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Seed Protein Profiling of Tomato (*Solanum lycopersicum* L.) Genotypes using SDS-PAGE

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A quantitative categorization of seed storage proteins profiles of 13 genotypes of *Solanum lycopersicum* L. was performed by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The banding patterns were characterized by 3 clear distinct zones viz., A, B and C. The unweighed pair group method using arithmetic average (UPGMA) analysis of 13 tomato genotypes was done and two major clusters obtained through seed protein analysis expressed better grouping of genotypes. The dendrogram showed that the genotype EC-519724 was most dissimilar from other genotypes.

Key Words: Cluster analysis, Germplasm, SDS-PAGE, *Solanum lycopersicum* L, Tomato

Tomato ((*Solanum lycopersicum* L.) popularly known as 'Love Apple' is one of the most widely grown and commercially important vegetable crops. Tomato is one of such crop which has received wider attention of vegetable breeders in various countries. Lot of diversity is found in growth and development pattern of tomato due to altitudinal variation in Uttarakhand. To conserve the genetic diversity, elucidation of genetic diversity is extremely necessary for the effective maintenance, evaluation and utilization of germplasm because it is the only source to be exploited for the development of new varieties during breeding programs. The problem of cultivar identification has been simplified to a great extent by the combined use of morphological, biochemical and molecular markers. Morphological markers are highly influenced by environment (Goodrich *et al*., 1985). The biochemical markers are proteins that can be isolated and their polymorphism identified through electrophoresis. Biochemical markers such as proteins and isozymes have served as important tools to detect genetic relationships in plants (Mukhlesur *et al.*,2004; Erum *et al*., 2011). The electrophoresis of proteins is a method to investigate genetic variation and to classify plant varieties (Isemura *et al.*, 2001). Its banding pattern is very stable and it is advocated for cultivars identification purpose in crops. It has been widely accepted that such banding patterns could be important supplemental method for cultivars identification (Tanksley and Jones, 1981; Thanh and Hirata, 2002). Numerous electrophoresis methods are available to identify cultivars by protein banding patterns. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) provides the best resolution. Analysis of SDS-PAGE is simple and inexpensive, which is an added advantage for use in practical plant breeding. Therefore, present study was undertaken to characterize 13 genotypes of Tomato through seed Protein banding pattern.

Seeds of 13 genotypes were procured from Pantnagar Centre for Plant Genetic Resources of G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. The SDS-PAGE is used for seed protein profiling as described by Laemmli, 1970 with slight modification. Each sample having 0.1g seed were crushed in 1 ml extraction buffer (1 M Tris-HCl pH 8.0, 2% SDS, 10% glycerol, 1mM PMSF- phenyl methyl sulphonyl fluoride and 2% mercaptoethanol). The mixture was heated at 65°C for 20 minute and then centrifuged it for 20 minute at 10,000 rpm at room temperature. The supernatant was collected and stored at 4°C for further use. Equal volume (20 µl) of supernatant i.e. protein sample and sample buffer were mixed. Then, it was heated at 60°C in water bath for 5 min. and after cooling samples were loaded to each well along with marker protein in one well with help of micro syringe. The SDS solubilised protein samples were then subjected to vertical slab SDS-PAGE with 12.5% separating gel and 5% stacking gel using Tris-glycine electrode buffer. The samples were electrophoresed at 80 V initially and

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increased upto 100 V with current 500 mA, when the tracking dye passed from the stacking gel. The run was stopped when the dye was approximately 0.5 cm away from the bottom of the gel, which took around 6 to 7 hours. The gel was removed with the help of spatula and dipped for overnight in staining solution. (0.2% coomassie brilliant Blue R 250, 60 g TCA, 180 ml methanol and 60 ml glacial acetic acid). Next day, destaining was performed; the gel was observed on Syngene Gel Documentation system and photographed. Protein bands were scored for their presence as 1 and absence as 0 from the de-stained gel. Presence and absence of the bands were entered in a binary data matrix. Based on results of electrophoretic band spectra, similarity index was calculated for all possible pairs of protein type's electrophoregrams. Coefficient of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by UPGMA(unweighed pair group method using arithmetic average) method by SAHN clustering function of NTSYS-PC(Numerical taxonomy and Multivariate analysis System programme) version 2.0 (Rohlf, 1987)

Seed Storage proteins are highly independent of environmental fluctuations. The high stability of seed protein profile and its additive nature makes it a promising tool for distinguishing genotypes of particular plant species. Therefore, in the present study an attempt has been made to give a blue print of the genetic diversity of genotypes of tomato through SDS-PAGE technique. The seed protein fragments (Plate1) exhibited appreciable polymorphism amongst the thirteen genotypes used for study and the diagrammatic representation has been depicted in Zymogram (Fig. 1). The degree of variation in the bands is interpreted as a measure of genetic divergence among cultivars (Siddiqui and Naz, 2009).

A total of 12 protein bands were obtained which were further categorized under 3 distinct Zones A, B, C depending on their decreasing molecular weight and increasing Rf values. The high molecular weight proteins were located in upper region and low molecular weight protein in the lower region of the gel. A standard medium range protein marker of known molecular weight (14.3 KD to 97.4 KD) was used along with samples. The protein bands were stacked according to their molecular weight i.e. high molecular weight proteins were located in upper region and low molecular weight proteins in the middle and lower region of the gel, respectively. Different protein electrophoretic pattern exhibited by the cultivars could be identified solely by the cultivar specific. For genotype discrimination the presence and absence of protein bands was the criteria for characterisation of germplasm differentiation.

Among 12 bands maximum number of bands was observed in PT-09-06 (10 Bands), EC-519724 (10

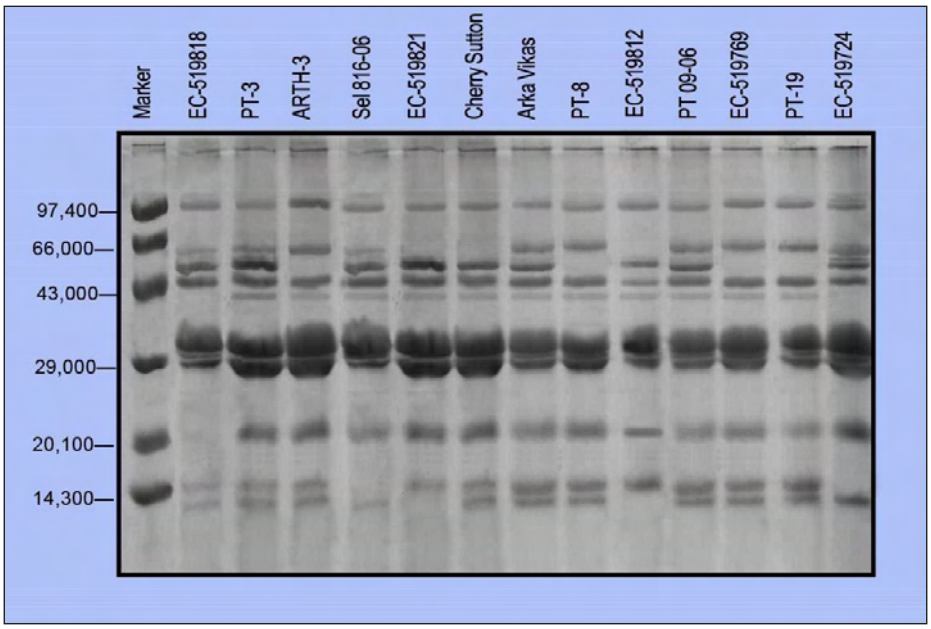

Plate 1. Protein profiling of Tomato through SDS-PAGE

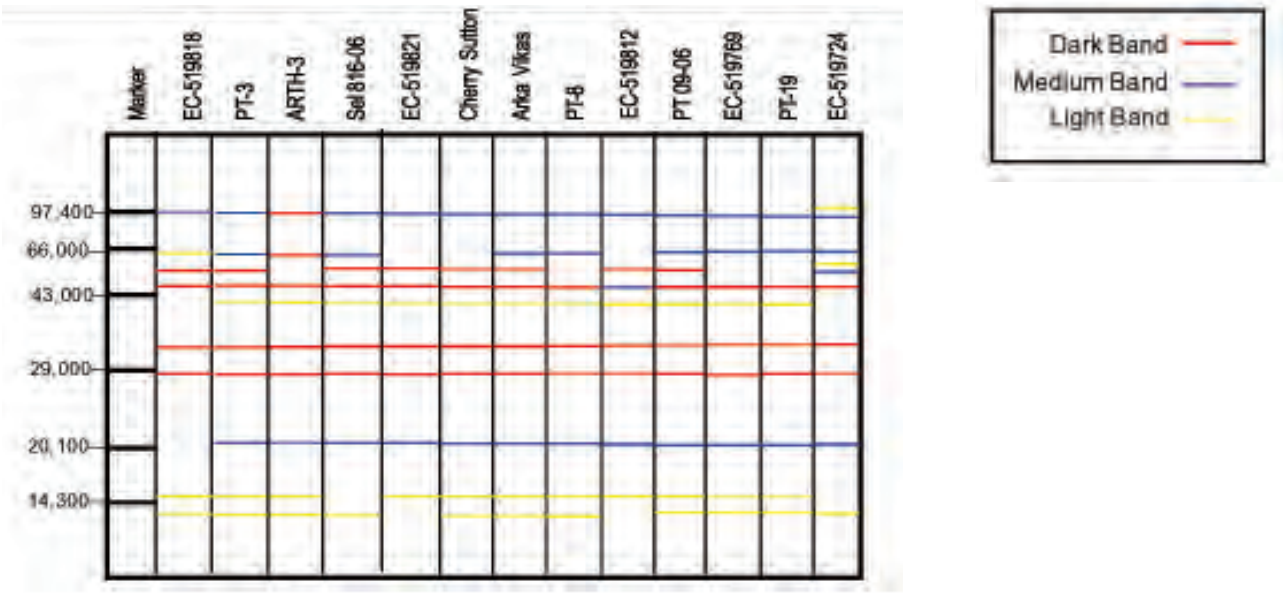

Fig. 1. Protein profiling of Tomato through SDS-PAGE

bands), Arka vikas (10 bands) and PT-3 (10 bands). In contrast to this result, Vishwanath *et al.* (2011) reported that Arka Vikas showed 14 bands.

Zone A representing the heaviest molecular weight protein ranges from 97 KDa to 43 KDa was subdivided into 7 distinct bands. The Subzone A1 was present only in EC-519724. The subzone A2 was present in all genotypes. The subzone A3 were present in ten genotypes and this zone was found absent in EC-519821, EC-519812 and Cherry Sutton. The subzone A4 was present only in EC-519724. The subzone A6 was present in all genotypes. Zone B representing mostly thick and dark bands subdivided into 2 bands i.e. B1 and B2. Bands B1 and B2 were present in all genotypes. The Zone C comprised of 3 Medium and light bands. These were designated as subzones C1, C2 and C3. The subzone C2 were present in 11 genotypes and this was found absent in Sel-816-06 and EC-519724. The subzone C3 present in 11 genotypes and this was found absent in EC-519821 and EC-519812 .Thus a total of 12 bands could be resolved across thirteen genotypes of tomato.

The cluster analysis distinguishes genotypes on the basis of their diversity and could be used as basis of selection of genotypes for crop improvement (Bharose *et al*., 2014). Cluster analysis (Fig. 2) after quantifying the protein bands using UPGMA procedure, indicated that broadly the genotypes were grouped into two major cluster at about 68% similarity index. The major cluster was further divided into two sub-clusters with approximately 78% and 88% similarity. Genotype namely Cherry Sutton showed 100% similarity with other genotypes of same sub-cluster namely EC 519821and EC-519812 in one group which expressed 100% genetic similarity within group. These genotypes were morphologically distinct with each other. The dendrogram showed that the genotype EC-519724 was most dissimilar from other genotypes. As a group genotype EC-519818 was dissimilar from that of PT-3, Arka Vikas, PT-09-06, ARTH-3, PT-8, E-519769, PT-19, Sel-816-06, EC-519821, EC-519812 and Cherry Sutton. Genotypes PT-3, Arka Vikas and PT-09-06 were related and formed one group. Another such group of related genotypes included ARTH-3, PT-8, EC-519769 and PT-19 was also related and formed the second group. Next similarity group was constituted by EC-519821 and EC-519812. One genotype as independent namely EC- 519724 was found most diverse among all genotypes and showed low similarity 75 % with the other genotypes of clusters.

Broadly, these clustering of genotypes to assess genetic diversity in tomato germplasm have also been reported (Hady *et al.*, 2010). The phylogenetic evolutionary tree developed from the analysis indicated that most of the tomato genotypes did not form distinct clusters; rather, the relatively close and small clusters of genotypes were distributed within one broad cluster as can be seen in the phenogram obtained in cluster 1 with 6 groups of 12 genotypes of tomato. The clustering

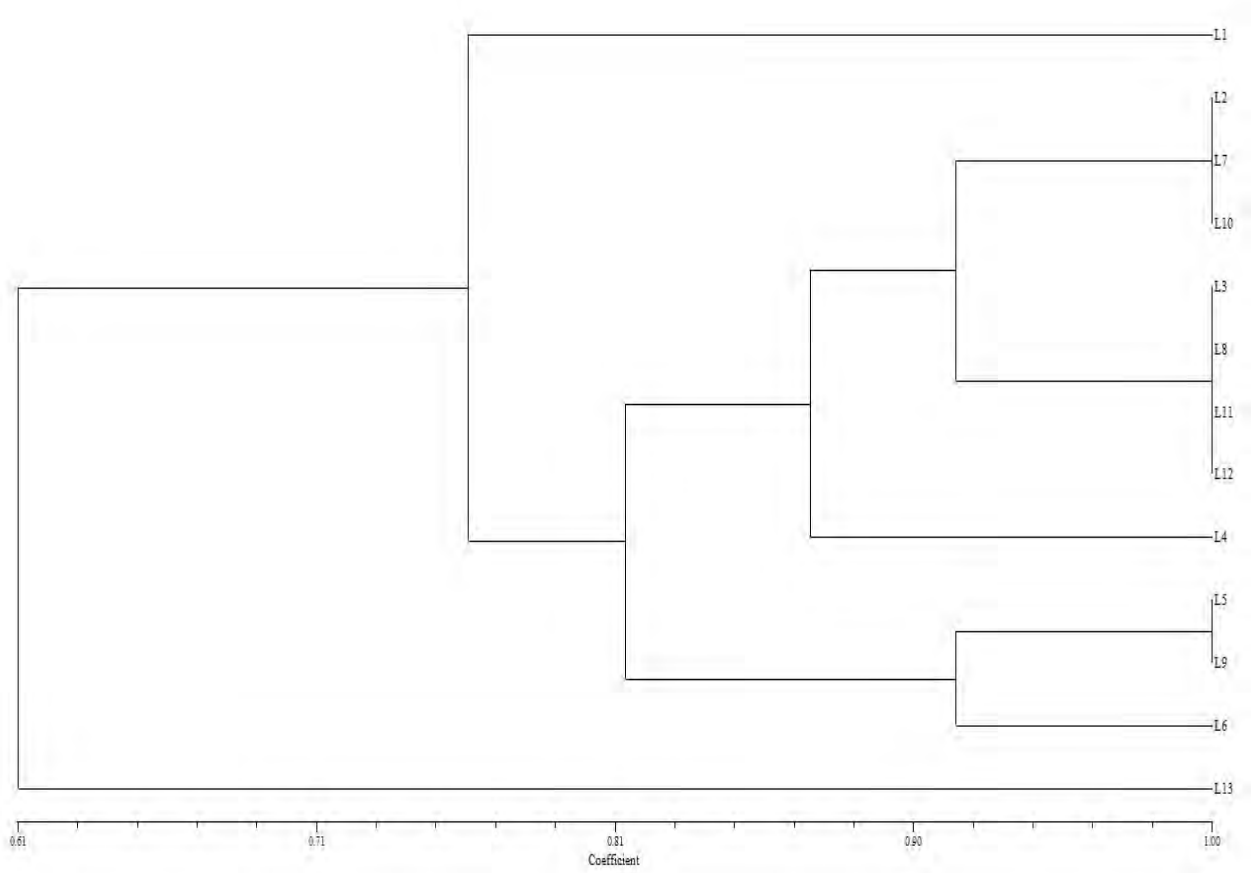

Fig. 2. Dendrogram of thirteen tomato genotypes, L1=EC-519818, L2=PT-3, L3=ARTH- 3, L4=Sel-816-06, L5=EC 519821, L6= Cherry Sutton, L7=Arka Vikas, L8= PT- 8, L9=EC-519812, L10= PT-09-06, L11= EC-519769, L12= PT-19, L13=EC- 519724

pattern indicates though, genotypes are morphologically different but at the genotypic level they are close to each other.Thus, the seed protein profiling emerges as a potent technique to generate wide array of polymorphism and as such it could serve valuable information for varietal identification and extent of genetic diversity. Further, an integrated approach utilizing both morphological and biochemical markers could help in proper characterization of tomato germplasm.

It's concluded from the study that EC 519818 and EC 519724 are most diverse among all genotypes and may be further utilized as potent genotypes in tomato breeding programme for the varietal development.

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