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Genetic Variation among Seedling of Pumpkins Genotypes through SDS-Page

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Abstract

Genetic diversity in 30 genotypes of Pumpkins was collected from unexplored mountainous areas of Khyber Pakhtunkhwa, Pakistan was investigated through biochemical characterization. For biochemical characterization, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis was carried out. The seed proteins were resolved on 7.5% and 15% polyacrylamide gel. A total of 35% genetic disagreement was observed in the collected lines with linkage distances ranging from 0.00 - 0.83 (percent disagreement). Similarly, cluster analysis sorted total germplasm on the basis of 12 bands (total bands) into eight clusters. Present study revealed a considerable amount of genetic diversity explored in pumpkin germplasm, Cluster analysis exhibited moderate level of genetic diversity; to broaden the gene pool. Further collection of the important germplasm is needed to be used in the development of improved cultivars with respect to quality and quantity.

Keywords

Genetic Diversity, SDS-PAGE, Pumpkin, Cluster Analysis

1. Introduction

Genetic diversity is the need for stabilizing production and increasing yields in the face of disease epidemic, environmental fluctuation, and utilization of these genetic resources to enhance the performance of a germplasm [1] [2] [3]. The knowledge of genetic diversity is a useful tool in planning experiments, as it facilitates sampling and utilization of germplasm, and helps in the establishment of core collections [4] [5]. Genetic divergence has been studied in many cultivated

plants [6] [7] and various other crops like maize, rice, sugarcane, Wheat, and legumes plants [8] [9] [10] [11]. Different techniques are adopted in germplasm evaluation for genetic diversity in desirable traits that may include morphological and agronomic evaluation (qualitative and quantitative), biochemical evaluation at protein level (SDS PAGE, Isozyme assay, isoelectric focusing) and at DNA level (Blotting and PCR based analysis [12] [13]. Electrophoresis (SDS-PAGE) is widely used to describe seed protein diversity of crop germplasm. The technique of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used for separation of seed storage proteins [14] [15] [16] and to investigate genetic variation and to classify plant varieties. Seed storage protein profiling based on SDS-PAGE can be employed for various purposes, such as characterization of germplasm [17] [18] varietal identification, biosystematics analysis, determination of phylogenetic relationship between different species and generation of pertinent information to complement evaluation [19]. Seed storage proteins have also been used to study the evolutionary and taxonomic relationship of several crop plants. SDS-PAGE analyses are simple and inexpensive, which are the major advantages for use in practical plant breeding [20] [21]. Among the Cucurbitaceous Pumpkin being an important vegetable cultivated under tropical, subtropical and temperate regions all over the world, and has a place of high value, excellent response to vegetable forcing, high nutritive estimates and better transport qualities, used at both mature and immature stages as a vegetable [22]. The fruits are sweetish and are used for preparing sweets, candy and fermented into beverages. Yellow or orange fleshed pumpkins are rich in carotene. The fruit contains 30 mg of phosphorus, 1.4 g of protein, 0.7 mg of iron, 10 mg of calcium, 2 mg vitamin C, and 50 μg of carotene in 100 g of edible portion [23]. Genetic analysis based on quantitative traits has been made in this crop in Pakistan [24] [25] [26] [27]. However, phenotypic characters have limitations as they are influenced by environmental factors and the developmental stages of the plant. In contrast, molecular markers, based on DNA sequence polymorphism are independent of the environmental conditions and show a higher level of polymorphism. Random amplified polymorphism DNA markers are more useful for the assessment of genetic diversity due to their simplicity, speed and relatively low cost compared to other molecular markers [28] [29]. SDS markers have been used extensively in cucurbits to classify accessions [30], to assess the genetic relationship among the different genotypes of pumpkin [31]. In the present study, SDS-PAGE and morphological markers were used to estimate genetic diversity among genotypes of pumpkin, collected from different zones of KPK and to explore genetic diversity on the basis of SDS-PAGE.

2. Materials and Methods

A total of 30 genotypes of Pumpkins (*C. maxima* and *C. pepo*) germplasm evaluated during the present study were obtained from different areas of Khyber Pakhtunkhwa Pakistan.

To explore the genetic diversity on the basis of protein SDS-PAGE was carried out. For SDS-PAGE analysis, seeds of each genotype were crushed into a fine powder with mortar and pestle. Protein extraction buffer (400 ml) was added to 0.01g of seed flour and mixed well with Automatic Lab-Mixer DH-10. The extraction buffer contained the following final concentrations: 0.5M Tris HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol [32]. Bromophenol blue (BPB) was added to the protein extraction buffer as tracking dye to watch the movement of protein in the gel. The molecular weight of the dissociation polypeptides was determined by using molecular weight protein marker MW-SDS-70 Kit (Sigma). The SDS-PAGE of total seed protein was carried out in the discontinuous buffer system according to the method of Acrylamide gel concentration 7.5% - 15% and 10 ml of sample were used for analyzing germplasm. After staining and destaining, the gel was dried using (Atto, Rapidery-Mini Japan) gel drier. In order to check the reproducibility of the method two separate gels were run under similar electrophoretic conditions. The data was scored on the presence (1) or absence (0) of protein bands and high intensity of glow was considered as major bands and low intensity as minor bands [33].

Data Analysis

Similarity indices of 30 genotypes were calculated for all possible pairs of protein types and used to construct dendrogram by the UPGMA by computer software STATISTICA ver-6.0.The binary data matrix was analyzed for cluster analysis and the genetic diversity and genetic homology calculated.

3. Results and Discussion

Protein electrophoresis is a most widely and powerful tool for population genetics [34] and the SDS-PAGE tool is particularly a reliable way because storage proteins are independent of environmental up down [35] [36]. Biochemical markers assess accurate genetic diversity index [37]. In the present study, the genetic diversity through SDS-PAGE of 30 genotypes of pumpkin were carried out, the protein profile of each cultivar was subjected to cluster analysis (**Figure 1**). The results of the cluster analysis were presented in the dendrogram on the basis of linkage distance. The dendrogram drawn for 30 pumpkins (*Cucurbita maxima* and *Cucurbita pepo*) germplasms showed two divisions. The distance 0.55 was divided into two lineages L-1 and L-2, while distance 0.29 was divided into eight clusters. The L-1 has a single cluster C-1, while the L-2 has seven clusters C2, C-3, C-4, C-5, C6, C7 and C8.

The dendrogram drawn for 13 pumpkins (*Cucurbita maxima*) germplasms showed division at linkage distance 0.38 and 0.190 (**Table 1**). The linkage distance 0.38 was divided into two lineages L-1 and L-2, while at 0.190 linkage distance was divided into five clusters. The L-1 has three clusters C-1, C2 and C3 while the L-2 has two clusters C4 and C5. The varieties which showed high linkage distance were including C3 collected from Buner of, C1 from Matkanai, C4

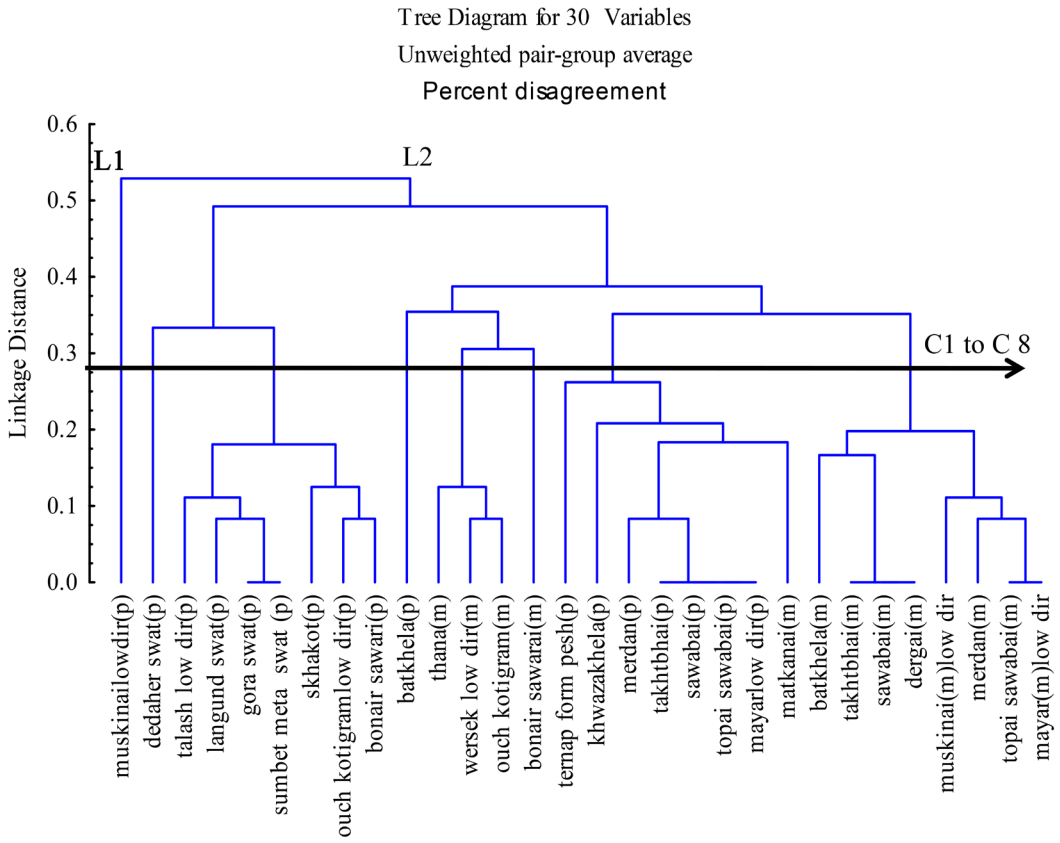

Figure 1. Genetic similarity of 30 pumpkin genotype based on SDS-PAGE analysis showing average linkage in pumpkin germplasm collected from different zone of KPK, Pakistan.

Table 1. Protein banding outline data of *C. maxima* based on linkage distance.

Locality	Mayar	Merdan	Topai	Dergai	Sawabai	Takhtbhai	Muskinai	Batkhela	Sawarai	Kotigram	Wersek	Thana	Matkanai
Mayar	0.00												
Merdan	0.08	0.00											
Topai	0.00	0.08	0.00										
Dergai	0.17	0.08	0.17	0.00									
Sawabai	0.17	0.08	0.17	0.00	0.00								
Takhtbhai	0.17	0.08	0.17	0.00	0.00	0.00							
Muskinai	0.08	0.17	0.08	0.25	0.25	0.25	0.00						
Batkhela	0.33	0.25	0.33	0.17	0.17	0.17	0.25	0.00					
Sawarai	0.33	0.42	0.33	0.50	0.50	0.50	0.25	0.50	0.00				
Kotigram	0.25	0.33	0.25	0.42	0.42	0.42	0.33	0.58	0.25	0.00			
Wersek	0.33	0.42	0.33	0.50	0.50	0.50	0.42	0.67	0.33	0.08	0.00		
Thana	0.17	0.25	0.17	0.33	0.33	0.33	0.25	0.50	0.33	0.08	0.17	0.00	
Matkanai	0.33	0.25	0.33	0.33	0.33	0.33	0.42	0.33	0.50	0.25	0.33	0.17	0.00

from Batkhela of and C2 from Thana. The varieties showed 100% similarities in their banding pattern were C4 collected from Takhtbhai, Swabi and Dergai and

C5 from Topai and Mayar.

The dendrogram drawn for 17 pumpkins (*Cucurbita pepo*) showed division at linkage distance 0.49 and 0.24 linkage distance. The distance 0.49 was divided into two lineages L-1 and L-2, while at 0.24 linkage distance divided into six clusters. The L-1 has two clusters C-1 and C2 while the L-2 has four clusters C3, C4, C5 and C6. The varieties showed high linkage distance including C1 of Batkhela, C2 of Muskinai, C3 of Dedaher, C5 from Tarnab farm Peshawar and C6 from Khwazakhela. The varieties showed 100% similarities in their banding pattern were C4 collected from Gora swat and Sumbet Mata of. Takht Bhai, Swabi, Topai and Mayar (**Figure 2**). The genetic distance was also estimated for the two varieties separately and combined form. The average genetic distance was calculated which showed that high genetic distances were found for *Cucurbita maxima* of the accessions from Mardan, Buner and Wersek. While high average genetic distances were calculated for *Cucurbita pepo* of the accessions from Buner Swari, Ouch kotigram, Sumber Mata Swat, Dedaher Swat, Langund Swat, Gora Swat and Muskinai Lower Dir. Our finding show similarity with [38], extracts of 11 protein lines of *Cucumis melo* representing different geographic regions showed considerable variation, the finding also showed agreement with Singh and Matta [39] and Ibrahim *et al.* [40] who reported that the genotypes within a same group showed divergence from each other than from the genotypes of different groups (**Table 2**). Our results do not conform to the findings of Mehrani [41] and Ghafoor and Ahmad [42], who reported low diversity for seed protein in pea and chickpea. Electrophoretic analysis of seed storage proteins is now widely recognized as a technique for cultivar identification in the breeding species. Such conclusions were reported in other local cultivars [43], and agree with the conclusions, that the diversity of protein bands between the varieties within the species are generally low [44]. The finding in the present study was found to be consistent with previous reports for recalcitrant plant tissues [45]. Cultivars included in the present study were referred to one genetic

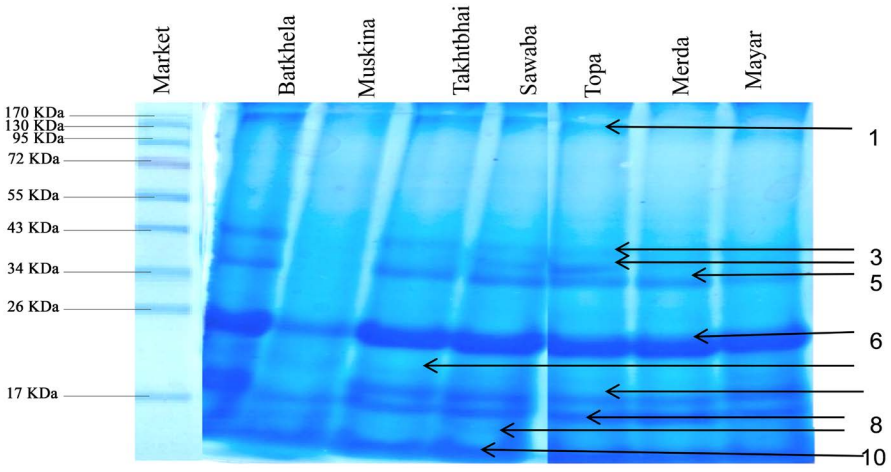

Figure 2. Electrophorogram of 7.50% polyacrylamide gel banding pattern showing diversity in total storage protein of *Cucurbita pepo.*

Table 2. Protein banding outline data of *C. pepo* based on linkage distance.

Locality	Mayar	Merdan	Topai	Sawabai	Takhtbhai	Muskinai	Batkhela	Sawari	Kotigram	Skhakot	Sumbet meta	Dedaher Swat	Ternap form pesh	Langund Swat	Gora Swat	Khwazakhela	Talash
Mayarlow dir	0.00																
Merdan	0.08	0.00															
Topai sawabai	0.00	0.08	0.00														
Sawabai	0.00	0.08	0.00	0.00													
Takhtbhai	0.00	0.08	0.00	0.00	0.00												
Muskinai	0.42	0.50	0.42	0.42	0.42	0.00											
Batkhela	0.50	0.42	0.50	0.50	0.50	0.42	0.00										
Sawari	0.42	0.50	0.42	0.42	0.42	0.50	0.42	0.00									
Kotigramlow	0.33	0.42	0.33	0.33	0.33	0.42	0.33	0.08	0.00								
Skhakot	0.25	0.33	0.25	0.25	0.25	0.50	0.42	0.17	0.08	0.00							
Sumbet Swat	0.42	0.50	0.42	0.42	0.42	0.50	0.42	0.17	0.08	0.17	0.00						
Dedaher Swat	0.67	0.58	0.67	0.67	0.67	0.58	0.50	0.42	0.33	0.42	0.25	0.00					
Ternap form pesh	0.25	0.17	0.25	0.25	0.25	0.67	0.58	0.33	0.42	0.33	0.50	0.58	0.00				
Langund Swat	0.50	0.58	0.50	0.50	0.50	0.42	0.33	0.25	0.17	0.25	0.08	0.33	0.58	0.00			
Gora swat	0.42	0.50	0.42	0.42	0.42	0.50	0.42	0.17	0.08	0.17	0.00	0.25	0.50	0.08	0.00		
Khwazakhela	0.17	0.25	0.17	0.17	0.17	0.58	0.67	0.42	0.33	0.25	0.25	0.50	0.25	0.33	0.25	0.00	
Talash	0.33	0.42	0.33	0.33	0.33	0.58	0.50	0.25	0.17	0.25	0.08	0.33	0.42	0.17	0.08	0.17	0.00

origin. On the basis of present investigations it is concluded that morphological traits, SDS-PAGE analysis revealed a considerable amount of genetic diversity explored in pumpkin germplasm. Important agro-morphological traits like greater yield potential, seeds cataloging and documenting the diversity of genotypes is essential for future pumpkin germplasm breeding programs.

4. Conclusion

It is concluded from the present study that pumpkin germplasm shows a significant amount of genetic diversity suggesting a broadened genetic pool among the genotypes. Further study needs to be carried out in comparison with other germplasm for selecting the best genotypes with high yielding and stress resistant capabilities.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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