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ASSESSMENT OF GENETIC DIVERSITY IN WATERMELON (*CITRULLUSLANATUS (THUNB.)* BY USING SDS-PAGETHROUGH GENETIC ANALYSIS SOFTWARE

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ABSTRACT

Seed storage Protein profiles of five varieties of *CitrullusLanatus* were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Total seed storage protein of *Citrullus Lanatus* were resolved on 10% SDS Polyarylamide gels showed variations in their banding pattern. The dendrogram constructed by NTSys PC software. The SAHN clustering based on UPGMA algorithms has put the all accession into different cluster groups. The Jaccord's coefficient also calculated. Maharaja Variety and Super dragon verity has close genetic relation. Shahanshah and Maharaja have highest variation among five cultivars.Jaccard's similarity shows that Maharaja and Shahanshah are 55% similar which is less similarity in all cultivars, Maharaja and Super Dragon are 73% similar, Maharaja and Durgesh has 73% similarity, Super Dragon and Durgesh are 71% similar. More work is needed to identify the specific genes in the cultivars, quantitative trait loci (QTL) works are carrying out to identify and correlate the genes for different traits using RAPD Technique.

KEY WORDS: *Citrullus Lanatus*, SDS-PAGE, Storage protein, Genetic diversity, Electrophoresis, NTSys PC, SAHN, UPUGMA, Jaccord's Coefficient.

INTRODUCTION

Citrullus genus belongs toCucurbitaceae family which includes about118 genera and 825 species. Citrullus is a member of subfamily calledCucurbitoidae, tribeBenincaseae, subtribeBenincasinae [1].Watermelon has been traced to tropicalAfrica, Asia, and the North American continent and widely cultivated throughout the world [2] The proteinprofiling of germplasm and use of geneticmarkers have been widely and effectively used todetermine the taxonomic and evolutionaryaspects of several crops [3] The electrophoresis of seed storage protein is a methodto investigate genetic variation or genetic diversity to classify plantvarieties. [4]Electrophoresis of seedprotein has been successfully used to reflect geneticaffinities within a family and between differentbiological entities [5,6]. Information about genetic diversity of germplasmusing marker isexquisite for gene bank managementand thus assist in designing breeding experimentsSDS-PAGE is one such simple, economical and extensively used biochemical techniques employedfor genetic structure analysis of germplasm [7]

MATERIALS & METHODS

2.1 Seed Sample

Total five seed samples were collected from different commercial companies of Hyderabad named

S.No.	Company Name	Variety of Hybrid.
1	Sagar Biotech Private Limited	Maharaja
2	Syngenta	Shahenshah
3	Syngenta	Supar Dragon
4	Durga Seed Farm	Durgesh 786
5	Garnier Seeds (India) Private Limited	GS-295

2.2 Protein Extraction

Protein was extracted by method given by Jensen and Lixue [8]. Protein was extracted from overnight presoaked seeds in protein solubilization solution (62 m M Tris –HCl, pH 6.8, 10% glycerol, 2% SDS, p- mercaptoethanol and traces of bromophenol blue) then transferred to 1.5ml tube and centrifuged at 15000 rpm for 1 minute. The supernatent was transferred to a fresh tube and placed into a boiling water bath for 5 minutes.

2.3 SDS-PAGE

SDS-PAGE was done by method suggested by Laemmli [9]. It was performed on a vertical slab gel. Bromophenol blue was added to the supernatant as tracking dye to watch the

movement of protein inthe gel. Seed protein was analyzed through slab type SDS-PAGE using 10% Separating gel and 5% Stacking gel.Protein Electrode buffer solution was poured into the bottom pool of the apparatus. Gel plates were placed in the apparatus carefully so as to prevent bubbles formation at the bottom of gel plated. Equal quantities of extracted protein from each sample were loaded with the micropipette into each wells of the gel. The apparatus was connected with constant electric supply. Electrophoresis was carried out at 20 mA current for 3-4 hours till the tracking dye reaches the bottom of the gel. After electrophoresis, the protein bands were visualized by staining with coomassie brilliant blue G-250 and destained with methanol, acetic acid and water (4:1:5).

2.4 Gel Documentation and Analysis

Finally gel was photographed. Molecular weight of protein bands were estimated by their relative mobility.

RESULTS AND DISCUSSION

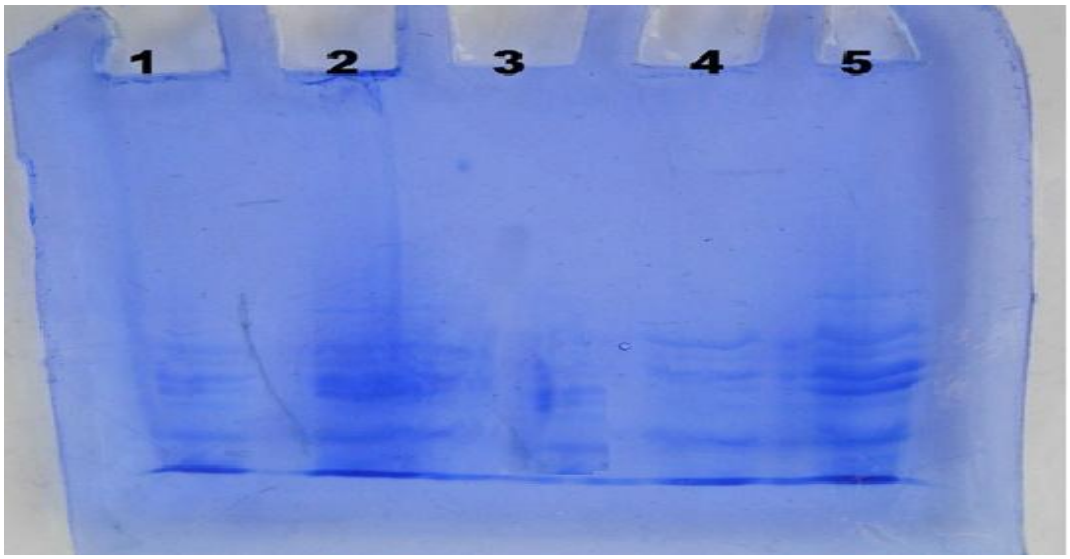

Figure 1. The SDS-PAGE result of seed proteins pattern of*CitrullusLanatus*varieties

1 Maharaja

2 Superdragon

3 Durgesh-786

4 GS-295

5 Shahensha

3.1 Dendrogram construction

The dendrogram constructed by NTSys PC software by the binary data as input. The SAHN clustering based on UPGMA algorithms has put the all accession into different cluster groups.

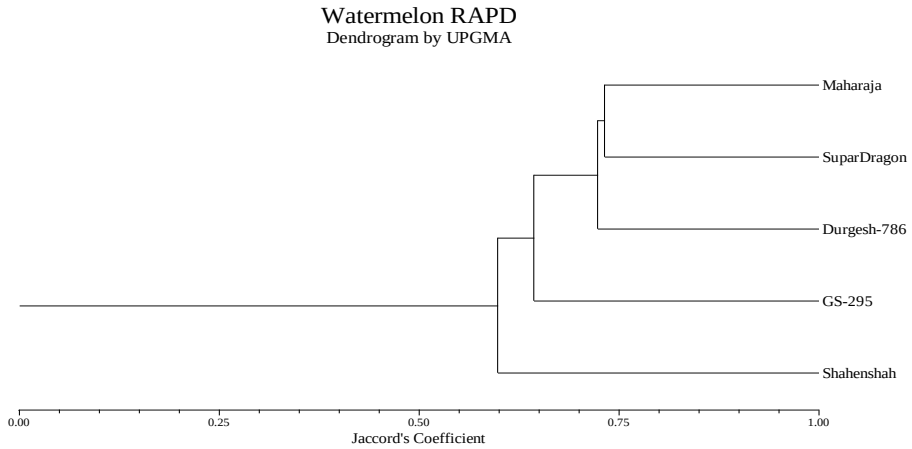

Figure 2. Dendrogram of the relationships among 5 cultivars of *Citrullus Lanatus* based on SDS-PAGE of seed storage proteins.

The dendrogram clearly shows that there is considerable range of genetic diversity in watermelon cultivars. In this Maharaja Variety and Super dragon verity has close genetic relation. Shahanshah and Maharaja have highest variation among five cultivars. Dendrogram initially separated into two clusters One Cluster has Shahanshah and other cluster is sub-clustered into two, first one has GS-295 and second sub-cluster further sub divided into sub-sub-clusters in first one has Durgesh-786 and second one further divided and has Maharaja and Super Dragon.

3.2 Jaccard`s similarity coefficient calculation

Maharaja	Shahenshah	SuparDragon	Durgesh-786	GS-295
1.00				
0.55	1.00			
0.73	0.60	1.00		
0.73	0.64	0.71	1.00	
0.60	0.58	0.66	0.66	1.00

Jaccard’s similarity shows that Maharaja and Shahanshah are 55% similar which is less similarity in all cultivars, Maharaja and Super Dragon are 73% similar, Maharaja and Durgesh has 73% similarity, Super Dragon and Durgesh are 71% similar.

CONCLUSION

Five local hybrid cultivars of watermelon which has high market demand were selected randomly. SDS-PAGE technique is used for determination of genetic diversity based on Seed storage protein. It could be concluded that the present results reveals that Seed storage proteins are able to distinguish the genetic diversity; these proteins are used for the identification of specific cultivar. The dendrogram constructed by UPGMA analysis reveals the genetic similarity and relations of different cultivars. More work is needed to identify the specific genes in the cultivars, quantitative trait loci (QTL) works are carrying out to identify and correlate the genes for different traits using RAPD Technique.

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