

## Comparative study of genetic variations as determined from marker systems in tomato *Lycopersicum esculentum*

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**Abstract:** Tomato (*Solanum lycopersicum* L.) is most important Solanaceous vegetable grown worldwide for its edible fruits. Various marker techniques have been successfully applied, either individually or in combination to study the genetic diversity of this crop. A Study to assess the usefulness of different markers system for analyzing the genetic diversity and relation between different varieties and to find out correlation between marker systems revealed that all tested tomato cultivars could be differentiated from each other based on either morphological/protein/RAPD markers individually, and can be applied for grouping of cultivars, pedigree analysis and genetic diversity analysis. However, markers system used in this study showed variations in understanding the genetic relation between studied varieties.

**Keywords:** Tomato, Morphological markers, RAPD, SDS-PAGE, Genetic Diversity.

### Introduction

Tomato (*Solanum lycopersicum* L.) is most important Solanaceae vegetable crop and widely grown on a large scale commercially for its fresh edible fruit and processed products. Its popularity in worldwide made the crop more commercial and crop industry developed many varieties and hybrids based on their requirements. Breeders developed improved varieties using wild accessions repeatedly which have resulted in narrowed genetic base and difficult to differentiate closely related varieties/hybrids. This resulted in duplicating of varieties and hence a technique to identify and to study the genetic relationship between varieties and also grouping of varieties are economically important.

To address the issues many studies have been conducted to assess the genetic diversity of tomato using morphological, biochemical and molecular markers (Powell *et al.*, 1996; Tam *et al.*, 2005; Terzopoulos and Bebeli, 2008; Vishwanath *et al.*, 2011). However, these studies were conducted on limited objective for genetic diversity. Each marker system has merits and demerits in studying the genetic relations. Hence the primary objective of this study was to assess the usefulness of different markers system for analyzing the genetic diversity of tomato to find out correlation between different marker systems.

### Material and Methods

Many quantitative morphological characters which are continuously variable are recorded on 1-9 scale according to UPOV, (1992) guidelines.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of total soluble seed protein was carried out by using 15 per cent polyacrylamide gel according to the method described by Laemmli (1970) with slight modifications. Proteins were separated on Vertical gel with resolving gel (15%) and stacking gel (4%) and stained with coomassie blue. Scoring was done based on the presence (1) and absence of a band (0) at predetermined level.

DNA was extracted by following the procedure as given by Meng *et al.*, (1998). PCR was carried out to screen 60 random primers of arbitrary sequence (Operon Technologies Inc.) to select primer that can amplify informative RAPD fragments and finally 18 primers were used for the study. The amplification profiles for all primers were compared with each other and unique bands and characteristic profiles were identified using 0,1 matrices.

Dendrogram was produced for 24 cultivars of tomato based on morphological, biochemical and molecular markers according to Ward's method. (Ward, 1963)

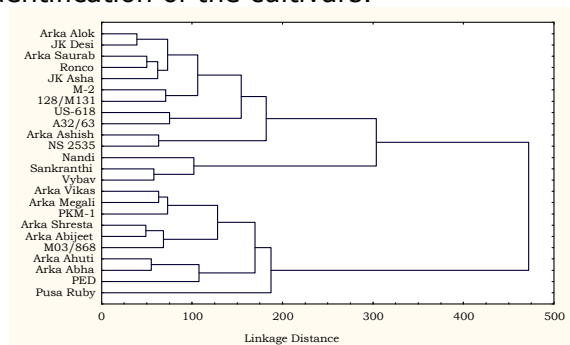
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### Results and Discussion

Dendrogram was constructed based on scoring data of qualitative morphological traits of 24 tomato cultivars (Fig 1 & Table 1), and cluster analysis divided cultivars in to two major clusters. The first cluster consisted of ten cultivars and remaining was grouped in to second major cluster. Morphologically most similar cultivars were Arka Alok and JK Desi with 84 per cent similarity between each other and least similar cultivars were Pusa Ruby and NS-2535 with only 10 per cent similarity. This showed that the some of the studied cultivars were closely related and some of selected cultivars might have related pedigree origin which was due to contentious breeding programme which necessitated us to relay up on other techniques like protein / isozyme / DNA markers for effective identification of the cultivars.

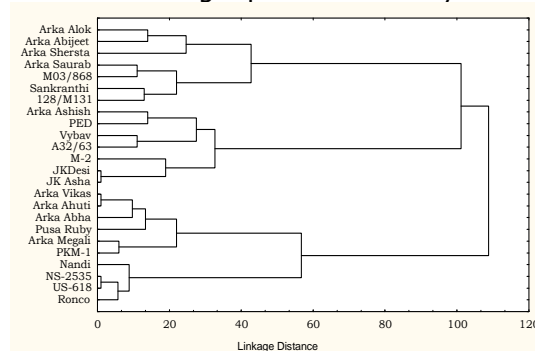


**Fig.1:** Dendrogram showing genetic relationship among 24 cultivars of tomato based on morphological markers according to Ward's method

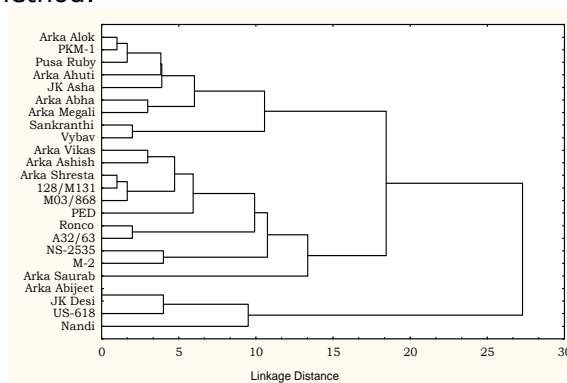
Whereas, based on protein analysis also most of studied cultivars were grouped in to two clusters. Among the cultivars Arka Vikas and Arka Ahuti, JK desi and JK Asha were closely related to each other (Fig.2 & Table.2). It was observed that, morphologically far cultivars NS-2535 and US-618 also showed 95 per cent similarity. M-03/868 and Arka Abha were most divergent cultivars with only 21 per cent similarity. Based on RAPD markers the cultivars were again grouped in to two major clusters (Fig 3 & Table 3) where only three varieties were grouped in to one cluster and all others under other cluster. Here cultivars Pusa Ruby and PKM-1 showed 84 per cent similarity while, least similarity was noticed in Arka Saurab and Mruthyunjaya-2.

Comparison revealed that clusters of morphological, protein and RAPD markers are not similar and showed difference in grouping

of varieties tested. However sub cluster of morphological and RAPD has similar cultivars. PKM-1 and Pusa Ruby which have similar morphological characters found to appear closely related in all marker systems. Similarly, Sankranthi and Arka Alok grouped into same sub cluster in both morphological and RAPD as they bears heart shaped fruits. However, there was no similarity for cluster analysis between different marker systems. Such non correlation between marker systems also noticed by Tam *et al.*, (2005). Similarly Genyi-Li and Quirus (2000) noticed more coefficient variation between the cultivars which are supposed to be sister lines selected from single parent in celery.



**Fig.2:** Dendrogram showing genetic relationship among 24 cultivars of tomato based on Protein markers according to Ward's method.



**Fig.3:** Dendrogram showing genetic relationship among 24 cultivars of tomato based on RAPD markers according to Ward's method

The difference in clusters between different markers might be due to difference in polymorphism. We do not believe difference in genetic relationship inferred by morphological /protein/RAPD can be attributed solely to difference in level of polymorphism detected by each marker system, but rather than that they reflect the complexity of the inheritance of different quantitative and qualitative characters.

Even though proteins are direct products of gene and should have similar pattern of clusters of RAPD there was no similar pattern found. RAPD marker has amplified limited segments of genome and that might have deviated the clusters.

In any case, we have to consider that the cause of dominant markers on heterozygous populations such as that of tomato cultivars included in this study. This limitation reduces the efficiency of detection of polymorphism. However, the use of relatively large number of markers, combined with pooling of plants per cultivars minimizes the potential problem. Cluster analysis obtained by using molecular markers is more appropriate than proteins and morphological markers. In conclusion, all tested tomato cultivars could be differentiated from each other based on either morphological / protein / RAPD markers, and are useful for grouping and to track their genetic relations with other varieties. However, relying on single marker system may not give truthful genetic relation between varieties.

### References

1. Genyi Li, Quiros, CF, Use of amplified fragment length polymorphism markers for celery cultivar identification, 2000, *Hort. Sci.*, 35: 726-728.
2. Laemeli UK, Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis, 1970, Nature (London), 227: 680.
3. Meng XD, Wei YY, MaH. Zhang, Li JR, Identification of wax gourd and chich-qua cultivars using RAPD markers, 1998, *Acta Agriculturae shanghai*, 12: 45-49.
4. Powell W, Morganate M, Andre C, Hanafev M, Vogel, Tingey C, Rafalski, A The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol Breeding*, 1996, 2: 225-238.
5. Tam SM, Mhiri C., Vogelaar A, Kerkveld M, Pearce SR, Marie-Angèle G, Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon-based SSAP, AFLP and SSR, 2005, *Theor Appl Genet*, 110, 819-831.
6. Terzopoulos PJ and Bebeli PJ. DNA and morphological diversity of selected Greek tomato (*Solanum lycopersicum* L.) landraces.2008, *Sci Hortic*, 116: 354-361.
7. UPOV, Guidelines for the conduct of tests for distinctness, homogeneity and stability. Tomato (*Lycopersicum esculentum* L.). TG/44/7, 1992, International Union for the Protection of New Varieties and Plants (UPOV), Geneva. 56p.
8. Ward JH, Hierarchical grouping to optimize an objective function, 1963, *J Am Stat Assoc*, 58: 236-244.
9. Vishwanath K, Prasanna, KPR., Pallavi HM, Rajendra Prasad S, Ramegowda, Devaraju PJ, Anantharayanan TV, Identification of Tomato (*Lycopersicon esculentum*) Varieties through Total Soluble Seed Proteins. 2011, *Res J Agril Sci*, 2(1): 08-12.

### Tables

**Table.1:** Pair wise similarity matrix of tomato genotypes based on morphological markers

Cvs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1	100																								
2	66	100																							
3	54	54	100																						
4	40	46	49	100																					
5	57	57	66	43	100																				
6	40	57	57	40	60	100																			
7	66	69	60	51	71	51	100																		
8	51	51	57	37	60	51	51	100																	
9	60	57	54	40	49	46	51	66	100																
10	51	63	46	37	43	40	54	37	37	100															
11	63	54	51	37	60	40	51	46	54	49	100														
12	49	57	49	46	60	54	60	49	57	51	66	100													
13	63	54	46	34	49	46	46	46	51	34	66	54	100												
14	57	49	49	40	49	51	51	40	40	34	46	46	63	100											
15	49	43	43	46	54	49	60	54	37	37	43	51	51	60	100										
16	43	43	71	60	51	57	54	49	49	31	40	51	49	46	60	100									
17	37	46	46	66	49	54	60	37	43	43	49	60	46	37	51	60	100								
18	57	46	49	54	51	37	54	46	57	51	49	57	43	43	46	46	51	100							
19	71	63	54	54	57	57	71	46	51	54	49	60	49	51	51	49	51	60	100						
20	51	46	49	49	46	51	51	49	51	31	54	54	57	40	43	57	54	51	60	100					
21	60	43	54	54	51	43	69	54	63	37	46	51	51	43	54	69	60	49	60	60	100				
22	57	49	46	54	43	43	49	49	46	46	43	46	57	51	46	54	51	60	54	49	54	100			
23	51	51	51	57	43	49	54	40	46	46	54	49	49	34	46	54	60	46	60	51	57	54	100		
24	54	54	54	43	57	40	63	66	63	43	60	60	49	37	46	60	60	46	43	54	71	46	54	100	

**Table.2:** Pair wise similarity matrix of tomato genotypes based on protein markers.

Cvs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1	100																								
2	79	100																							
3	74	95	100																						
4	58	53	58	100																					
5	58	79	74	47	100																				
6	58	58	63	47	74	100																			
7	68	47	53	58	42	68	100																		
8	63	47	53	47	37	58	84	100																	
9	68	58	58	47	58	68	74	68	100																
10	68	79	74	53	84	74	53	42	53	100															
11	58	63	58	68	53	42	53	37	47	68	100														
12	53	53	47	47	63	68	58	42	47	63	53	100													
13	47	42	42	42	37	42	42	32	37	47	37	58	100												
14	63	58	58	53	53	53	63	53	53	58	58	58	58	100											
15	42	47	47	37	47	37	21	21	21	53	42	42	68	53	100										
16	47	63	63	32	53	53	47	42	47	42	47	68	63	58	58	100									
17	37	47	53	53	37	32	42	37	26	32	42	32	47	53	53	63	100								
18	42	58	58	26	47	47	42	37	42	42	37	42	63	58	53	95	58	100							
19	42	47	47	53	42	32	42	47	37	37	42	37	47	58	37	58	68	53	100						
20	42	47	47	53	42	32	42	47	32	37	42	37	53	63	42	63	68	58	95	100					
21	53	58	58	37	53	63	58	53	58	47	42	42	58	68	47	79	58	74	58	63	100				
22	53	47	47	53	42	53	58	47	47	58	53	63	79	58	68	53	63	58	63	74	58	100			
23	47	58	58	37	53	53	68	63	63	53	53	47	53	63	37	79	47	74	58	63	74	63	100		
24	47	26	26	32	21	42	74	58	58	32	37	42	42	47	21	47	37	53	37	32	47	47	58	100	

**Table.3:** Pair wise similarity matrix of tomato genotypes based on RAPD Markers

Cvs	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1	100																								
2	79	100																							
3	72	73	100																						
4	82	73	60	100																					
5	77	72	62	75	100																				
6	77	75	72	75	73	100																			
7	70	68	78	62	60	67	100																		
8	75	70	60	77	82	75	58	100																	
9	82	67	63	83	75	78	65	80	100																
10	83	82	72	78	73	80	77	72	72	100															
11	68	73	73	67	75	72	68	80	73	68	100														
12	75	73	77	63	72	68	75	70	67	85	73	100													
13	77	68	72	72	70	83	67	75	72	83	65	82	100												
14	83	65	68	75	70	77	67	82	82	80	72	82	80	100											
15	78	70	73	70	68	78	72	77	70	85	67	83	88	85	100										
16	72	60	70	70	58	68	72	73	80	72	70	73	72	85	70	100									
17	67	58	52	72	60	70	50	68	68	60	58	52	67	67	58	75	100								
18	70	55	68	68	60	73	67	65	82	67	65	65	70	77	68	82	60	100							
19	80	65	62	82	73	80	60	78	82	77	68	72	80	83	78	75	73	73	100						
20	83	68	65	78	70	77	63	75	75	83	65	78	80	90	85	75	67	70	87	100					
21	87	68	68	82	70	77	73	78	85	80	72	75	73	90	78	78	63	77	83	83	100				
22	80	68	78	68	67	73	73	72	75	73	68	72	77	83	82	72	57	70	70	73	83	100			
23	75	73	60	80	72	85	55	80	80	75	67	67	82	75	77	67	72	65	85	75	75	72	100		
24	67	62	62	68	60	77	57	62	68	70	58	62	70	73	72	72	73	70	80	77	70	67	75	100	

- 1: A. Alok                      5: A. Abha                      9: A. Abijeet                      13: Nandi                      17: Mruthyunjaya-2                      21: Ronco
- 2: A. Vikas                      6: A Megali                      10: P. Ruby                      14: Sankranthi                      18: US -618                      22: A-32/63
- 3: A. Ahuti                      7: A. Saurab                      11: PED                      15: Vybhav                      19: JK Desi                      23: 128/M131
- 4: A. Ashish                      8: A. Shresta                      12: PKM-1                      16: NS-2535                      20: JK Asha                      24: M-03/868

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