MULTIVARIATE ANALYSIS OF ELEVEN GENOTYPES OF TOMATO AT SEEDLING STAGE UNDER DROUGHT IMPOSED BY POLYETHYLENE GLYCOL

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***ABSTRACT*:** *Growth and yield of tomato (Solanum lycopersicum L.) is being seriously constrained by many abiotic factors including drought in arid and semi-arid regions of the world. A better understanding about the mechanism of water stress tolerance provides basic strategies for crop breeding for drought tolerance. In this study, seeds of 11 local and exotic lines of tomato were allowed to germinate at varying levels of polyethylene glycol (PEG_{8000}) induced water stress (2.5%, 5.0% and 7.5%) for two weeks in full strength Hoagland nutrient solution along with control (only nutrient solution). Significant amount of genetic variability was found in all attributes of 11genotypes of tomato. Consistent decrease in seed germination percentage and seedling growth was recorded by increasing PEG_{8000} concentrations in the growth medium (water stress). 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 11 lines; 4 genotypes were ranked as tolerant while 2, 3 and 2 were ranked as 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. Conclusively, lines 'Lyallpur-1', 'CLN1767', '10584/G' along with wild genotypes were found to be water stress tolerant at least at early growth stages. Moreover, these genotypes may be a potential target in studying stress-responsive genes study.*

KEY WORDS: Polyethylene glycol, Drought, Tomato, Seedlings, Multivariate analysis

1. INTRODUCTION

Efficient and fair management of water resources chiefly depends on sustainable economic and social development while, agriculture consumes large amounts of water for irrigation, hence it is imperative to screen the germplasm under water stress condition to decrease the number of irrigation. Drought is the major abiotic stress reducing yield by 50-70% thus hampering the needs of food requirement in developed and developing countries of the world [1-4]. The production of new cultivars of crops with higher yields under water limited conditions can be achieved through germplasm screening, breeding or through advanced molecular biology techniques [5-7]. It has been reviewed many times that water stress tolerance of a crop species depends on its ability to access soil water and to use it efficiently. However, abilities to access soil water and use efficiencies depends on type of crop species, type of cultivar, time and duration of water availability and type of agricultural conditions [7,8]. Another important issue is the degree of drought tolerance varies with growth and development in most plant species. Thus, there is a need to assess the overall drought tolerance of a plant species for farmers' standpoint [9-11]. The improvement of drought tolerance in tomato cultivars for farming in hot and dry environments with lesser number of irrigation, water is a continuing research intention in developing countries [12,13]. Physiological and genetic investigations conducted during last few decades indicated that utmost traits for abiotic stress tolerance are multifaceted. Since more than one gene is involved in controlling such traits and greatly influenced due to environmental differences [14]. Seed germination is first critical and the best sensitive period in lifespan of plants. However, both seed germination along with initial seedling development phases are usually considered more sensitive to water stress. The first and notable consequence of water stress is decreased germination and poor stand establishment [15,16]. Screening and Selection of genotypes which can be cultivated for profitable yield under drought situations represent eternal as well as complementary resolution in order to curtail consequence of drought. Besides, improvement in crop yield under water stress is dependent on selection: one of significant aspect of plant breeding [17,18]. A variety of other physiological indicators are also available such as accumulation of proline [19] ABA production [20] relative water content, cell membrane stability, quantum yield of PSII [21] root growth [22].Under water stress situation maximum root development indicated plants capacity to cope and live in stressful environment. Root length was significantly longer in resistant tomato cultivar 'TM 0126' than in 'Kyokko' under control and different levels of water stress [23]. Though a variety of physiological indicators are available, still it has been felt that for rapid improvement in this field, there is a need to develop a simple, non-destructive criteria. Moreover, genetic variation in traits responsible for drought tolerance is pre-requisite. Inter-intra-specific, intra-cultivar variation for physiological or biochemical traits provides scope for selection and similar study was conducted for cultivated and wild species (150 lines) of tomato under drought stress in order to select lines with high yield and drought tolerance potentials along with high heritabilities [24]. Whereas, 101 tomato genotypes were also evaluated for stress condition out of which nineteen were ranked as promising lines. While evaluating tomato germplasm collected from different sources (breeding lines/cultivars introduced from AVRDC, Taiwan, CATIE, Costa Rica and ZIGUK, Germany as well as indigenous material from IIHR, Bangalore and NBPGR, New Delhi) evaluated in 3 seasons

that none of the lines/cultivars survived under drought conditions, except T1147, German accession of *L. pimpinellifolium* with long internodes and small fruits [25]. Nevertheless 124 tomato genotypes, evaluated under stress conditions out of which only two genotypes performed best [26]. Foolad and coworkers also evaluated 30 accessions of tomato for seed germination rate under specific set of laboratory conditions in order to explore the drought stress potential of germplasm [27,28]. It was reported that both genotype and severity of stress predict the seedling growth responses. As systematic study of genotypic analysis is of great importance in germplasm screening, hence based on various morpho/physiological and biochemical attributes, Kenya tomato germplasm including 26 land-races and 9 market cultivars was screened to delineate genetic variability [29]. Likewise, when different germplasm of tomato was screened by exposing them to different levels (0, 20, 40, 60 g/L) of polyethylene glycol imposed water stress, they observed mutant hybrid of tomato and its derivatives were successful to maintain root growth at every level of water stress [30,31]. Predictive parameters that can be useful for screening experiments at initial growth phases of crop like tomato hence, 55 genotypes were screened under control and different levels of osmotic stress. They concluded that out of 55 genotypes; 8 were tolerant, 7 were most susceptible, while, 36 responded gradually between stress tolerant and sensitive genotypes [32]. Another study performed reveled that *L. esculentum* viz. Moneymaker and Edkawi were more tolerant than *L. cheesmanii*, LA1401 and *L. Pennellii* recorded as more tolerant than *L. peruvianum*. For effective exploration of intra-varietal, study of drought at different stages of growth with maximum number of local as well as exotic genotypes is crucial. Most drought tolerant lines might be used directly to dry locale or may be crossed with other cultivar to augment their drought tolerance [33].

Multivariate analysis procedures are very attractive tool and can be utilized to describe phenotypic variations among the genotypes. It is used as a management tool for discovering underlying data grouping and relationships. Two complimentary procedures namely Cluster Analysis (measures similarities and dissimilarities in order to determine the cluster numbers that explained in data) and Principle Component Analysis (PCA: explains principle variables contributing to the data relationship) were used in this study because they allow the data to group itself. Cluster analysis not only grouped together genotypes with greater morphological matches, but also avoid grouping of genotypes from the identical origin or nearby spots. Scientists also opined that the association between morphological 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 program. 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 [34,35].

2. METHODOLOGY

Seeds of hundred and eight local/exotic genotypes of the species were obtained from Plant Genetic Resource Institute, National Agriculture Research Center (PGRI, NARC), Islamabad. The exotic germplasm of twelve different tomato genotypes was also obtained from Tomato Genetics Resource Center California, United States of America. Seed germination and seedling evaluation were performed using PEG_{8000} (Sigma-Aldrich Co., Life Science. 2KG- Avg. Molecular Weight 8000, EC 203-473-3) at Department of Botany, PMAS-Agriculture University Rawalpindi, Pakistan. The experiment was laid out with four treatments, in triplicate plus 11 genotypes using completely randomized design (CRD).

2.1 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 in Petri dishes, to ensure adequate moisture for the seeds [36]. Seed samples of 11 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; three replicates four treatments and 11 genotypes, in growth cabinets [37].

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 [38]. A seed was considered germinated when both plumule and radicle has emerged $\geq$ 5 mm [39]. 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.

2.2 Seedlings Evaluation

Pre-germinated seeds of 11 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 [36]. 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:

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

2.3 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 [40,41]. For comparing genotypes for drought tolerance; all the data were transformed into drought tolerance indices i.e., means of each parameter of drought stressed plants divided by the means of their respective controls [42]. 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 [43].

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}. The phenogram was constructed in order to support the grouping of the 11 tomato genotypes under drought stress condition. 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.

3. RESULTS AND DISCUSSION

In order to maintain, evaluate and utilize germplasm efficiently under drought stress conditions, it is important to investigate the extent of genetic variability it contains. High variance was observed for seeds shoot fresh and dry weight, root fresh and dry weight and relative water content. For germination percentage and rate; shoot and root length low variance was observed and hence low genetic variability seemed to restrict the scope of selection for these traits in the present germplasm collection. Polyethylene glycol (PEG) is frequently used as drought inducing external osmotica to examine effects of water deficit conditions on plant development. Polymers of PEG molecules, molecular weight greater than 6000 e.g., PEG_{8000}, are recommended as they formulate almost water-resistant chains due to its inert as well as non-ionic makeup [44-46]. Hence, these chains are less absorbed by plants and failed to penetrate the pores of cell wall and apoplast. In order to sustain the uniform osmotic potential of nutrient solution with least physiological damage, PEG is successful to pull out water both from cell and cell wall. Also solutes are not infiltrating through the lignified cell wall [47,48]. The results of 13 morphometric traits presented here clearly exhibited that there was absence of consistent relationship in the sample of 11 cultivars/accessions of tomato to PEG_{8000} induced water stress. The genotype '*L. pennellii*' found to gain more germination percentage whereas '*L. chiliness*' gained germination less than other tolerant genotypes. But the rate of germination of these both genotypes was less than other members of tolerant group. Contrary, both of these genotypes were recorded with more shoot dry weight and relative water content (Figure1: a, and c). Generally it is assumed that genotypes with greater biomass were found to be more efficient during water stress than those with lower biomass. Higher biomass accumulation in water stress tolerant cultivars of tomato may have been due to better plant water status. However, water uptake being a physiological process directly affects the dry matter accumulation and yield [49-51]. Plants uptake water through roots and water is transported from roots to shoots through xylem [52]. Dye uptake studies showed that in plants exposed to drought, a large majority of xylem vessels are not functional in water transport.

Moreover, it was also found that drought sensitive cultivars had small root length, less number of protoxylem vessels with lower diameter than those of resistant cultivars [53]. Similarly, it was also suggested that water stress modulates root anatomical features that enable plant to take up more water [54]. Such kind of water stress-induced changes in root anatomy were more pronounced in drought stress tolerant genotypes. Reduced-irrigation treatment significantly altered number and width of functional xylem elements in the fruit pedicel, especially in the abscission zone. This indicates that drought modifies xylem architecture and, thus, environmentally produced change in the hydraulic property of pedicel may affect fruit development [55].

Moreover, according to classical work of various plant scientists, efficacy of PCA has been documented as an attractive technique because it was found very useful in removing interrelationships between components so reduces the data and renewal of germplasm with new clusters and variation in components of yield depends on crop tolerance under less water irrigation. Hence, multivariate analysis is an effective method to deal with germplasm collection and average linkage cluster and principle component analyses, proved that the utility of such results in preservation and utilization of germplasm [56-60]. Nonetheless, it has been reported that tomato genotype with different origin, depicted the products variability and relationships among variables by computing correlation analysis and multidimensional data analysis techniques (principal component analysis and hierarchical classifications). Three variables were selected, with the aim of classifying the collection of samples in a way consistent with the classification obtained with the first principal components [61]. Genetic diversity among tomato

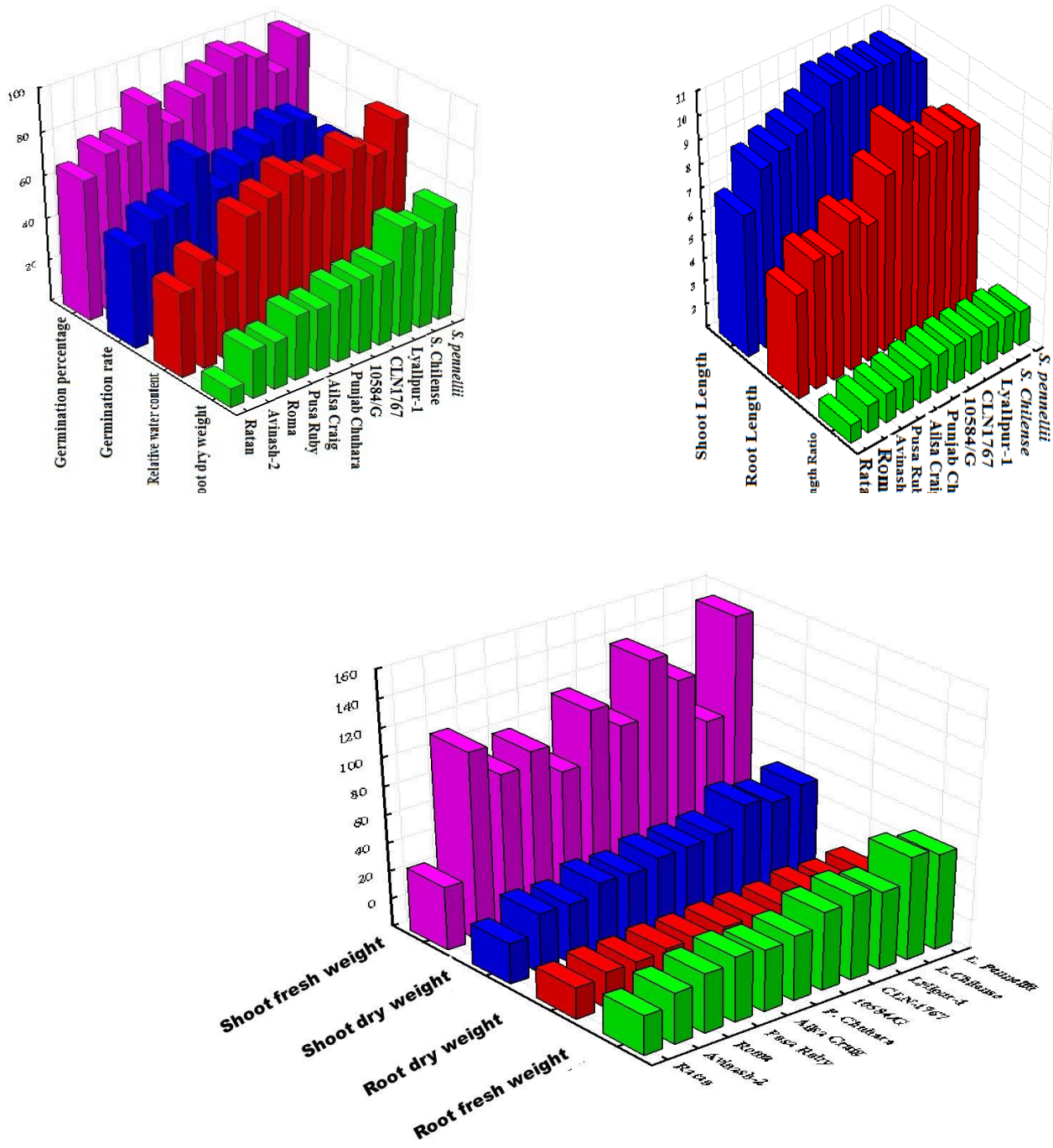

Figure 1: Comparison among mean values of (a) percentage of germination, germination rate, relative water content and shoot dry weight (b) shoot length, root length and shoot-to-root length ratio (c) fresh and dry biomass of 11 selected tomato genotypes under varying levels (2.5%, 5.0% and 7.5%) of PEG_{8000} imposed water stress.

hybrids was also explained by clusters formation and reported that even though the clusters II and III were solitary, but possessed important hybrids having special features which could be better exploited by double cross hybrids or their derivatives for future selection [62].

Therefore, in order to attest the degree of water stress tolerance and their ranking, phenogram of 11 genotypes was constructed because cluster analysis based on complete linkage correlation coefficient distance allows the data to group itself according to their linkage distance (Figure 2a). This phenogram can be allocated into two separate groups 'A' and 'B'. Group 'A' was further divided into two clusters. Cluster 1 consisted of five genotypes: '*L. pennellii*', 'Lyallpur-1', '*L. chiliness*', 17889 (CLN-1767) and '10584/G'. Second cluster consisted of three genotypes: 'Punjab Chuhara', 'Ailsa Craig' and 'Pusa Ruby'. Third cluster was included in Group number B, showed three genotypes: 06233 (Roma), 'Avinash-2' and 'Ratan'.

According to their clusters number, members of cluster 1 were designated as tolerant while, cluster II and III were ranked as moderately sensitive and drought sensitive groups. First two components which contributed 70.94% and 13.04% respectively of the total variance were plotted graphically as presented in scree plot (Figure 2b) and scattered diagram (Figure 3c) to observe the relationship between three clusters. When Factor 1 was plotted against Factor 2, there was complete separation between sub-sample of 11 tolerant and sensitive tomato genotypes. Projection of 13 variables on the factor-plane (1 × 2) for 11 tomato genotypes under varying levels (2.5%, 5.0% and 7.5%) of PEG_{8000} induced water stress depict that the parameters like germination rate, dry weights of shoot or root and shoot length contributed more therefore, could be reliable indicators in screening or classificatory techniques for tomato genotypes particularly under drought (Figure 3). Finally the tomato genotypes which have been ranked after data analysis are presented in Table 1. Similar results were also reported in a current study that morphometric traits may be used selection criteria for germplasm selection under water deficit condition [63].

Table 1: Tomato genotypes finally ranked in four groups (tolerant, moderately tolerant, moderately sensitive and sensitive) on the basis of 13 traits at various levels (2.5%, 5.0% and 7.5%) of PEG_{8000} imposed water stress according to phenogram.

No	Taxon	Accessions	Ranking
1	*S. pennellii*	LA0716	Tolerant
2	*S. Chilense*	LA0458	Tolerant
3	Lyallpur-1		Tolerant
4	CLN1767		Tolerant
5	10584/G		Moderately tolerant
6	Punjab Chuhara	017865	Moderately tolerant
7	Ailsa Craig	LA2711	Moderately sensitive
8	Pusa Ruby	017860	Moderately sensitive
9	Roma	006233	Moderately sensitive
10	Avinash-2	017867	Sensitive
11	Ratan	017870	Sensitive

4. CONCLUSION AND SUMMARY

This analysis depicts that proposed tomato genotype (Table 1) may be use in the QTL analysis linked with water stress. Therefore, it can be concluded that water stress tolerance in plants is mainly coupled with maintenance of plant water status. Thus, seed germination, root length and leaf water content can be used as growth parameters for rapid selection of water stress tolerant genotype. Nevertheless, this information may be helpful in understanding both mechanisms of drought tolerance and improvement for stress tolerance in tomato through selection and breeding programs.

ACKNOWLEDGEMENT

Tomato local and exotic germplasm was kindly provided by 'National Agriculture Research Center' (PGRI) Islamabad, Pakistan and C.M. Rick, Tomato Genetics Resource Center (TGRC), University of California, UC. Davis, USA.

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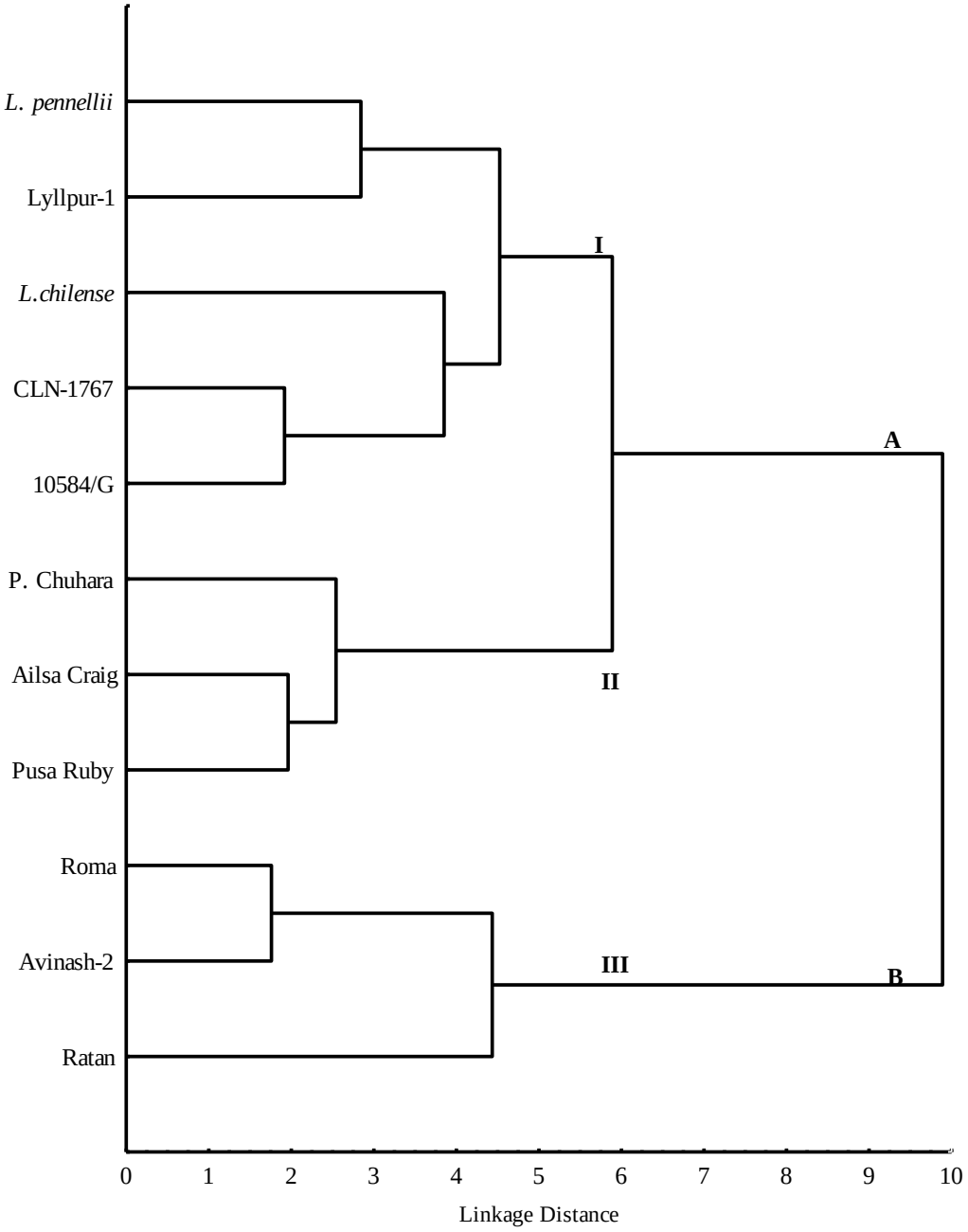
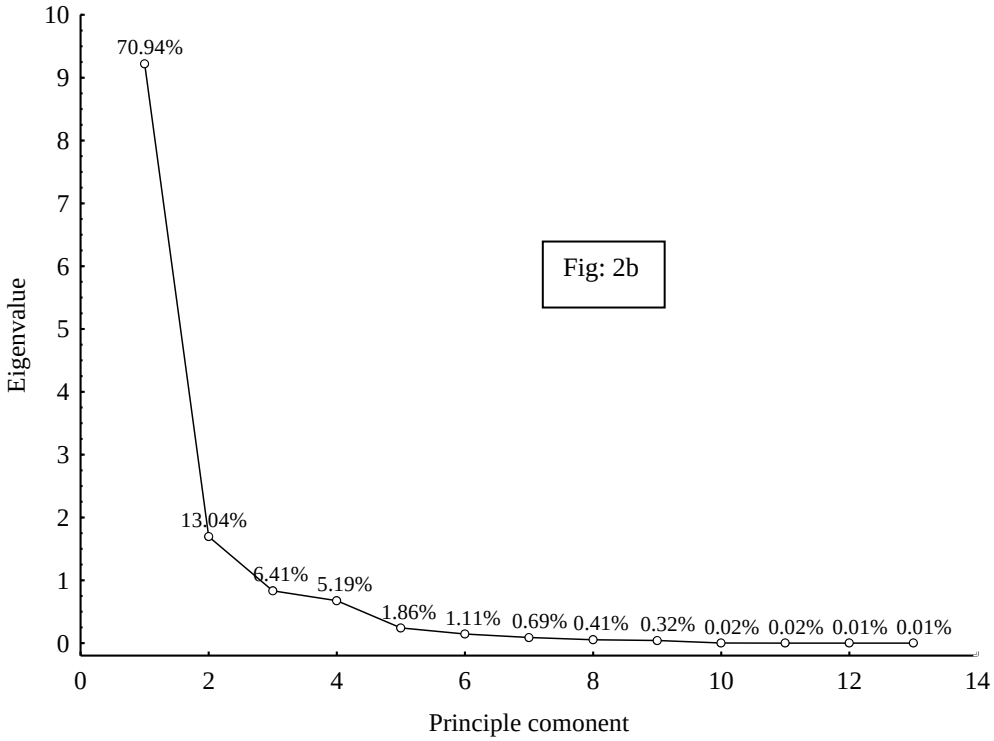


Figure 2(a): Phenogram of 11 selected tomato genotypes for 13 traits constructed under varying levels (2.5%, 5.0% and 7.5%) of PEG_{8000} imposed water stress.



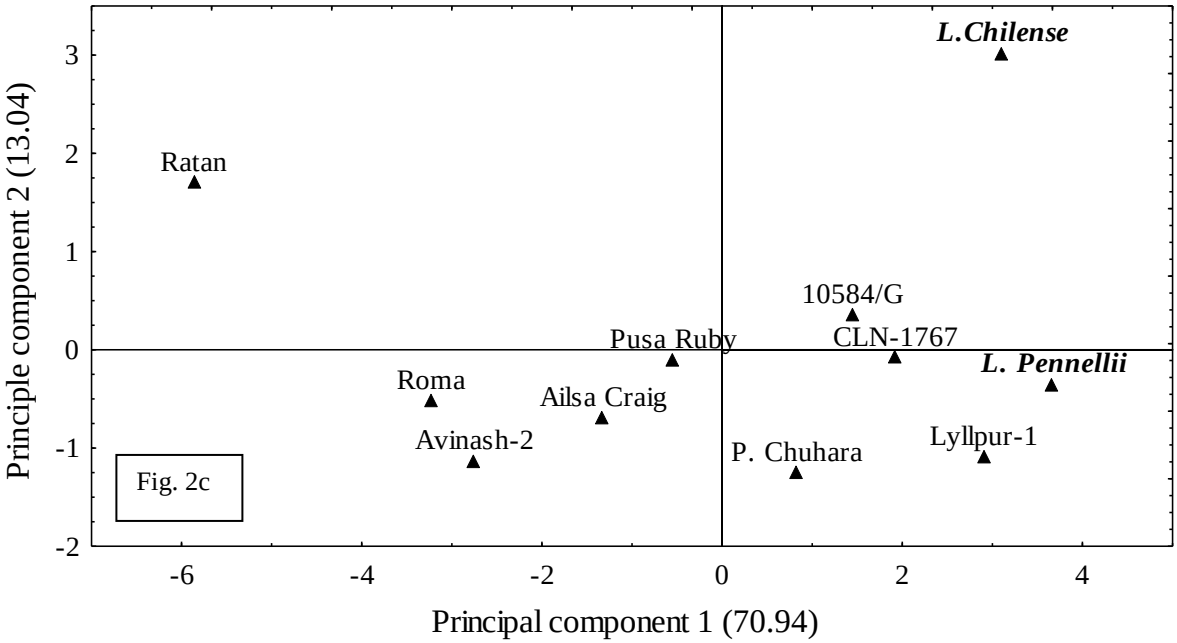


Figure 2: (b) Scree plot and (c) Scatter diagram on average cluster analysis for first two PCs of 11 selected tomato genotypes under varying levels (2.5%, 5.0% and 7.5%) of PEG_{8000} imposed water stress.

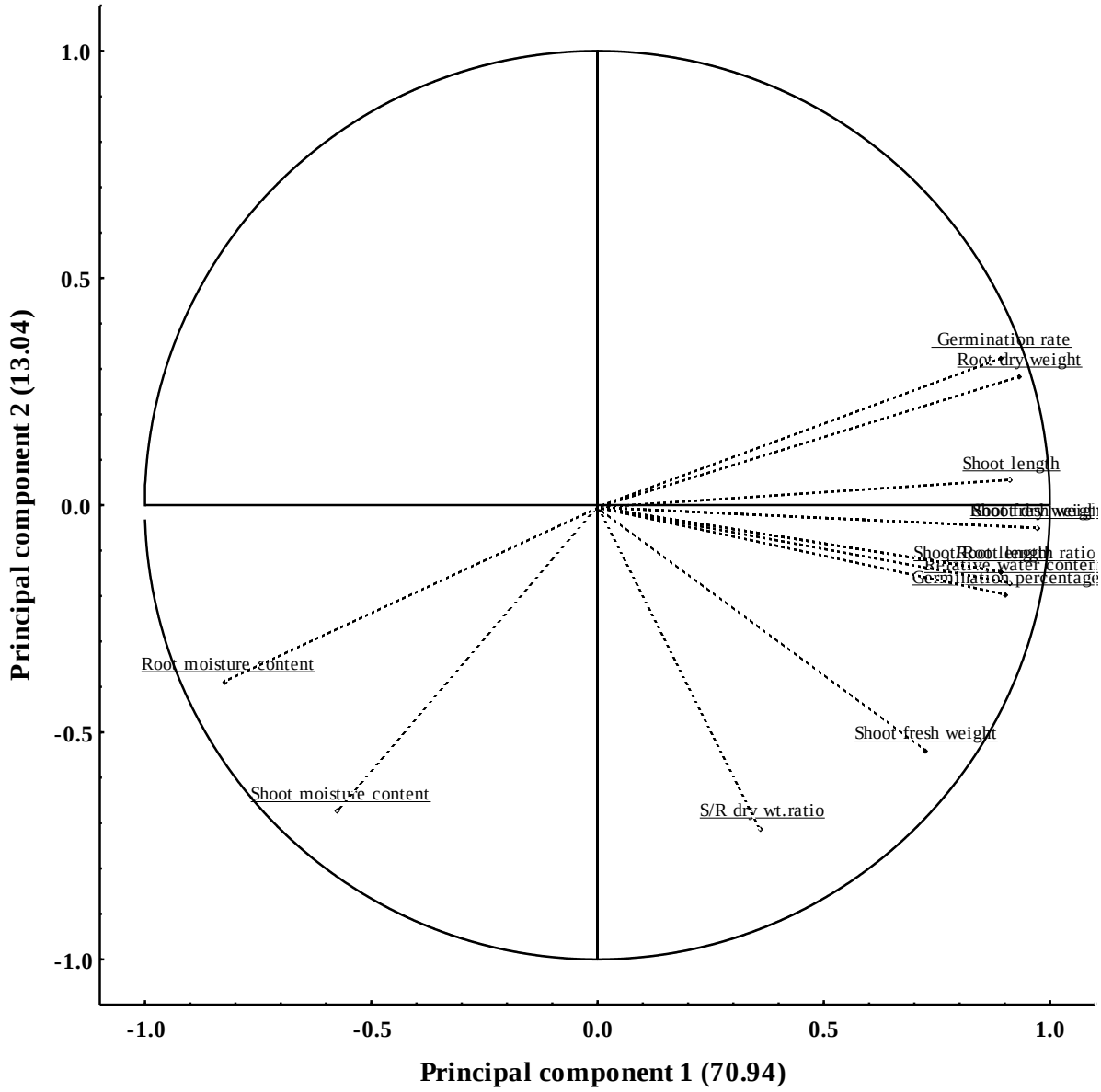


Figure 3: Projection of 13 variables on the factor-plane (1 × 2) for 11 selected tomato genotypes at varying levels (2.5%, 5.0% and 7.5%) under drought imposed by different concentration of PEG_{8000}.