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EVALUATION AND ESTIMATION OF GENETIC DIVERGENCE OF TOMATO HYBRIDS BY USING PRINCIPLE COMPONENT ANALYSIS AND CLUSTER ANALYSIS UNDER HIGH TEMPERATURE

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ABSTRACT

Increasing temperature is a major limiting factor for crop productivity. However, Tomato (*Solanum lycopersicum* L.) is highly sensitive to increasing temperature as a result major yield loses. Thus understanding the mechanism of high temperature become crucial for tomato improvement programme because it depends on the genetic variation which are present in the genome of tomato. Therefore, an experiment was conducted at the field of Department of Plant Breeding and Genetics using a randomized complete block design with two treatments and each treatment has three replications to determine the high temperature tolerant genotype on the base of phenological, physiological and morphological parameters. The genetic material proposed the considerable amount of diversity for all the studied parameters. Results shows that cumulative variation of first six principal components is 83.671 % and their eigen value greater than 1under normal treatment, while cumulative variation of first four principal components under high temperature stress is 86.690% having eigen value greater than 1. Under normal temperature PC1 contributed maximum variation 0.873% for Number of days to first fruit set, PC3 and PC4 contribute minimum variation -0.059 and -0.094% for fruit diameter and pericarp thickness respectively. While under high temperature PC1 contribute the maximum variation 0.968 and 0.969% for Flesh thickness and Shelf life respectively, and PC2 contribute minimum variation -0.057 and -0.075 for fruit length and fruit diameter respectively. According to the score plot under normal treatments genotypes Tom-15 and Cchaus were close to each other and quit away from all other genotype while under high temperature Anna quit away from other genotypes and show the maximum variation. Biplot graph show that individual fruit weight, fruit length and number of days to 50% flowering have the large variability and stem diameter and plant height have the lowest variability under normal treatments and under high temperature stress number of days to first flowering, number of flowers/cluster and number of days to 50% flowering have the maximum variability, while fruit length, fruit diameter, flesh thickness, pericarp thickness, shelf life and yield per plant had showed minimal variation. All the hybrids were grouped into 3 clusters. Maximum number of genotypes was quartered in cluster I and II under stressed and normal treatment respectively. Maximum distance to centroid in cluster I (55.669) and minimum distance to centroid in cluster III (00) under stressed treatment while under normal treatments maximum distance to centroid in Cluster II (302.087) and minimum distance to centroid in cluster I (68.957). Therefor it is suggested that cluster I has the maximum divergence or variation which is suitable for future breeding programme for the development of temperature tolerant genotypes.

***Keywords*:** Tomato, Temperature, Variation, Principle Component Analysis, Cluster Analysis

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1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the agricultural product and essential part of several people 's daily use. It belongs to *Solanaceae* family which includes approximately 98-102 genera with 2700-3000 species (Olmstead and Bohs, 2007). Tomato was globally cultivated on an acreage of 5.16 million hectares having production of 189.30 million tons (FAO, 2021). China was the leading producer followed by India and Turkey accounting for a share of 35.7%, 11.19% and 6.92 % in the global produce with overall yields of 67.53, 21.18 and 13 million tons, respectively, whereas Pakistan contribute 0.8% in a total production (FAO, 2021). In Pakistan tomato was cultivated on 0.15 million hectares which produced 0.80 million tons with an average yield of 5.34 tons per hectare which is pretty low with respect to other nations (FAO, 2021). Tomato is utilized as a fresh, cooked and after processing by canning, it is used for making the sauces, juice, paste and pulp.(Zhang et

al. 2016). Utilization of tomatoes exercise positive effects on human health and is recognized for anti-mutagenic, anti-inflammatory, anti-proliferative anti-genotoxic, and chemo preventive activities (Feng et al. 2010). Tomatoes are an ample source of vitamin A, C, and lycopene, and their increased utilization is found to reduce incidences of cardiovascular disease (Sesso et al. 2003). The lycopene of tomato also has anti-oxidative and anti-cancerous properties. Due to the nutritional values, tomato production and consumption have been increasing continuously (Raiola et al. 2014). Under the current global warming scenario, temperature is considered as an important factor threatening agriculture and related sectors with serious consequences on quality and food production (Gourdji et al. 2013). Amrutha and Beena (2020) conclude that in last few years' increasing food demand and global climate change are largest challenges of the world, as they badly affect plant growth and development.

Abiotic stress, mostly revelation to heat stress (HS), significantly decrease quality, yield and output (Aleem et al. 2021). Temperatures below or above the optimum cause stress for plant (Wahid et al. 2007). High temperatures disturb many characteristics of plant physiology, morphology, biochemical and molecular levels, as a results decrease plant yields (Hasanuzzaman et al. 2013). The optimum temperature of tomatoes is normally deliberated to be 25–30°C during day and 20°C at night (Liu et al. 2018). For economic characters successful breeding programme depend on the accessibility of germplasm that show a maximum diverse genetic origin and has key role in strengthening and sustaining the food and nutritional value of the nation. In hybridization programme of tomato assessment of genetic distance is one of suitable tools for parental selection. Understanding about patterns and levels of genetic diversity is very significant for assorted applications in plant breeding. Such study focuses on the degree of similarities and dissimilarity in genetic resources leading to established up organization of gene banks and isolation of best parental combinations (Rashid et al. 2008; San-San-Yi et al. 2008). Resulting hybridization for these parental combinations can possibly produce progenies with maximum genetic variability, in that way increasing chances of making superior genotypes with traits of interest (Crossa and Franco, 2004). In tomato, yield is the cumulative effect of many character contributing discretely to yield (Bernousi et al. 2011). Different characteristics viz., number of flowers cluster-1, days to first fruit ripening, fruit weight, fruit length, fruit width play vital role for maximum genetic divergence targeting to develop high yielding tomato varieties or hybrids. The most commonly used processes for this purpose, are principal component analysis, canonical variable analysis, and clustering methods (Sudré et al. 2007). Prior to cluster analysis principal component analysis is repeatedly used to estimates the relative significance of different variables of classification (Jackson, 1991). Principal component analysis helps breeders to differentiate significantly associated traits. The main advantage of using PCA over cluster analysis is that each genotype can be assigned to one group only. Hybridization programme involve genetically diverse parents belonging to different clusters that would provide an opportunity for bringing together gene constellations of diverse nature (Crossa and Franco, 2004). The genetic improvement of tomato mainly depends upon the amount of genetic variability present in the population. Hence the aim of present study was to estimate the genetic divergence and evaluate the 16 hybrid of tomatoes through clustering pattern and principle component analysis under normal and temperature stress.

2. MATERIALS AND METHODS

2.1. Experimental Location and Plant Materials

The 16 hybrid of tomatoes with different characteristics were provided by the store house of Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan. The experiment was conducted at the Vegetable Research Area of Institute of Horticultural Sciences, University of Agriculture, Faisalabad (latitude 31°25' North, longitude 73°4' East with an altitude of 184.4 m above sea level). During the year 2017-18 tomatoes hybrid were sown into a randomized complete block design (RCBD), replicated thrice under the split-plot arrangement with 2 treatments. All the studies hybrid which were used in the experiment given in Table 1.

2.2. Treatments and Traits Evaluation

From 2 treatments, one treatment sown under normal condition and other was sown under stressed condition. In the stressed condition late sowing was done to evaluate the material under high temperature. The experiment was carried out in two phases, as once the genotypes were transplanted under normal field conditions on 1st February 2018 whereas in the next phase the same material was evaluated during the summer period and was transplanted in field on 17th April 2018. Twelve plants per genotype were transplanted in each replication having 50 cm plant to plant distance, on the both sides of 4.5×44 feet raised beds with 4 feet distinguishing path between genotypes having bed to bed distance of 2 feet. All recommended agronomic and cultural practices for tomato cultivation were followed throughout the whole experiment. Data was recorded from eight plants out of the total 12 transplanted plant from each replication and average values were calculated for each genotype.

2.3. Data Collection

Parameters included in this study are number of days to first flower, number of days to 50% flowering, number of days to first fruit set, number of clusters per plant, number of flowers per cluster, number of fruits per cluster, number of locules per fruit and number of days to first harvesting are measured manually by simple counting. Moreover, plant height and fruit length was measured by meter rod into centimeter, stem diameter, fruit diameter, pericarp thickness and flesh thickness was measured by using Vernier caliper. Individual fruit weight and fruit yield

per plant was measured by weight balance into grams. Total soluble solids (TSS) was measured by refractometer, chlorophyll content was measured by SPAD-502 meter. Shelf life and Cell membrane thermo-stability (CMT) were measured by following procedures:

Table 1: Studies Hybrids

Sr. No	Hybrids	Sr. No	Hybrids
1	ANNA	9	RIO GRANDE
2	CCHAUS	10	ROMA
3	LAIYALPUR-I	11	AVR-I
4	MONEY MAKER	12	T-837
5	NAQEEB	13	TG-9
6	PAKIT	14	TG-25
7	PEGASO	15	T-5 (88572)
8	PONY EXPRESS	16	TOM-15

2.4. Shelf Life

The samples of tomato genotypes with three different temperatures which were 25°C, 35°C and 45°C stored in incubator and their water content of different samples were measured for every 3 days until 14 days, to find out storage time and temperature depend on the measurement strategies to calculate the shelf life of food material that was given by (Asiah et al. 2018). The biggest R2 score of selected order reaction were analyzed by the results of sample water content. The estimation of shelf life was measured from the reaction rate K score at certain temperature and it calculate by putting the score of 1/T (oK) of temperature into the Arrhenius equation:

$$t = \frac{Q - Qo}{k}$$

Explanation:
T: Time (Shelf Life)
Q: Parameter of final storage quality
Qo: Parameter of first storage quality
K: The reaction rate at certain temperature

This method to calculate the shelf life was first conceded out by making data plot on the association between the observation time (t day) and quality scores (Qt) for each temperature according to the reaction order 0 and 1. Additionally, depend on the Arrhenius equations, the reaction rate constant/degradation (kt) score can be compare and obtained with the association score. Then the most suitable reaction order can also be estimated. Subsequently, the estimation of the shelf life can be obtained by concluding the storage temperature to the Arrhenius equation (Desva et al. 2023).

2.5. Cell Membrane Thermo-stability (CMT)

Cell membrane thermo-stability (CMT) was determined from the both treatment samples by succeeding the procedure of Sullivan (1972). Using punch machine, after removing the uppermost leaves 0.75 cm in diameter rounded leaf discs were made. 10 leaf discs were taken in two sets of 50 ml glass tubes, and washed gradually three times with de-ionized refined water to eliminate surface adhered electrolytes from the sample. Then put the washed leaf disc into the glass tubes and filled with 10ml distilled water. From these two sets, one set of test tube was located in a water bath at 45°C for 1 hour and other remained normal at room temperature 25°C. After a one hour both the test tube were exposed to air conditioned room at 22°C temperature for an overnight. Then next day, after shaking it well of the test tubes with samples LF 538 EC meter were used to measured electrical conductivity of sample from both test tubes. Then at 15 Ibs pressure and 121°C temperature for 15 min both test tube with samples were autoclaved to assassinate the leaf tissues, which were endorsed 12hours to cool down at 22°C temperature. Consequently, second time electrical conductivity were recorded from both test tubes. Under stress, the amount of membrane integrity allowed to measure of membrane stability to electrolyte leakage.

2.6. Statistical Analysis

2.6.1. Principle Component and Cluster Analysis

Principal Component Analysis based on 20 quantitative traits was computed in to order find out the comparative importance of different parameters in capturing the genetic variation. The principal component analysis method explained by Harman (1976) was followed in the extraction of the components. The percentage of variance explained by each component were determined (Harman, 1976; Sharma, 1996; Tadesse and Bekele, 2001). Principal component analysis, loading plot, biplot graphical display and the factors correspond to 20 PCs were subjected to cluster analysis based on Euclidean distances and wards minimum variance using Agglomerative hierarchical clustering were performed using XLSTAT Version 2019.2.2 software for all the studies traits of tomatoes hybrid.

3. RESULTS

PCA (principal component analysis) is basically a multivariate statistical approach which helps in the extraction of results from a given data set in a quite valuable, meaningful and simplified form. In order to distinguish and find out variational pattern, principal component analysis was simultaneously performed for all the variables under consideration. PCA depicted genetic variation and diversity among genotypes under both temperature treatments. Principal component studies under normal temperature treatment revealed cumulative variation of 83.671 % by first six principal components having eigen value > than 1 according to the (Table 2), while on the other hand a cumulative variation of 86.690 was illustrated by first four principal components under heat stress conditions with an eigen value > unity according to the (Table 5).

4.1. PCA and Cluster Studied under Normal Temperature Circumstances

Principal component studies under normal temperature treatment revealed cumulative variation is 83.671 % by first six principal components having eigen value > than 1 which are presented in Table 2 and Fig. 1. Out of first six axis having eigen value more than 1, the first principal component (PC-I) nearly contributed 34.230 % in the total variation. The variability on PC-I was primarily due to positive loadings of number of days to first flower, number of days to 50% flowering, number of days to first fruit set, number of days to first harvesting and negative loadings of individual fruit weight, fruit length, fruit diameter, pericarp thickness, number of locules per fruit, chlorophyll content and yield per plant. PC-II accounted for about 18.103 % of the overall variation, which was largely due to positive contribution of number of clusters/plant, number of flowers/cluster and relative cell injury % and negative contribution of shelf life. Third principal component (PC-III) was responsible for approximately 10.241 % of the entire variability, which was mainly caused by the positive loading stem diameter and negative contribution of flesh thickness. PC-IV accounted for about 8.251 % of the overall variation and was primarily due to only positive contribution of plant height. The fifth axis contributed 6.544 % to the entire variation and which was largely because of only positive loading of total soluble solids %. At last the final, meaningful and sixth principal component contributed 6.303 % of the total variation and number of fruits/cluster was the only negatively contributing variable which were represented in Table 2.

4.2. Score Plot

Scatter plot for principal component analysis shows that the genotypes which are adjacent to each other were alike as if ranked on the basis of variables. Therefore, the genotypes Anna, T-5, TG-25 and Rio Grande while the genotypes TG-9, Roma, Laiyalpur-I, and T-837 were quite adjacent to the both principal axis PC-I and PC-II, respectively. The genotypes Pakit and Money Maker in the first quadrant (+, +), Anna in the second quadrant (–,+), Pony Express and Pegaso in the third quadrant (–,–), and AVR-I, TOM-15 and Cchaus in the fourth quadrant (+, –) are quite away from each other as well as from the other genotypes. Moreover, the genotypes Tom-15 and Cchaus were quite close to each other in the fourth quadrant according to the Fig. 3.

4.3. Biplot

Each genotype under consideration was plotted and variables were represented in biplot with their respective vectors, where the distance of each genotype from the center of origin shows the amount of variation for that particular genotype and little resemblance with other genotypes. The specific length of vector for each single variable shows the amount of variability as more the length of vector greater will be the variability and vice versa. Characters such as number of flowers/cluster, yield/plant, pericarp thickness, individual fruit weight, fruit length, number of days to 50% flowering and number of days to first fruit set have depicted large proportion of variability, whereas plant height, relative cell injury %, flesh thickness and stem diameter showed minimal variation according to the Fig. 4.

4.4. Clustering

All of the factors which were correspondent to 15 principal components were used for cluster analysis and the respective analysis was worked out by adopting the Agglomerative hierarchical clustering on the Euclidean distance matrix using Ward's linkage method and 3 distinct-clusters were found in the resulting dendrogram Fig. 5 and Table 4. It was found that the among all three clusters, second cluster (cluster-II) was the main and the biggest cluster having 9 tomato genotypes *viz*. Money Maker, Pakit, TG-9, Rio Grande, Roma, T-5 (88572), Laiyalpur-I, Naqeeb, Anna, which was been followed by the first cluster (Cluster-I) constituting5 genotypes of tomato such as: Cchaus, TOM-15, AVR-I, TG-25, T-837, whereas on the contrary third cluster (cluster-III) has the least and only two genotypes Pegaso and Pony Express according to the Table 3. It was quite evident from the results that the respective genotypes in the first cluster (Cluster-I), depicted highest mean values for various quantitative traits such as number of days to first flower, number of days to 50% flowering, number of days to first fruit set, number of days to first harvesting and stem diameter. Genotypes present in the second cluster (cluster-II) were categorized on the basis of high means for number of clusters per plant, number of flowers per cluster, total soluble solids, number of fruits per cluster, plant height and relative cell injury. Similarly, the third cluster (cluster-III) showed highest means for the traits individual fruit weight, fruit length, fruit diameter, pericarp thickness, flesh thickness and number of locules per fruit Table 3.

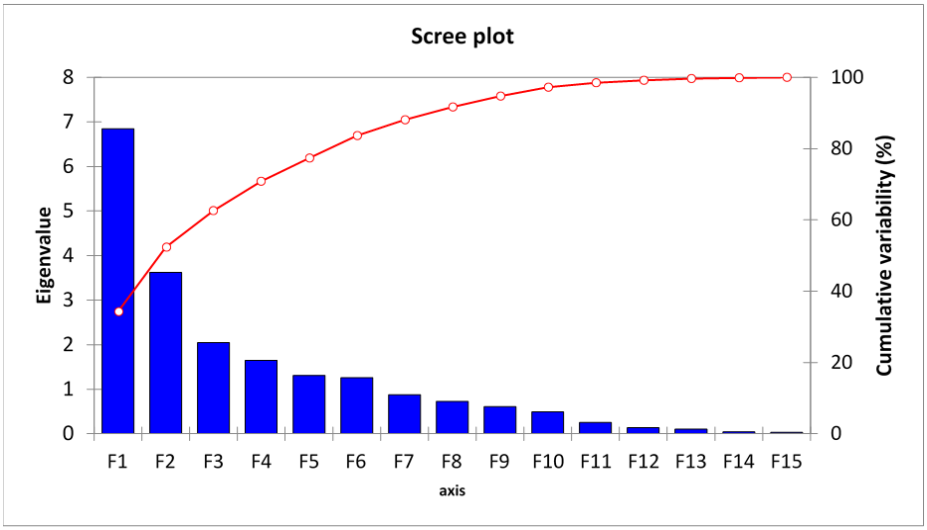

Fig. 1: Percentage of variability explained by main principal components under normal temperature treatment

Table 2: Eigen value, variability, cumulative variability and factor loadings of first six principal component axis to variation in tomato genotypes under normal temperature

Parameter	PC-I	PC-II	PC-III	PC-IV	PC-V	PC-VI
Eigen value	6.846	3.621	2.048	1.650	1.309	1.261
Variability (%)	34.230	18.103	10.241	8.251	6.544	6.303
Cumulative %	34.230	52.332	62.573	70.824	77.368	83.671
Number of days to first flower	0.831	-0.484	0.006	-0.034	-0.084	0.123
Number of days to 50% flowering	0.830	-0.480	0.055	-0.036	-0.019	0.114
Number of days to first fruit set	0.873	-0.288	-0.207	-0.127	-0.037	0.081
Number of clusters per plant	-0.184	0.618	-0.054	-0.564	0.205	0.112
Number of flowers per cluster	0.558	0.673	0.104	-0.026	-0.095	0.325
Number of days to first harvesting	0.666	-0.412	-0.526	0.073	0.151	-0.135
Individual fruit weight	-0.775	-0.400	-0.052	0.245	-0.268	0.223
Fruit length	-0.724	-0.552	0.299	-0.042	-0.187	0.120
Fruit diameter	-0.556	-0.221	-0.059	0.028	0.266	0.482
Pericarp thickness	-0.800	-0.302	0.286	-0.094	-0.157	0.211
Flesh thickness	-0.394	-0.206	-0.572	0.570	-0.174	-0.025
Number of locules per fruit	-0.624	-0.152	-0.398	-0.314	0.219	-0.312
Total soluble solids	-0.101	0.346	-0.016	0.288	0.696	0.401
Chlorophyll content	-0.640	0.145	-0.357	-0.188	-0.084	0.201
Number of fruits per cluster	-0.292	0.603	-0.358	0.038	-0.088	-0.448
Shelf life	-0.270	-0.606	-0.359	-0.076	0.525	-0.037
Stem diameter	0.209	-0.162	0.701	0.100	0.329	-0.311
Plant height	0.355	0.382	-0.034	0.759	0.052	0.062
Relative cell injury %	0.302	0.431	-0.352	-0.175	-0.241	0.338
Yield per plant	-0.679	0.409	0.219	0.265	0.040	-0.181

Table 3: Cluster means of 20 quantitative traits of *Solanum lycopersicum* genotypes under normal temperature

Characters	Cluster-I	Cluster-II	Cluster-III
Number of days to first flower	64.583	56.236	54.617
Number of days to 50% flowering	66.800	57.889	55.667
Number of days to first fruit set	74.917	67.014	63.834
Number of clusters per plant	23.499	33.185	28.870
Number of flowers per cluster	5.462	6.195	4.162
Number of days to first harvesting	105.077	98.080	95.535
Individual fruit weight	52.505	55.435	109.725
Fruit length	4.721	4.469	6.633
Fruit diameter	4.105	3.955	5.330
Pericarp thickness	4.671	4.766	6.360
Flesh thickness	25.573	27.007	30.264
Number of locules per fruit	2.593	2.786	3.392
Total soluble solids	6.663	6.943	6.373
Chlorophyll content	0.067	0.073	0.091
Number of fruits per cluster	2.020	2.841	2.367
Shelf life	8.337	6.943	8.383
Stem diameter	15.117	14.235	12.800
Plant height	85.242	91.902	80.600
Relative cell injury	7.385	9.460	4.968
Yield per plant	270.062	523.822	531.810

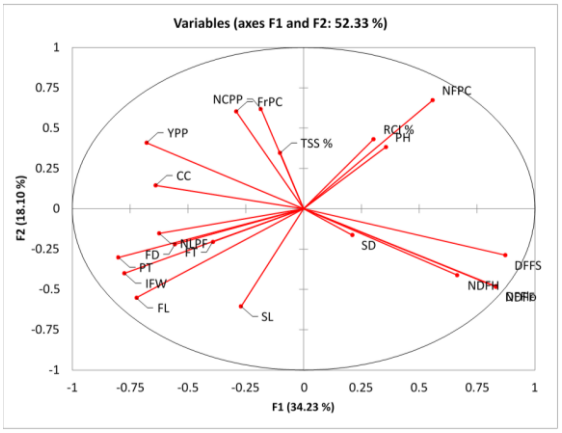

Fig. 2: Loading plot of 20 morphological characters under normal temperature treatment.

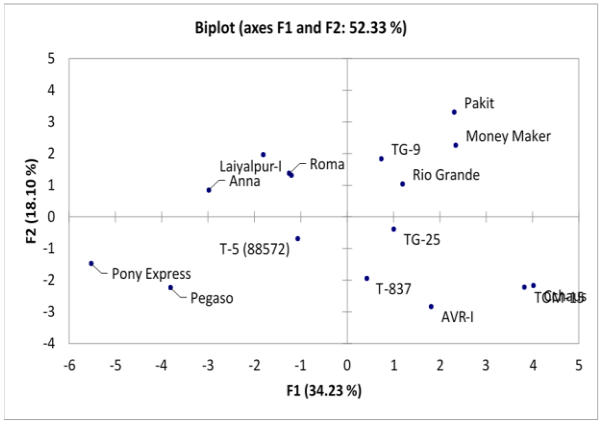

Fig. 3: Principal component bi-plot for 16 *Solanum lycopersicum* genotypes under normal temperature.

4.5. PCA and Cluster Studied under Sub-optimal Temperature Circumstances

Principal component studies revealed cumulative variation of 86.690 % by first four PCS (principal components) having eigen value > than 1 under sub optimal temperature regime as presented in Table 5 and Fig. 6. Among first four principal axes, having eigen value more than 1, the PC-I (first principal component) nearly contributed 62.936 % in the total variation. The variability on PC-I was primarily due to positive loadings of number of days to first fruit set, number of clusters per plant, number of days to first harvesting, individual fruit weight, fruit length, fruit diameter, pericarp thickness, flesh thickness, number of locules per fruit, total soluble solids, number of fruits per cluster, shelf life, plant height and yield per plants while negative loadings of number of days to first flower and number of days to 50% flowering. PC-II accounted for about 8.725 % of the overall variation, which was largely due to only positive contribution of chlorophyll content and relative cell injury %. Third principal component (PC-III) was responsible for approximately 7.895 % of the entire variability, which was mainly caused by the positive loading of stem diameter and negative contribution of number of flowers per cluster. PC-IV accounted for about 7.134 % of the overall variation according to the Table 6 and Fig. 7.

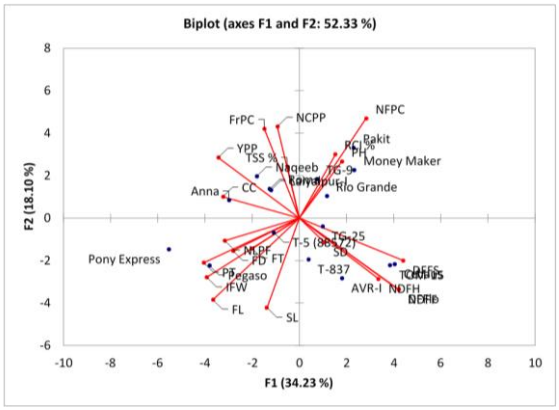

Fig. 4: Distribution of various traits and genotypes across two principal axes on biplot under normal temperature.

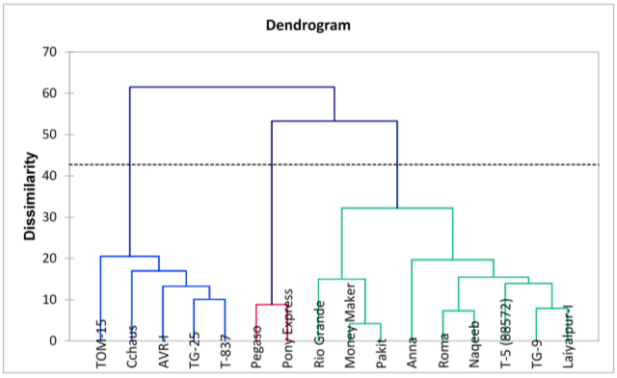

Fig. 5: Dendrogram showing clustering pattern of 16 *Solanum lycopercium* genotypes on the basis of morphological traits under normal temperature.

Table 4: Clustering pattern of 16 *Solanum lycopersicum* genotypes under normal temperature

Class	Cluster-I	Cluster-II	Cluster-III
Objects	5	9	2
Sum of weights	5	9	2
Within-class variance	2804.168	28377.214	115703.014
Minimum distance to centroid	24.568	18.023	240.523
Average distance to centroid	43.649	134.020	240.523
Maximum distance to centroid	68.957	302.087	240.523
	Cchaus	Money Maker	Pegaso
	TOM-15	Pakit	Pony Express
	AVR-I	TG-9	
	TG-25	Rio Grande	
	T-837	Roma	
		T-5 (88572)	
		Laiyalpur-I	
		Naqeeb	
		Anna	

Table 5: Eigen value, variability, cumulative variability and factor loadings of first six principal component axis to variation in tomato genotypes under sub-optimal temperature

Parameter	PC-I	PC-II	PC-III	PC-IV
Eigen value	12.587	1.745	1.579	1.427
Variability (%)	62.936	8.725	7.895	7.134
Cumulative %	62.936	71.661	79.556	86.690
Number of days to first flower	-0.717	-0.340	-0.008	0.116
Number of days to 50% flowering	-0.824	-0.296	0.104	0.134
Number of days to first fruit set	0.662	-0.106	-0.401	-0.406
Number of clusters per plant	0.618	0.361	0.051	-0.556
Number of flowers per cluster	-0.027	0.338	-0.681	0.484
Number of days to first harvesting	0.861	-0.118	-0.259	-0.127
Individual fruit weight	0.965	-0.075	0.010	0.127
Fruit length	0.961	-0.057	0.094	0.112
Fruit diameter	0.976	-0.116	-0.103	0.096
Pericarp thickness	0.962	-0.107	0.113	0.156
Flesh thickness	0.968	-0.115	-0.044	0.058
Number of locules per fruit	0.980	-0.050	-0.020	0.025
Total soluble solids	0.868	-0.019	-0.205	0.085
Chlorophyll content	0.197	0.599	0.239	0.583
Number of fruits per cluster	0.924	-0.201	-0.007	0.148
Shelf life	0.969	-0.121	0.068	-0.022
Stem diameter	0.383	0.250	0.734	-0.234
Plant height	0.722	0.525	0.207	0.178
Relative cell injury %	-0.231	0.668	-0.391	-0.382
Yield per plant	0.946	-0.154	0.024	-0.061

Table 5: Cluster means of 20 quantitative traits of *Solanum lycopersicum* genotypes under sub-optimal temperature

Characters	Cluster-I	Cluster-II	Cluster-III
Number of days to first flower	39.160	36.758	32.400
Number of days to 50% flowering	41.889	38.722	34.667
Number of days to first fruit set	14.556	62.722	59.667
Number of clusters per plant	11.772	15.879	16.000
Number of flowers per cluster	4.535	4.702	4.093
Number of days to first harvesting	0	76.167	71.330
Individual fruit weight	0	7.027	20.253
Fruit length	0	1.095	3.277
Fruit diameter	0	1.088	2.320
Pericarp thickness	0	0.552	1.800
Flesh thickness	0	3.528	7.017
Number of locules per fruit	0	0.389	0.833
Total soluble solids	0.066	0.740	1.727
Chlorophyll content	0.034	0.034	0.042
Number of fruits per cluster	0	0.173	0.467
Shelf life	0	0.818	1.887
Stem diameter	10.438	10.882	13.393
Plant height	34.698	39.687	63.670
Relative cell injury	55.222	54.245	29.243
Yield per plant	0	41.306	83.103

Table 6: Clustering pattern of 16 *Solanum lycopersicum* genotypes under sub-optimal temperature

Class	Cluster-I	Cluster-II	Cluster-III
Objects	9	6	1
Sum of weights	9	6	1
Within-class variance	1235.896	911.417	0
Minimum distance to centroid	16.619	14.107	0
Average distance to centroid	30.238	25.999	0
Maximum distance to centroid	55.669	39.044	0
	Cchaus	T-837	Anna
	TOM-15	Pakit	
	AVR-I	Roma	
	TG-25	T-5 (88572)	
	Money Maker	Laiyalpur-I	
	TG-9	Naqeeb	
	Rio Grande		
	Pegaso		
	Pony Express		

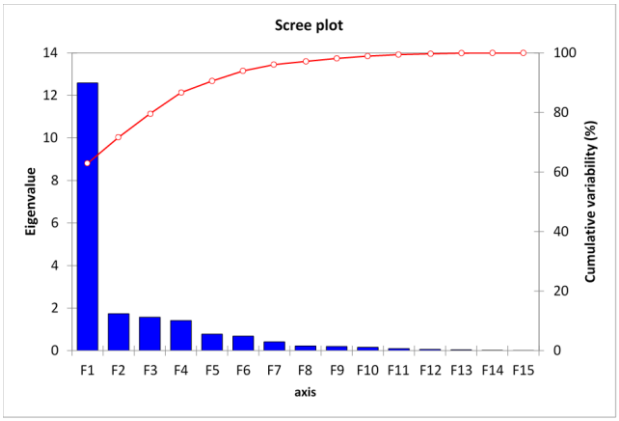

Fig. 6: Percentage of variability explained by main principal components under sub-optimal temperature.

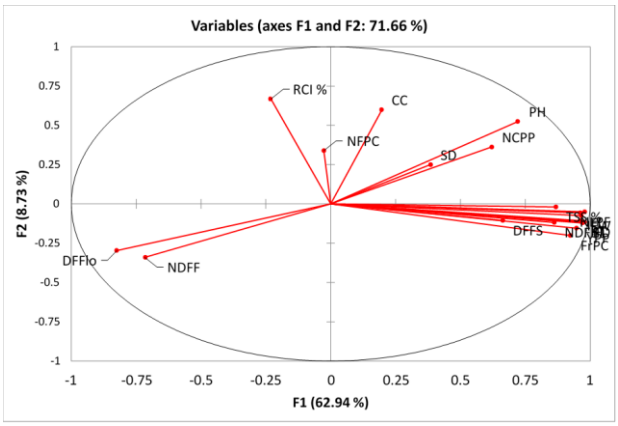

Fig. 7: Loading plot of 20 morphological characters under sub-optimal temperature,

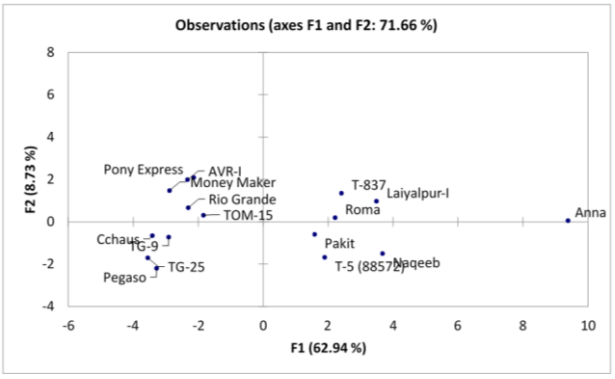

Fig. 8: Principal component bi-plot for 16 *Solanum lycopersicum* genotypes under sub-optimal temperature

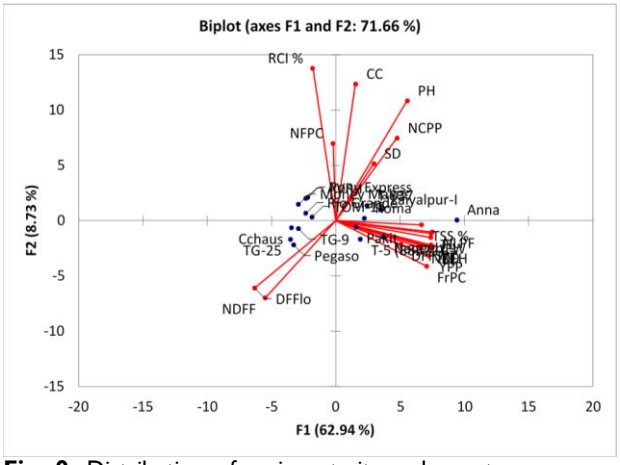

Fig. 9: Distribution of various traits and genotypes across two principal axes on biplot under sub-optimal temperature

4.6. Score Plot

Scatter plot for principal component analysis shows that the genotypes which are adjacent to each other and the respective principal axis were alike as if ranked on the basis of variables. Therefore, the genotypes Anna, Roma, Tom-15, Cchaus and Pakit were quite adjacent while the genotypes T-837, AVR-I and T-5 were nearly close to both principal axis PC-I and PC-II, respectively. The genotypes Anna in the first quadrant (+, +), Pony Express in the second quadrant (–,+), Pegaso in the third quadrant (–,–), and Naqeeb in the fourth quadrant (+, –) are quite away from each other as well as from the other genotypes. Moreover, in the first quadrant the genotype Anna was quite away from all other genotypes Fig. 8.

4.7. Biplot

Each genotype under consideration was plotted and variables were represented in biplot with their respective vectors, where the distance of each genotype from the center of origin shows the amount of variation for that particular genotype and little resemblance with other genotypes. The specific length of vector for each single variable shows the amount of variability as more the length of vector greater will be the variability and vice versa. Traits such as number of clusters/plant, plant height, chlorophyll content, relative cell injury %, number of days to first flowering, number of flowers/cluster, number of days to 50% flowering and number of fruits/cluster have depicted large proportion of variability, whereas stem diameter, number of days to first fruit set, number of locules per fruit, total soluble solids, number of days to first harvesting, individual fruit length, fruit diameter, flesh thickness, pericarp thickness, shelf life and yield per plant had showed minimal variation Fig. 9.

4.8. Clustering

All of the factors which were correspondent to 15 principal components were used for cluster analysis and the respective analysis was worked out by adopting the Agglomerative- clustering on the Euclidean distance matrix using Ward's linkage method, and 3 distinct-clusters were found in the resulting dendrogram Fig. 10 & Table 6. It was found that the among all three clusters, first cluster (Cluster-I) was the main and the biggest cluster having 9 tomato genotypes namely Cchaus, TOM-15, AVR-I, TG-25, Money Maker, TG-9, Rio Grande, Pegaso, Pony Express which was been succeeded by the second cluster (cluster-II) containing 6 genotypes of tomato *viz*. T-837,

Pakit, Roma, T-5 (88572), Laiyalpur-I, Naqeeb whereas on the contrary third cluster (cluster-III) has the minimal and only one genotype Anna Table 6.

It was quite evident from the results that the respective genotypes in the first cluster (Cluster-I), depicted highest mean values for various quantitative traits such as number of days to first flower, number of days to 50% and relative cell injury %. Genotypes present in the second cluster (cluster-II) were categorized on the basis of high means for number of days to first fruit set, number of flowers/cluster and number of days to first harvesting. Similarly, the third cluster (cluster-III) showed highest means for the traits number of cluster/plant, individual fruit weight, fruit length, fruit diameter, pericarp thickness, flesh thickness, number of locules/fruit, total soluble solids, chlorophyll content, number of fruits per cluster, shelf life, stem diameter, plant height and yield per plant according to Table 5.

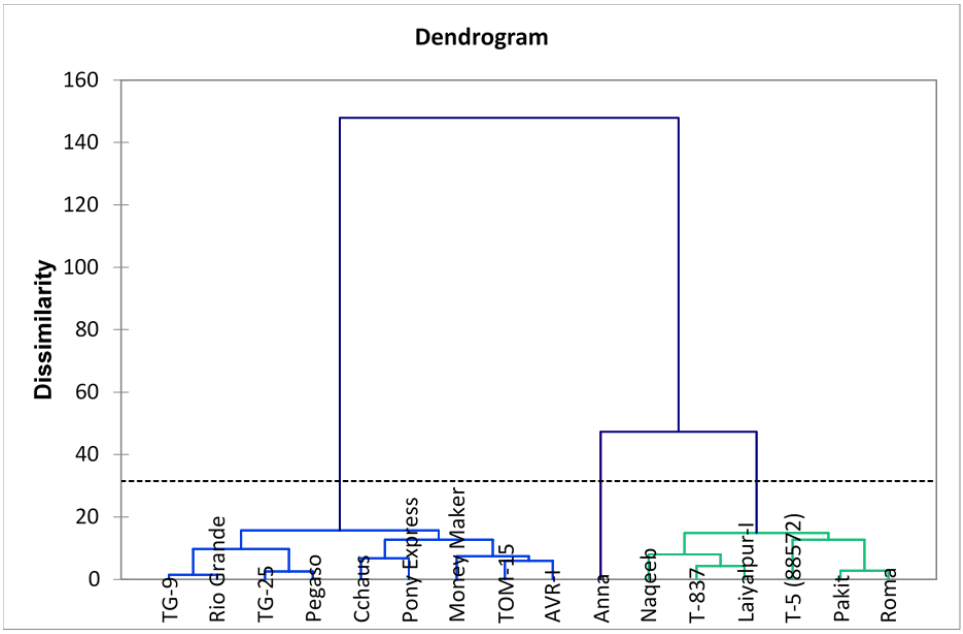

Fig. 10: Dendrogram showing clustering pattern of 16 *Solanum lycopercium* genotypes on the basis of morphological traits under normal temperature

5. DISCUSSION

Variation among various quantitative characters was assessed in this investigation under both temperature regimes. Principal component studies were carried out and a total of 15 PCs were found. Under optimal and sub-optimal temperature treatments, first two principal components accounted for 52.33% and 71.66%, respectively. Emami and Eivazi (2013) illustrated that 97% of the overall present variability by PC-I and PCII, among 25 genotypes of tomato. Similarly, Iqbal et al. (2014) also reported that 81.72% of overall variation for various characters by the first three principal component axis between forty-seven *Solanum lycopersicum* varieties. Moreover, Henareh et al. (2015) reported that 71.6% cumulative variability by the PC-I, PC-II and PC-III of the 97 studied tomato lines. Kumar et al. (2018) also demonstrated that 77.61 % variability by first two principal axes. Chernet et al. (2014) assessed the performance of 36 *Solanum lycopersicum* lines by using PCA and obtained 6 PCs which explained 83.03% of the overall variation present. Similar type of findings was also reported by Mitul et al. (2016) who found 79.16% variability from the first three PCs. These findings evidently showed that principal component analysis in correspondence to classification of genetic-resources also displayed specific characters for desired able selection in tomato breeding programs. Similar outcomes were obtained in previous studies carried out by Krasteva and Dimova (2007).

In order to additionally support our results, Merk et al. (2012) stated the findings of PC-I and PC-II which were 28% and 16.2%, respectively of the overall present variability and was primarily due the yield attributing traits such as fruit length, fruit weight, fruit width and shape. Principal component analysis is one of the BLUPs (Best Linear Unbiased Predictors) which helps in the assessment of variation among the studied material that have a desirable character under. Under both temperature regimes with principal axis flowering and fruit related traits contributing in the variability. Thus, PC-analysis assists the plant breeders for the improvement of genetic makeup of crop plants especially yield related characters having quite lower heritability or during the initial generations through procedure of indirect selection for yield improving traits (Leilah and Al-Khateeb 2005, Golparvar et al. 2006).

Moreover, selection of breeding material having maximum fruit yield/plant and yield related components were highly recommended for the improvement of genetic makeup of tomato crop. Similar results were also demonstrated by (Ahmadizadeh and Felenji 2011; Bernousi et al. 2011; Krasteva et al. 2014). Biplot has been applied in numerous plant breeding programme by various plant breeder namely Sethuraman et al. (2007) employed in studied on sweet potato, Ahmadizadeh and Felenji (2011) used in potato to screen out and choose the stable, high yielding and best performing genotype out of the examined clade of genotypes.

Cluster studies of the genotypes in 3 different cluster having variation among clusters on the basis of different traits under consideration at both temperatures. Reddy et al. (2013) also proposed that maximum inter-cluster variation between 19 tomato lines during examining the genetic variability. Sharma et al. (2006) found highest cluster-mean for yield/plant during studying the performance of sixty tomato elite lines. Our findings are also in line with the findings of (Singh et al. 2008; Kumar et al. 2018). It is quite clear from the present investigation that cluster analysis could be used an effective statistical in order to classify various genotypes and extracts reliable results for the selection of breeding material to carry out future tomato hybridization programs as previously reported by (Bernousi et al. 2011; Iqbal et al. 2014). Mitul et al. (2016) found five clusters of tomato genotypes on the basis of various morphological, biochemical and flowering traits such as low yield and late ripening, early flowering types, bug fruited with high yielding capability, lower ascorbic acid content and little fruited with early maturing capability in C-I, C-II, C-III, C-IV and C-V, respectively.

Conclusion

All 16 genotypes of tomato have evaluated using randomized complete block design for the estimation of genetic divergence. Genetic diversity is an effective way to estimates the genetic variation among the hybrids. Diversity not only induces variations but also provides new combinations of genes. Thus, knowledge on the degree and nature of genetic divergence commonly helps in the selection of suitable genotypes for the efficacy of future breeding program. According to PCA first six principle component show the maximum variability under normal temperature and first 4 principle component show the maximum variation under high temperature stress. Anna, Tom-15 and Cchaus genotypes show the maximum variation. The genotypes with maximum variation help the breeder for the effective selection of desirable traits. Considerable diversity between and within the clusters was conclude among the genotypes. It was depicted that Cluster I is the biggest cluster with maximum genetic divergence under sub-optimal temperature having 9 tomato genotypes namely Cchaus, TOM-15, AVR-I, TG-25, Money Maker, TG-9, Rio Grande, Pegaso and Pony Express. So these genotypes are used in future tomato breeding programme for the development of high temperature tolerant genotypes.

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