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SCREENING AND SELECTION OF TOMATO GENOTYPES/CULTIVARS FOR DROUGHT TOLERANCE USING MULTIVARIATE ANALYSIS

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Abstract

Drought is one of the most important abiotic stresses reducing crop growth and yield of tomato. Development of water stress tolerant cultivars through screening and selection is one important strategy to overcome this problem. In the present study, seeds of 120 local and exotic lines of tomato were allowed to germinate at varying levels of polyethylene glycol (PEG_{8000}) induced water stress (PEG_{8000} 0, 2.5%, 5.0% and 7.5%) for two weeks. Increasing PEG concentrations in the growth medium (water stress) caused a consistent decrease in seed germination percentage and seedling growth of all tomato cultivars. Moreover, a significant amount of genetic variability was found in all attributes of 120 genotypes of tomato. All lines/cultivars of tomato were ranked on the basis of relative water stress tolerance using 13 morphometric traits and categorized in four groups (tolerant, moderately tolerant, moderately sensitive, and sensitive) through multivariate analysis. Of 120 lines, 18, 25, 29 and 48 lines were ranked as tolerant, moderately tolerant, moderately sensitive and sensitive respectively. The germination percentage or speeds of germination were not found as effective indicator of genotypic differences for water stress at the seedling stage. Moreover, degree of water stress tolerance at the germination and seedling growth stage did not maintain in all tomato lines. Thus, it is not certain whether such variation is detectable at the later vegetative or reproductive growth stages. This needs to be further investigated. Overall, lines 19905, 19906, LA0716, and LA0722 were found to be water stress tolerant at least at early growth stages.

Introduction

At present, drought is a major threat to sustainable food production, which reduces the crop yields up to 70 percent. Hence, of all the abiotic stresses, drought (complex nature) is considered as the supreme destructive (Gosal *et al*., 2009). In order to meet the rising demand of food especially vegetables for the growing population, rainfed production of vegetables is the need of hour. Yield is frequently limited by scarcity of water besides, universal environmental variations accompanying growing struggle for water resources, forms the genomic advancement of crop for water use efficiency, a progressively more significant objective (Parry *et al*., 2002). Previously, it has been described that genetic improvements of tomato not only influenced by the background of germplasm but also depends on the stable improvement of more meticulously adapted genotypes appropriate to confined environments (Agong, 2001). The scope of plant genetic improvement through the manipulation of available genetic variability under stress and non-stress condition is still equally believed by all plant scientists. For enhancement of drought tolerance potential in a crop either through selection or breeding basic important criteria is presence of genetic variability for stress resilience (Dias, 2010 & 2014). The knowledge of genetic variability is useful tool in order to maintain (gene bank management), evaluate and utilize germplasm effectively under control and water stress condition (Cuartero & Flower, 1992). The material from diverse geographical origin of the crop species can help to ensure conservation of co-adapted gene complexes, because genetically heterogeneous populations produce more and stable yield than genetically homogenous lines (Simmonds, 1979; Samovol, 1996). The application of genetic variation can also be manipulated either selecting superior genotypes or to be utilized as parents for the development of future cultivars through hybridization (Frankel *et al*., 1995; Goncalves *et al*., 2009). Systematic characterization and evaluation of plant genetic resources are prerequisites for the efficient use of the material through conventional methods or modern techniques. Growth and development of crops plants depend on judicious management of intrinsic (genetic, hereditary) and extrinsic (environment) factors. Tomato germplasm has been evaluated in different countries into different groups based on their latitudes of adaptations but the different planting time in the tropic causes complication. The behavior of tomato genotypes within groups varies from season to season and even in a single season, so the identification of the group can be lost. No systematic attempt has so far been made, to screen/evaluate the tomato germplasm under water deficit conditions and generate database in Pakistan. Characterization and evaluation of tomato genotypes in gene banks is necessary to determine their genetic diversity. Classification methods are being used to quantify the genetic divergence in many crops thus quantification of degree of divergence under drought would be of help in choosing suitable genotypes for tomato breeding programme (Dasgan *et al*., 2002; Kulkarni and Deshpande, 2007).

Multivariate analyses of verbatim/precise data procedures are helpful to describe phenotypic variations among the genotypes. It is used as a management tool for discovering underlying data grouping and relationships. Results reported by various researchers showed multivariate analysis as a valid system to deal with germplasm collection. Cluster Analysis (measures similarities and dissimilarities in order to determine the cluster numbers that explained in data) was used in this study because it allows the data to group itself. Though cluster analysis grouped together genotypes with greater morphological similarity, the clusters did not necessarily include all the genotypes from the same origin or nearby sites. Gunathilake *et al*. (2004) while working on tomato also reported the association between morphololological characters and geographic origin was absent. The genotypes have been grouped in a particular cluster on the basis of morphological trait similarities, thus representative genotypes from a cluster of particular group could be chosen for hybridization programme. Ghafoor *et al*. (2009) and Nikolic *et al*. (2010) reported that grouping pattern of the genotypes suggested no parallelism between genetic divergence and geographical distribution of the genotypes. Some potentially important traits have been identified and these can be exploited for specific trait improvement and assemblage of core collection from a bulk genetic stock.

With this aim in mind the present study was undertaken to run a classificatory analysis on the tomato genotypes by means of multivariate analysis which facilitated us to classify the available germplasm into distinct clusters on the basis of their genetic potential. The information, thus obtained, could be further utilized to develop an effective tomato-breeding programme. Therefore, 120 tomato genotypes were screened at germination and seedling stage on hypothesis that the germplasm performs different due to genetic variability under control and water deficit conditions. Also, to decipher the extent of genetic variability and drought tolerance potential under water deficit conditions in tomato germplasm and to identify the promising drought tolerant lines among tomato germplasm for future research.

Materials and Methods

Seeds of 108 local/exotic genotypes of tomato were obtained from Plant Genetic Resource Institute, National Agriculture Research Center (NARC), Islamabad, while 12 exotic genotypes of tomato obtained from TGRC (Tomato Genetics Resource Center) California, USA. These experiments were conducted in the Stress Physiology Laboratory of the Department of Botany, Pir Mehr Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan.

Germination assays: Germination trials were conducted in Petri dishes double lined with filter paper. Growth media contained four osmotic levels (0, 2.5%, 5.0% and 7.5% of PEG in full strength Hoagland nutrient solution (Hoagland and Arnon, 1950) in Petri dishes, to ensure adequate moisture for the seeds. Seed samples of 120 tomato genotypes were initially surface sterilized in 3% solution of sodium hypochlorite for 10 minutes and were rinsed three times with sterile water to eliminate residual chlorine, using muslin cloth. Fifteen surface sterilized seeds of tomato genotypes were spread in Petri dishes which were arranged in a completely randomized design with three replicates, four treatments and 120 genotypes, in growth cabinets. The seeds were examined daily and five ml of appropriate treatment solution was applied on alternate days for 14 days to each Petri dish after dripping out the previous solution. Seeds were not fully flooded in the solution to avoid anoxic conditions. Numbers of seeds germinated were observed and counted and germination data was recorded daily until the completion of two weeks (Anonymous, 1996). A seed was considered germinated when both plumule and radicle has emerged $\geq$ 5 mm (Chartzoulakis and Klapaki, 2000). Rate of germination (1/t50, where t50 is the time to 50% of germination) was computed from untransformed data. Total germination was expressed as percent of that in control treatment for each tomato genotype and then data were arcsine transformed for the statistical analysis.

Seedlings evaluation: Pre-germinated seeds of 120 tomato genotypes were planted in plastic containers of 200x100cm size with 25cm depth. Ten seedlings of same size of each genotype were transplanted hydroponically. Growth media contained four osmotic levels (0, 2.5%, 5.0% and 7.5%) of PEG_{8000} in full strength Hoagland nutrient solution (Hoagland and Arnon, 1950). Containers were arranged in a completely randomized design with three replicates. After about two weeks morphological parameters like shoot and root length, fresh and dry biomass and relative water content of each genotype were recorded. Plant material was dried at 70 ^{o}C and dry weights measured. Leaf relative water content was calculated using the equation below:

$$\text{Relative water content (\%)} = \frac{\text{Leaf fresh weight} - \text{Leaf dry weight}}{\text{Leaf turgid weight} - \text{Leaf dry weight}} \text{ X } 100$$

Ranking of tomato genotypes for drought tolerance: The joint analysis of variables of different types (continuous and nominal/binary type) can provide intensify and inclusive information about a set of genotypes thus became an interesting substitute for both breeders and gene bank curators for a better quantification of genetic variability in tomato (Sudre *et al*., 2007 and Gonclaves *et al*., 2009). For comparing genotypes for drought tolerance; all the data were transformed following Zeng *et al*. (2002) into drought tolerance indices i.e., means of each parameter of drought stressed plants divided by the means of their respective controls. The cultivars were ranked in different groups by frequency distribution. Usually, number of groups and class intervals set based on range of observations and general trend class intervals were determined as the difference between high and low drought tolerance indices. Furthermore, cluster group ranking numbers were also assigned to cluster groups based on cluster means and used to score genotypes. The cluster analysis was based on Wards minimum variance cluster analysis of the averages of the drought tolerance indices for all parameters (Ward, 1963). Tomato genotypes were ranked on the basis of Euclidean dissimilarity coefficient matrix based on phenograms, constructed on thirteen traits of genotypes under 2.5%, 5.0% and 7.5% of PEG_{8000}. All the phenograms were constructed in order to support the grouping of the 120 tomato genotypes under drought stress condition. A sum was obtained by adding the number of cluster group ranking at each level in each genotype. The genotypes were finally ranked on the basis of sum, such that those with smallest and largest sums were ranked as the tolerant and sensitive genotypes, respectively in terms of relative drought tolerance. All the traits were analyzed by cluster analysis and principal component analysis with the help of software program ‘Statistca’ v 6.0 and ‘SPSS’ v 12.0 for windows.

Results

Cluster analysis based on 13 morphometric plant growth parameters: The results of our study revealed considerable phenotypical (and presumably genetic) diversity among tomato genotypes. The descriptive statistics for plant growth parameters in 120 tomato genotypes under control and varying levels (2.5%, 5.0% and 7.5%) of PEG_{8000} induced water deficit conditions is presented in Table 1. Phenograms constructed was based on thirteen biological traits of 120 tomato genotypes imposed by the use of PEG_{8000}; maintaining three levels of osmotic stress (2.5%, 5.0% and 7.5 %) are presented in Figs. 1, 2 and 3 respectively. Their sum which was calculated by adding the number of cluster group, were used to rank at each level in every genotype is represented in Table 2. Comparison of genotypes indicated that all the characters studied upto seedling stage was considerably affected by water stress. It can be seen that the less water conditions depressed all the characters of all the genotypes. It was also observed from the data under drought conditions, the genotypes having substantial variation for growth attributes, were statistically different from each other for dry matter production. According to final ranking of genotypes based on clusters numbers four groups was made (Table 3), their means and standard deviation are presented in Table (4), principle component matrix is given in Table 5, whereas correlation matrix for growth parameters of the tomato genotypes under PEG_{8000} induced water stress is given in Table 6.

Tolerant group (group 1): In group 1, eighteen genotypes were placed which were 15.00 % of the total genotypes. It has been observed that except for two characters root dry weight (66.16) and shoot-to-root ratio; group 1 performed best for all parameters like germination percentage (69.3), germination rate (7.73), shoot length (0.92), root length (42.74), shoot fresh weight (34.59), shoot dry weight (12.13) root fresh weight (1.03) and relative water content (55.81) hence the group members designated as tolerant group, thus this group could be used for crop improvement under water deficit conditions.

Moderately tolerant group (group 2): Group 2 accounts for 20.83 % of the total population and includes 25 genotypes. This group was very close to the tolerant group and has germination percentage (62.5), germination rate (7.45), shoot length (0.87), root length (31.13), shoot fresh weight (27.26), shoot dry weight (6.37) root fresh weight (0.87), root dry weight (74.68) and relative water content (50.48) hence the group members designated as moderately tolerant/intermediate group under drought stress conditions.

Moderately sensitive group (group 3): Group 3 represents 24.17 % of the population and comprised of twenty nine genotypes. The genotypes from this group were with second highest shoot length (0.90) but their germination rate (5.48), root length (13.68), shoot fresh weight (16.40) shoot dry weight (3.43) root dry weight (76.38) were minimum with relative water content (35.56) thus, this group was intermediate/moderately sensitive in its performance.

Sensitive group (group 4): Group 4 contributed 40.00 % to the population and comprising of forty eight genotypes. This group shows no significant performance for any trait and has less germination percentage (42.9), germination rate (5.51), shoot length (0.83), root length (16.86), shoot fresh weight (17.60), shoot dry weight (3.44) root fresh weight (0.86), root dry weight (80.77) and relative water content (32.13).

Table 1. Descriptive statistics for plant growth parameters in 120 tomato genotypes under control and varying levels (2.5%, 5.0% and 7.5%) of PEG_{8000} induced water deficit conditions.

Plant growth parameters	Minimum value	Maximum value	Mean ± Standard error
Germination percentage	61.1	98.3	79.7 ± 0.8
Germination rate (% day^{-1})	2.1	5.5	3.3 ± 0.1
Shoot fresh weight (mg)	15.6	155.8	58.0 ± 3.6
Root fresh weight (mg)	9.7	106.8	38.3 ± 2.1
Shoot dry weight (mg)	3.6	57.6	16.4 ± 1.1
Root dry weight (mg)	3.3	53.0	14.3 ± 1.0
Shoot/Root ratio	0.7	1.7	1.0 ± 0.01
Shoot Length (cm)	3.9	10.0	6.5 ± 0.1
Root Length (cm)	4.1	10.9	7.6 ± 0.2
Shoot/Root length ratio	0.7	1.3	0.9 ± 0.01
Shoot moisture content	43.7	90.1	72.4 ± 0.7
Root moisture content	43.4	89.6	69.6 ± 0.6
Relative water content (%)	34.9	82.3	52.0 ± 1.0

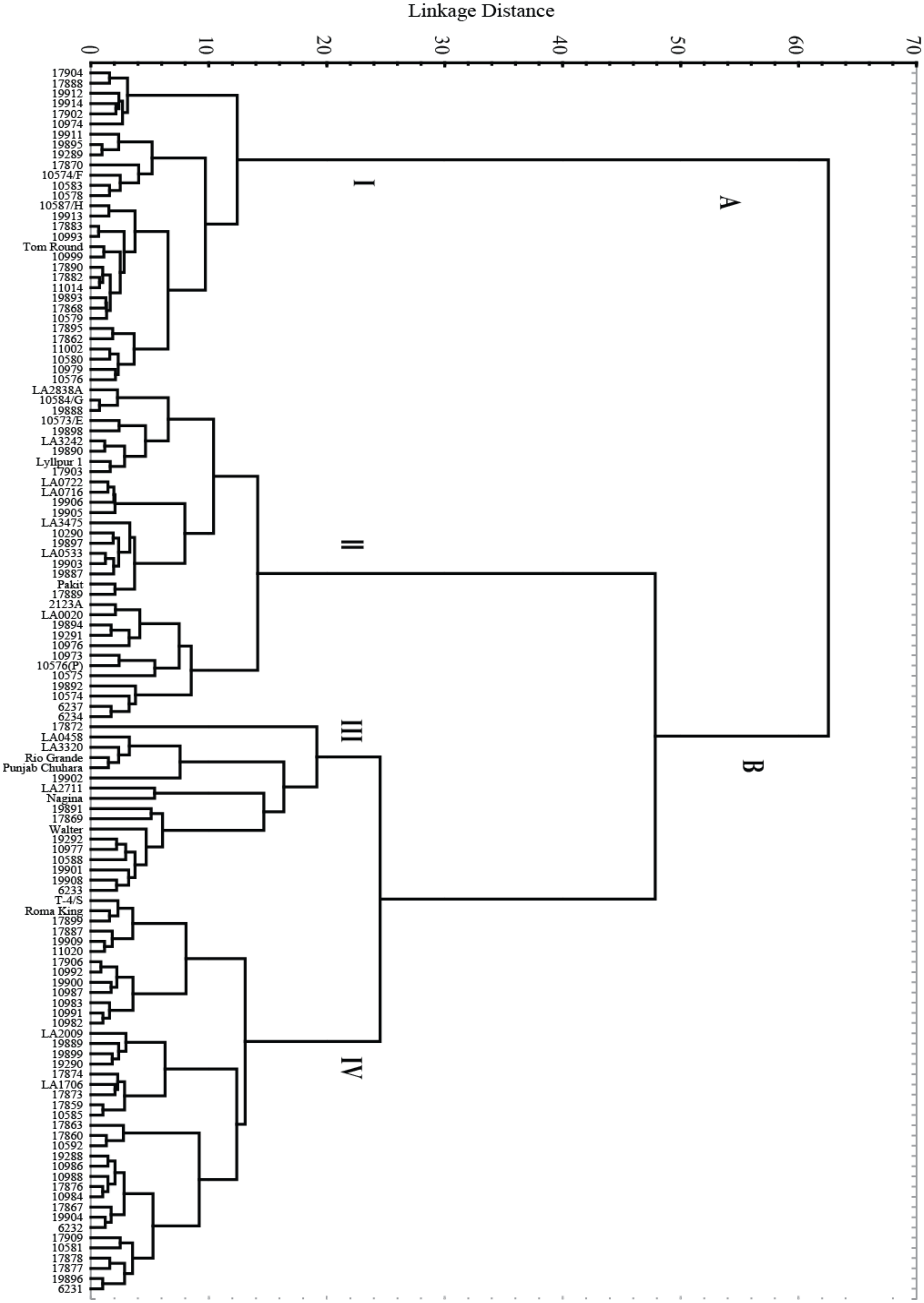

Fig. 1. Phenogram of 120 tomato genotypes based on 13 plant growth parameters at the seed germination and seedling stages under 2.5% of PEG_{8000}.

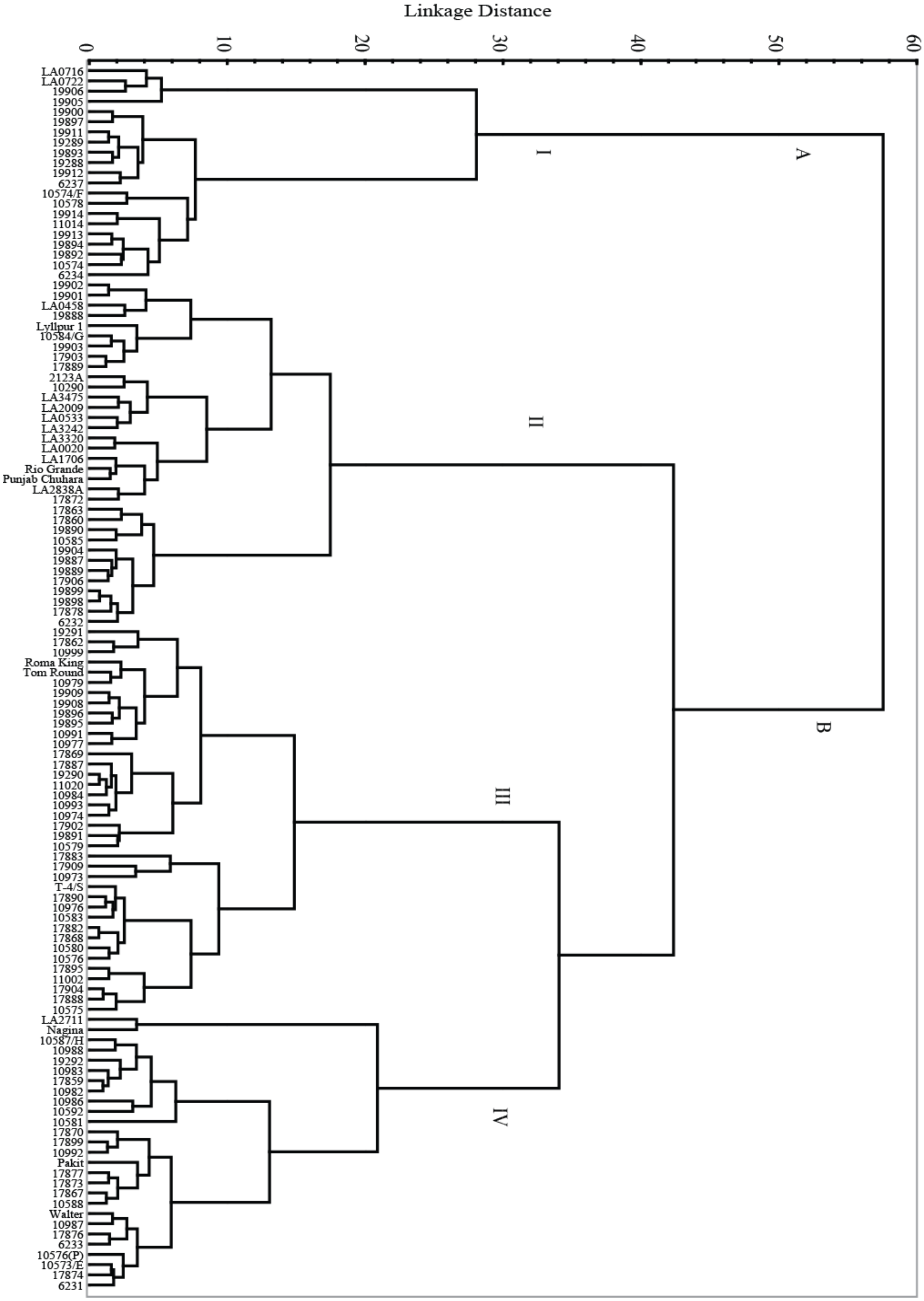

Fig. 2. Phenogram of 120 tomato genotypes based on 13 plant growth parameters at the seed germination and seedling stages under 5.0% of PEG_{8000}.

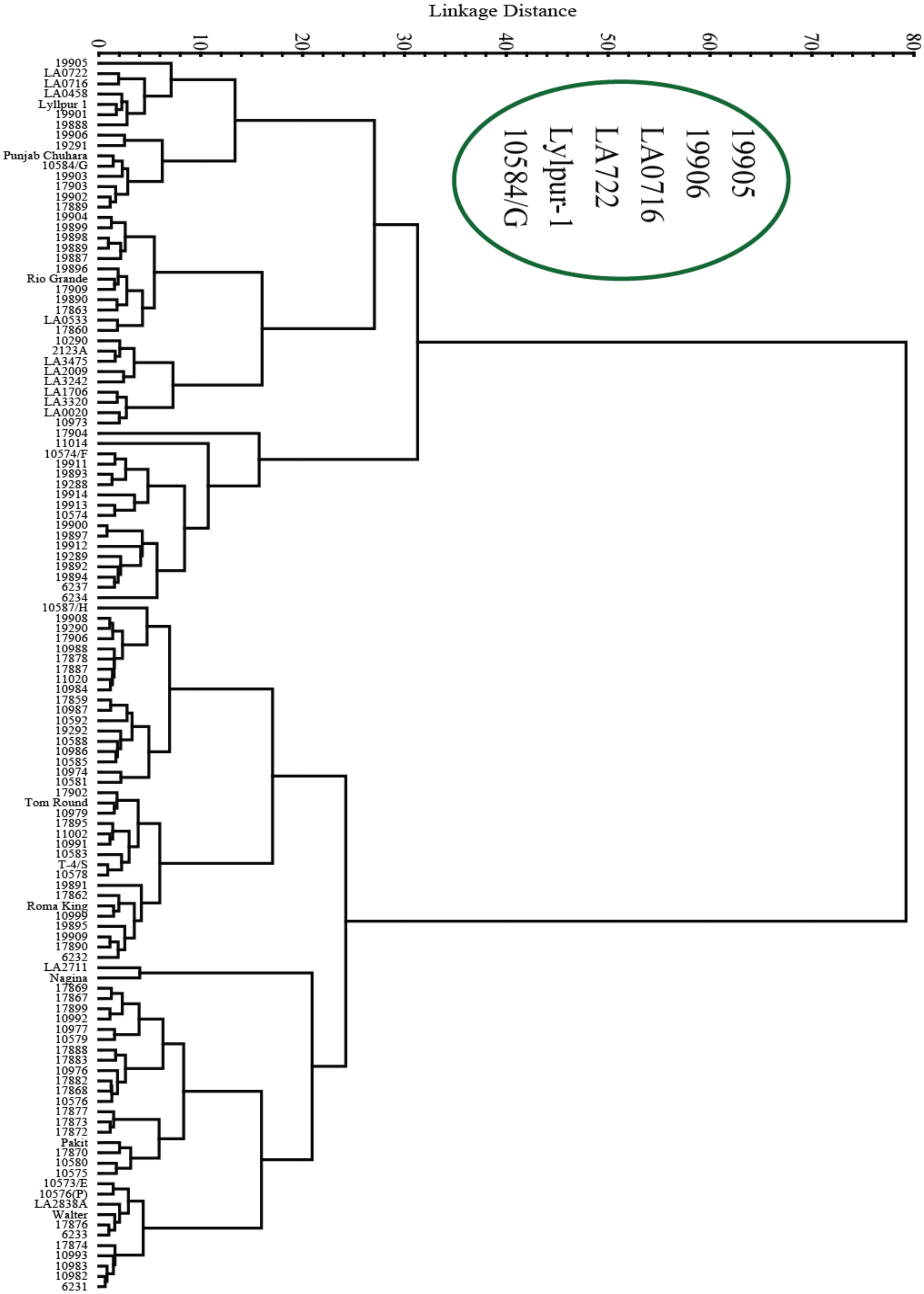

Fig. 3. Phenogram of 120 tomato genotypes based on 13 plant growth parameters at the seed germination and seedling stages under 7.5% of PEG_{8000}.

Table 2. Ranking of the tomato genotypes for their relative drought tolerance of plant growth parameters at germination and seedling stages based on cluster analysis (Ward's method).

Genotypes	PEG_{8000} levels			Drought tolerance rank ($)
	2.5%	5.0%	7.5%	
19905	2	1	1	Tolerant
19906	2	1	1	Tolerant
LA0716	2	1	1	Tolerant
LA0722	2	1	1	Tolerant
11014	1	1	3	Tolerant
17889	2	2	1	Tolerant
17903	2	2	1	Tolerant
19289	1	1	3	Tolerant
19888	2	2	1	Tolerant
19893	1	1	3	Tolerant
19903	2	2	1	Tolerant
19911	1	1	3	Tolerant
19912	1	1	3	Tolerant
19913	1	1	3	Tolerant
19914	1	1	3	Tolerant
10574/F	1	1	3	Tolerant
10584/G	2	2	1	Tolerant
Lyallpur-1	2	2	1	Tolerant
6234	2	1	3	Moderately tolerant
6237	2	1	3	Moderately tolerant
10574	2	1	3	Moderately tolerant
10578	1	1	4	Moderately tolerant
19291	2	3	1	Moderately tolerant
19887	2	2	2	Moderately tolerant
19890	2	2	2	Moderately tolerant
19892	2	1	3	Moderately tolerant
19894	2	1	3	Moderately tolerant
19897	2	1	3	Moderately tolerant
19898	2	2	2	Moderately tolerant
19901	3	2	1	Moderately tolerant
19902	3	2	1	Moderately tolerant
P. Chuhara	3	2	1	Moderately tolerant
LA3242	2	2	2	Moderately tolerant
LA0533	2	2	2	Moderately tolerant
LA0020	2	2	2	Moderately tolerant
LA3475	2	2	2	Moderately tolerant
10973	2	3	2	Moderately tolerant
17904	1	3	3	Moderately tolerant
Rio Grande	3	2	2	Moderately tolerant
LA3320	3	2	2	Moderately tolerant
10973	2	3	2	Moderately tolerant
17904	1	3	3	Moderately tolerant
Rio Grande	3	2	2	Moderately tolerant
LA3320	3	2	2	Moderately tolerant
10576	1	3	4	Moderately sensitive
10579	1	3	4	Moderately sensitive
10580	1	3	4	Moderately sensitive
10583	1	3	4	Moderately sensitive
10974	1	3	4	Moderately sensitive
10979	1	3	4	Moderately sensitive

Table 2. (Cont'd.).

Genotypes	PEG$_{8000}$ levels			Drought tolerance rank ($)
	2.5%	5.0%	7.5%	
10993	1	3	4	Moderately sensitive
10999	1	3	4	Moderately sensitive
11002	1	3	4	Moderately sensitive
17860	4	2	2	Moderately sensitive
17862	1	3	4	Moderately sensitive
17863	4	2	2	Moderately sensitive
17868	1	3	4	Moderately sensitive
17882	1	3	4	Moderately sensitive
17883	1	3	4	Moderately sensitive
17888	1	3	4	Moderately sensitive
17890	1	3	4	Moderately sensitive
17895	1	3	4	Moderately sensitive
17902	1	3	4	Moderately sensitive
19288	4	1	3	Moderately sensitive
19889	4	2	2	Moderately sensitive
19895	1	3	4	Moderately sensitive
19899	4	2	2	Moderately sensitive
19900	4	1	3	Moderately sensitive
19904	4	2	2	Moderately sensitive
Tom Round	1	3	4	Moderately sensitive
LA2838A	2	2	4	Moderately sensitive
LA2009	4	2	2	Moderately sensitive
LA1706	4	2	2	Moderately sensitive
10576	1	3	4	Moderately sensitive
10579	1	3	4	Moderately sensitive
10580	1	3	4	Moderately sensitive
10583	1	3	4	Moderately sensitive
10974	1	3	4	Moderately sensitive
10979	1	3	4	Moderately sensitive
10993	1	3	4	Moderately sensitive
10999	1	3	4	Moderately sensitive
11002	1	3	4	Moderately sensitive
17860	4	2	2	Moderately sensitive
17862	1	3	4	Moderately sensitive
17863	4	2	2	Moderately sensitive
17868	1	3	4	Moderately sensitive
17882	1	3	4	Moderately sensitive
17883	1	3	4	Moderately sensitive
17888	1	3	4	Moderately sensitive
17890	1	3	4	Moderately sensitive
17895	1	3	4	Moderately sensitive
17902	1	3	4	Moderately sensitive
19288	4	1	3	Moderately sensitive
19889	4	2	2	Moderately sensitive
19895	1	3	4	Moderately sensitive
19899	4	2	2	Moderately sensitive
19900	4	1	3	Moderately sensitive
19904	4	2	2	Moderately sensitive
Tom Round	1	3	4	Moderately sensitive
LA2838A	2	2	4	Moderately sensitive
LA2009	4	2	2	Moderately sensitive

Table 2. (Cont'd.).

Genotypes	PEG_{8000} levels			Drought tolerance rank ($)
	2.5%	5.0%	7.5%	
LA1706	4	2	2	Moderately sensitive
10575	2	3	4	Sensitive
10976	2	3	4	Sensitive
17870	1	4	4	Sensitive
17872	3	2	4	Sensitive
17909	4	3	2	Sensitive
19896	4	3	2	Sensitive
10587/H	1	4	4	Sensitive
6232	4	2	4	Sensitive
10576(P)	2	4	4	Sensitive
10585	4	2	4	Sensitive
10977	3	3	4	Sensitive
17905	4	2	4	Sensitive
17906	4	2	4	Sensitive
19891	3	3	4	Sensitive
19908	3	3	4	Sensitive
10573/E	2	4	4	Sensitive
Pakit	2	4	4	Sensitive
6233	3	4	4	Sensitive
10588	3	4	4	Sensitive
10984	4	3	4	Sensitive
10991	4	3	4	Sensitive
11020	4	3	4	Sensitive
17887	4	3	4	Sensitive
19290	4	3	4	Sensitive
19292	3	4	4	Sensitive
19909	4	3	4	Sensitive
Roma King	4	3	4	Sensitive
T-4/S	4	3	4	Sensitive
Walter	3	4	4	Sensitive
Nagina	3	4	4	Sensitive
LA2711	3	4	4	Sensitive
6231	4	4	4	Sensitive
10581	4	4	4	Sensitive
10592	4	4	4	Sensitive
10982	4	4	4	Sensitive
10983	4	4	4	Sensitive
10986	4	4	4	Sensitive
10987	4	4	4	Sensitive
10988	4	4	4	Sensitive
10992	4	4	4	Sensitive
17859	4	4	4	Sensitive
17867	4	4	4	Sensitive
17873	4	4	4	Sensitive
17874	4	4	4	Sensitive
17876	4	4	4	Sensitive
17877	4	4	4	Sensitive
17899	4	4	4	Sensitive

Legend $;
Tolerant, sum of clusters at three levels of PEG_{8000} is 4 and 5
Moderately tolerant, sum of clusters at three levels of PEG_{8000} is 6 and 7
Moderately sensitive, sum of clusters at three levels of PEG_{8000} is 8 and 9
Sensitive, sum of clusters at three levels of PEG_{8000} is > 9

Table 3. Groups membership based on cluster analysis from relative data in 120 tomato genotypes.

Group No.	Frequency	Genotypes			
		19905	19906	LA0716	LA0722
Group 1	18	11014	17889	17903	19289
		19888	19893	19903	19911
		19912	19913	19914	10574/F
		10584/G	Lyllpur-1		
Group 2	25	6234	6237	10574	10578
		19291	19887	19890	19892
		19894	19897	19898	19901
		19902	P.Chuhara	LA3242	LA0533
		LA0020	LA3475	LA0458	10290
		2123A	10973	17904	Rio Grande
		LA3320			
Group 3	29	10576	10579	10580	10583
		10974	10979	10993	10999
		11002	17860	17862	17863
		17868	17882	17883	17888
		17890	17895	17902	19288
		19889	19895	19899	19900
		19904	Tom Round	LA2838A	LA2009
		LA1706			
Group 4	48	10575	10976	17870	17872
		17909	19896	10587/H	6232
		10576 (P)	10585	10977	17869
		17878	17906	19891	19908
		10573/E	Pakit	6233	10588
		10984	10991	11020	17887
		19290	19292	19909	Roma King
		T-4/S	Walter	Nagina	LA2711
		6231	10581	10592	10982
		10983	10986	10987	10988
		10992	17859	17867	17873
		17874	17876	17877	17899

Table 4. Tolerance-rank wise means for morphometric parameters recorded under PEG_{8000} induced stress.

	Tolerant	Moderately tolerant	Moderately sensitive	Sensitive
Growth parameters	Mean	Mean	Mean	Mean
Germination percentage	$68.3_{(1.45)}$	$62.5_{(1.29)}$	$45.9_{(0.91)}$	$42.9_{(0.62)}$
Germination rate	$7.73_{(1.98)}$	$7.45_{(1.46)}$	$5.48_{(1.58)}$	$5.50_{(1.09)}$
Shoot length (cm)	$0.92_{(0.11)}$	$0.87_{(0.15)}$	$0.90_{(0.15)}$	$0.83_{(0.14)}$
Root length (cm)	$42.74_{(37.52)}$	$31.13_{(23.58)}$	$13.68_{(9.09)}$	$16.86_{(7.82)}$
Shoot-to-root length Ratio	$14.67_{(14.50)}$	$6.24_{(3.05)}$	$3.15_{(2.06)}$	$3.20_{(1.41)}$
Shoot fresh weight (mg)	$34.59_{(21.25)}$	$27.26_{(10.49)}$	$16.40_{(8.15)}$	$17.60_{(6.39)}$
Shoot dry weight (mg)	$12.13_{(9.83)}$	$6.37_{(2.49)}$	$3.43_{(2.21)}$	$3.44_{(1.49)}$
Root fresh weight (mg)	$1.03_{(0.36)}$	$0.87_{(0.15)}$	$0.87_{(0.14)}$	$0.86_{(0.11)}$
Root dry weight (mg)	$66.16_{(11.99)}$	$74.68_{(10.14)}$	$76.38_{(6.05)}$	$80.77_{(5.06)}$
shoot-to-root ratio	$69.42_{(7.39)}$	$76.11_{(10.21)}$	$81.73_{(5.09)}$	$83.58_{(4.49)}$
Shoot moisture content	$49.87_{(18.48)}$	$42.30_{(13.61)}$	$33.73_{(9.02)}$	$31.07_{(6.58)}$
Root moisture content	$10.94_{(3.26)}$	$10.27_{(2.19)}$	$8.39_{(1.48)}$	$8.94_{(1.08)}$
Relative water content (%)	$55.81_{(14.33)}$	$50.48_{(25.95)}$	$35.56_{(20.69)}$	$32.13_{(21.42)}$

Table 5. Principal Component Analysis of 120 tomato genotypes for thirteen biological attributes at seed germination and seedling stages under varying levels (2.5%, 5.0% and 7.5%) of PEG$_{8000}$.

		PC1	PC2
Eigen values		7.44	1.90
Proportion of variance		57.19	14.61
Cumulative variance		57.19	71.80
Growth parameters	**Communalities**	**Eigenvector**	
Germination percentage	0.81	0.89	-0.15
Germination rate	0.81	0.87	0.22
Shoot Length	0.44	-0.15	-0.65
Root Length	0.80	0.88	0.17
Shoot -to-root length ratio	0.85	0.90	-0.19
Shoot fresh weight	0.84	0.91	0.05
Shoot dry weight	0.88	0.91	-0.24
Root fresh weight	0.32	0.56	0.08
Root dry weight	0.72	-0.46	0.71
Shoot -to-root ratio	0.77	-0.54	0.69
Shoot moisture content	0.77	0.85	0.20
Root moisture content	0.91	0.89	0.34
Relative water content	0.44	0.55	0.37

Table 6. Correlation matrix for growth parameters of the tomato genotypes under PEG$_{8000}$ induced water stress.

Growth parameters	Germination percentage	Rate of germination	Shoot length	Root length	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Shoot-to-root ratio
Germination rate	0.80**								
Shoot length	0.72**	0.79**							
Root length	0.72**	0.77**	0.84**						
Shoot fresh weight	0.65**	0.74**	0.75**	0.79**					
Shoot dry weight	0.65**	0.81**	0.74**	0.79**	0.89**				
Root fresh weight	0.64**	0.77**	0.81**	0.84**	0.89**	0.92**			
Root dry weight	0.65**	0.78**	0.73**	0.78**	0.86**	0.96**	0.93**		
Shoot moisture content	-0.12	-0.17	-0.16	-0.10	0.16	-0.19	-0.08	-0.20	-0.13
Root moisture content	-0.39**	-0.40**	-0.28**	-0.22	-0.22	-0.40**	-0.18	-0.44**	-0.02
Relative water content	0.56**	0.67**	0.66**	0.59**	0.55**	0.65**	0.62**	0.57**	0.39**

* = Significant at the 0.05 probability level
** = Significant at the 0.01 probability level

Principle component analysis based on plant growth parameters: The variation among genotypes was also computed through Principle Component. Eigenvalues of thirteen principle components drought stress potential is shown in scree plot (Fig. 4) and principle component matrix/analysis is given in (Table 5). The data revealed that under drought stress (mean values) two principle components having greater than 1 Eigenvalues contributed 71.80 % of the total variation among 120 genotypes of tomato. It was found that two Principle Component 1 (PC1) contributed 57.19%, whereas, PC2 contributed 14.61% of the total variation. Ten biological characters which contributed more positively to PC1 were shoot fresh and dry weight (0.91), germination percentage (0.89), root length (0.88), and rate of germination (0.87). Moreover, root fresh weight and relative water content contributed (0.56) and (0.55), respectively. While shoot length and root dry weight under present study in PC1 contributed less than 0.5, which showed non-significant genetic variance. This indicated that the populations with greater PC1 values produced more shoot fresh and dry weighs under different levels of PEG$_{8000}$ and exhibited maximum germination percentage and rate of germination thus seedling maintained root length and fresh weight of roots. Maximum genetic variance to PC2 was contributed by only two traits: root dry weight (0.71) and shoot-to root ratio (0.69) which contributed more positively. Relative water content, germination rate, root length, root and shoot fresh weight contributed positively but not significant. Germination percentage, shoot dry weight and shoot length in this component have negative association but non-significant. It is evident that under water deficit conditions maximum biological traits contributed more positively to PC1 (57.19%) and hence could be given considerable importance for the four groups based on thirteen traits of tomato genotypes. When PC1 was plotted against PC2, although there was not complete separation of four groups but maximum separation was between group 2, 3 and 4 but maximum mixing up of groups was in moderately sensitive and sensitive groups (3 and 4). Moreover, maximum mixing was also exhibited by groups 1 and 2 presented in scatter diagram (Fig. 5). The correlation coefficients among plant growth parameters were computed and are presented in (Table 6). The results of present investigation revealed water stress tolerance was positively correlated with growth attributes. So, scatterplot among (120) tomato genotypes was developed in order to determine stress tolerance.

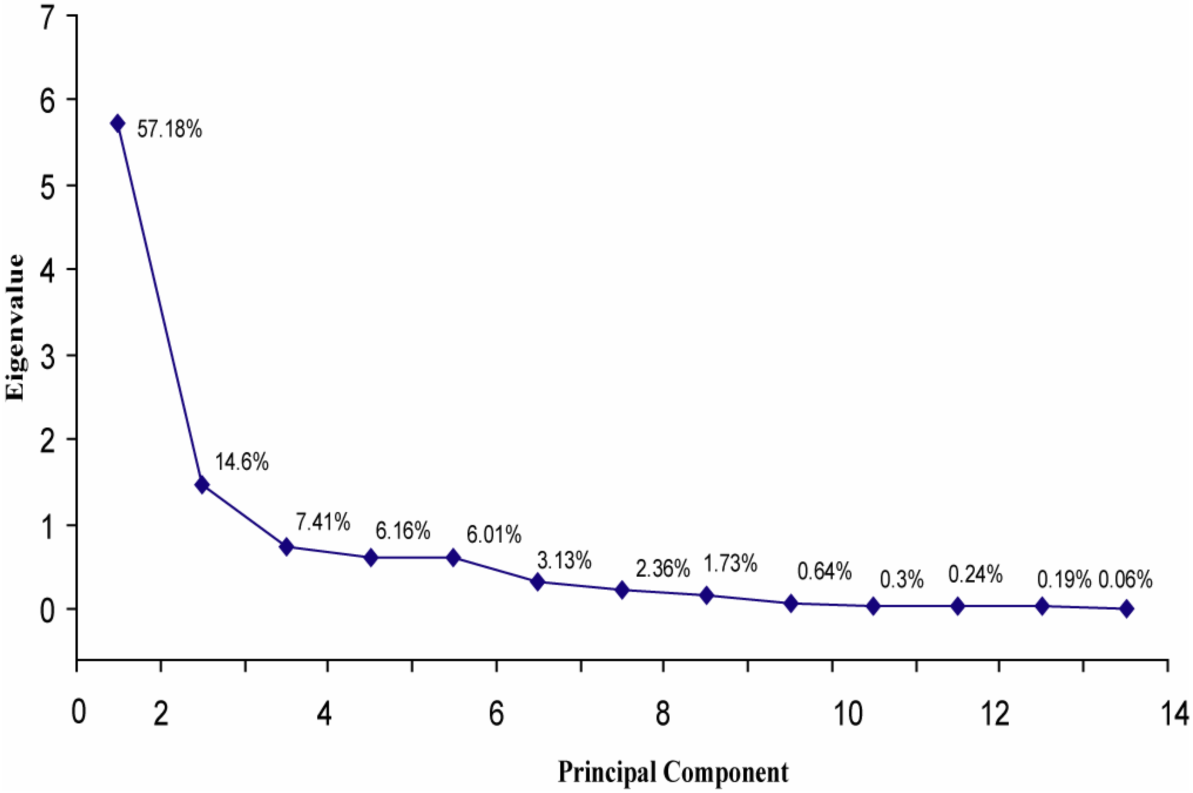

Fig. 4. Scree plot for 13 Principle Components in 120 tomato genotypes at seed germination and seedling stages under varying levels (0, 2.5%, 5.0% and 7.5%) of PEG_{8000} induced water stress.

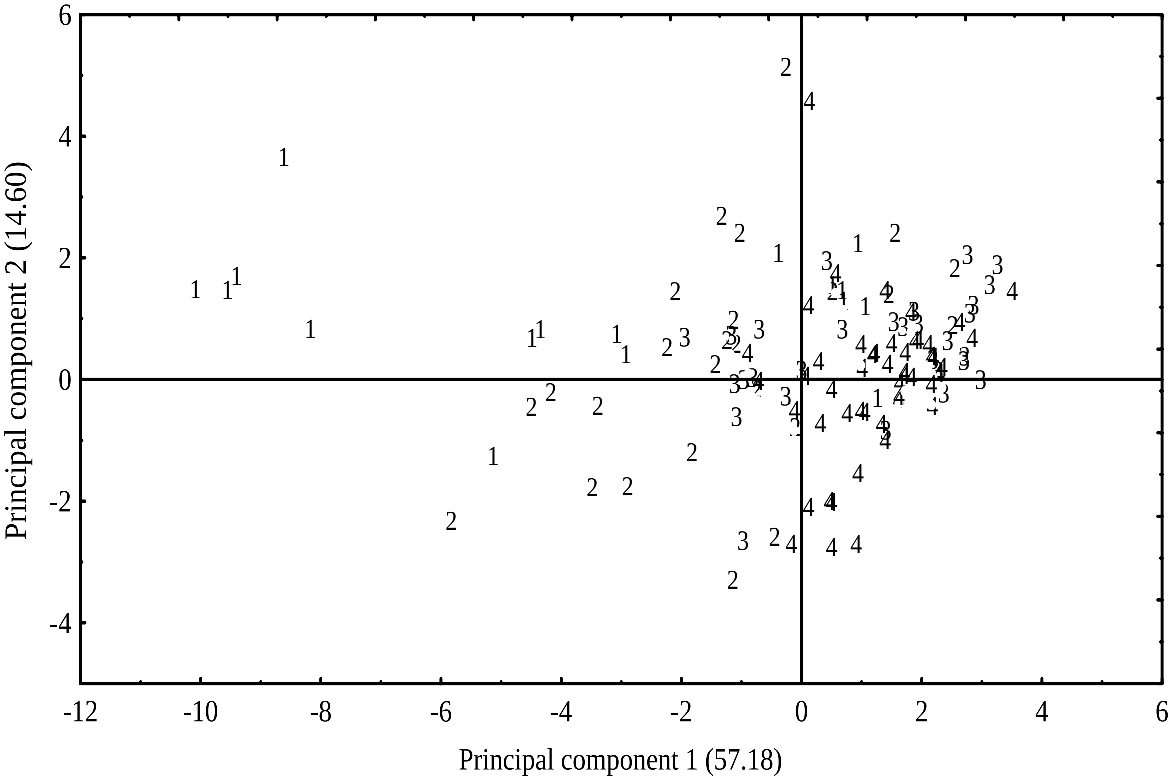

Fig. 5. Scatter diagram on average of four cluster diversity for first two PCs of tomato genotypes at seed germination and seedling stages under varying (0, 2.5%, 5.0% and 7.5%) of PEG_{8000} induced water stress.

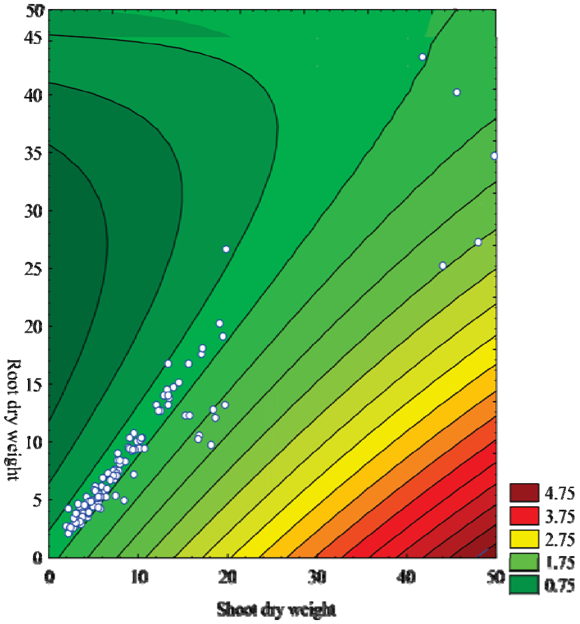

Fig. 6. Shoot and root dry weight correlation under PEG_{8000} at three different concentrations (2.5%, 5.0% and 7.5%). Shoot-to-root dry weight ratio, the legend shows that most of the genotypes were above the 1:1 line suggests greater root dry weight at shoot, few genotypes fells below the line and were tolerant.

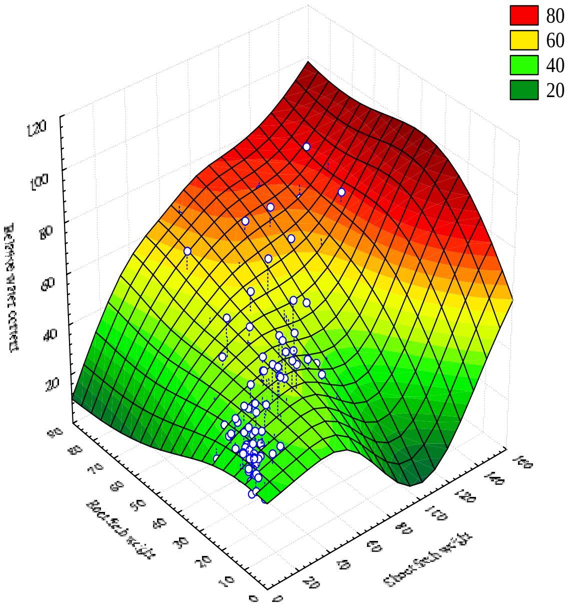

Fig. 7. Comparison of 120 tomato genotypes on the basis of shoot and root fresh weights with relative water content at three different concentrations (2.5%, 5.0% and 7.5%) of PEG_{8000} imposed water stress. Leaf relative water content, the legend shows that most of the genotypes have 40% (green color) leaf water content. While tolerant genotypes tend to maintain this percentage (60%) or have more shoot and root fresh weights with maximum water content (80%). Under water stress root fresh weight is less affected as compared to shoot fresh weight hence a deep growth depression is present in shoot fresh weight.

Discussion

Drought tolerance is a developmentally regulated, stage specific phenomenon; depend on genotype and the severity of the stress applied. Thus drought tolerance at one stage of plant development may not be related to adult stage; hence seedling stage was reported as more sensitive stage of water stress compared with total germination in tomato (Shtereva *et al*., 2008). Current findings of our study also have strong agreement with the previous findings of several other workers who reported no consistent correlation between germination and seedling stage. For instance, genotypes 10576 (P), 19900 and LA0020 were slower for total germination percentage had high seedling biomass production in PEG_{8000} concentrations. Similarly, the genotypes 19904, 19892, 006232 and LA0533 were successful to gain maximum germination percentage but 19904, 06232 and LA0533 became intermediate whereas, 19892 proved sensitive for biomass accumulation at seedling stage. Germplasm of 120 tomato genotypes successfully attained germination percentage above 70 percent but there was much variation regarding the points of shoot dry weight. Similar results were also determined by Shtereva *et al*. (2008). The tolerance observed in those genotypes with high biomass production at the seedling stage was also conferred at the germination stage. Moreover, the highly tolerant genotypes (high biomass producing) differ from the intermediate and sensitive genotypes in both total germination percentage and rate of germination. If parallels are drawn between data for two initial growth stages, the tolerance observed in nine genotypes, 17889, 19903, 19905, LA0716, LA0722, LA0458, Lyallpur-1, 19898 and 19902 at the seedling stage was also conferred at the germination stage (Table 5). Because these genotypes were highly tolerant at the seedling stage for dry mass accumulation and also differ from the intermediate and sensitive accessions in total germination percentage and rate of germination.

Likewise, our findings are also in close conformity with early reports of Prodriguez *et al*. (1997) who opined that root growth of tomato is less sensitive to stress than leaf. In contrast, our data disagree with findings of Dasgan *et al*. (2002) who considered that during plant growth development under water stress conditions the dry weight of root and shoot were independent of stress tolerance. However, if we look on the data for biomass shoot to root ratio, a trend is evident that tolerant genotypes tend to sustain the ratio whereas susceptible genotypes have a tendency to achieve less shoot to root dry weight ratio (Fig. 6). Rahman *et al*., (1999) also of the view that water stress tolerant genotypes has low shoot to root dry weight ratio under water deficit conditions and vice versa. One of the important inherited traits in plants is water content of leaf (Chaudhry *et al*., 1999) that may have been used as an index of water stress tolerance to determine plant water status (Sinclair and Ludlow, 1985; McCaig and Romagosa, 1991). In the current envisaged experiment tolerant genotypes were able to maintain water content or less reduction was noted. Besides, genotypes showed different response for relative water content may have been due to genotypic differences for uptake water from soil. Also, a growth depression was observed in the current research under water stress condition as depicted in (Fig. 7).

Therefore, drought tolerance of five tolerant included two wild; '*Lycopersicon pennellii*' and '*Lycopersicon chilense*' 'Lyallpur-1', '17889' and '10584/G', four moderate, 'Punjab Chuhara', 'Pusa Ruby' and 'Ailsa Craig' and three sensitive, 'Roma', 'Avinash-2' and 'Ratan' were confirmed on the basis of biomass production at seedling stage. In these selected eleven genotypes it was observed that there was absence of consistent relationship between germination and seedling stage. *L. pennellii* was successful to gain 98 percent germination percentage but *L. chilense* scored germination percentage less than other tolerant genotypes. Similarly both rate of germination of wild genotypes was less than other tolerant group. But maximum shoot dry weight and relative water content recorded in *L. pennellii* and *L. chilense* along with tolerant genotypes. Similarly, this subset of 11 tomato genotypes was also exhibited variation in shoot, root length and fresh weight. Tolerant genotypes attained more biomass under stress conditions. However, since the degree of water stress tolerance varies with the change in developmental stage, it is suggested to assess water stress tolerance in selected 11 genotypes at the adult stage. This will definitely help in introducing new varieties with higher yield in different environmental conditions of low water supply.

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