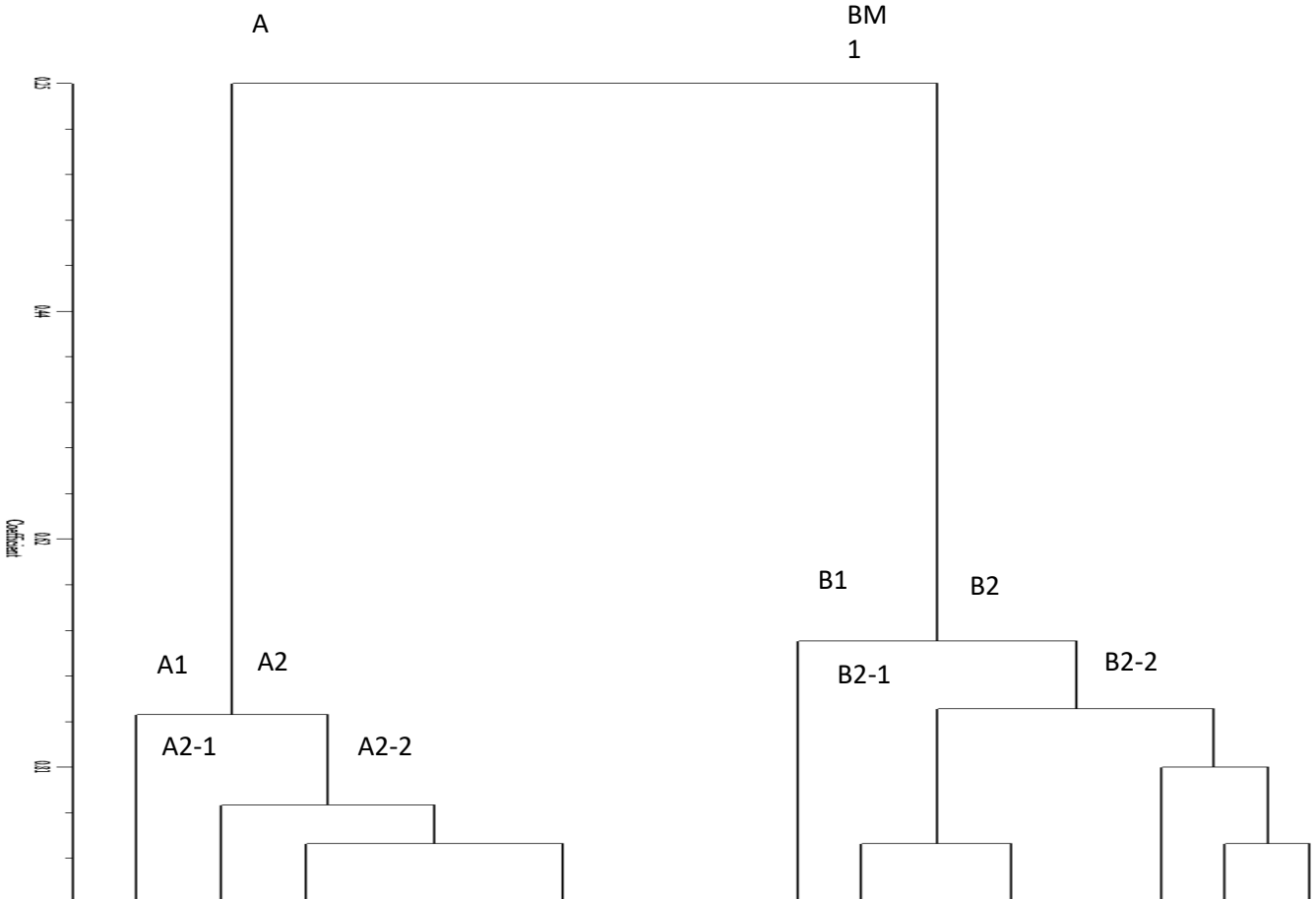

A
BM
1
0.12
0.04
0.00
0.01
Coefficient
A1
A2
A2-1
A2-2
B1
B2
B2-1
B2-2

Figure 6. UPGMA of thirty chilli genotypes based on total seed protein obtained by SDS-PAGE.

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