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IMPACT OF POLYETHYLENE GLYCOL ON PROLINE AND MEMBRANE STABILITY INDEX FOR WATER STRESS REGIME IN TOMATO (*SOLANUM LYCOPERSICUM*)

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Abstract

Drought is one of the most important constraints worldwide for crop growth including tomato. It adversely affects germination and seedling that ultimately reduces crop development and economic yield. Polyethylene glycol (PEG) gives an indication to abiotic stresses and has been used throughout world in various crops for successful screening and breeding against stresses. Contrarily proline protects plant tissues against stress through preventing molecular denaturation, scavenges reactive oxygen species and interacts with phospholipids. Present paper presents the results on PEG and proline estimation in tomato. The PEG screening reduced the experimental material and finally 20 genotypes (6232, 6233, 6234, 10584, 10587, 17889, 17902, 17904, 19288, 19289, 19290, 19291, 19893, Avinash-2, Feston, Nagina, Punjab Chohara, Ratan and T-4) from diverse origin were investigated for proline estimation, chlorophyll contents and membrane stability index that gave a clear reference for drought tolerance in tomato. All the techniques (PEG, Proline, MSI) related to drought screening were employed and their interactive interpretation will enable us to design future breeding strategies for tomato development under drought that is still a dream for man. Among 20 genotypes, "19291" possessed the highest proline contents hence was tolerant to drought conditions, although needs verification under actual drought for adaptability and yield potential. High MSI under stress was observed for Punjab Chuhara, Chuhara, Avinash-2, Ratan, 19893, 19291 and 6233.

Key words: Tomato, Polyethylene glycol, Stress, Proline, Membrane stability.

Introduction

Tomato, *Solanaceae* plant is one of the world's most popular vegetables and is antioxidant in nature that could be categorized as health food (Oliveira *et al.,* 2013). In Pakistan, it is grown all over the country throughout year depending upon the climatic requirements and growth conditions (Bibi *et al.,* 2012). Tomato is susceptible to environmental stresses, including elevated saline conditions, water scarcity, high temperatures, excessive water conditions and mineral toxicity (Rick, 1990). Water scarcity adversely affect germination and seedling growth rates thus enhancing cell elongation sensitivity to damages induced under stressed conditions (Selote & Khana-Chopra 2004; Delachiave & Pinho, 2003; Nakashima *et al*., 2000). Many plants including tomato are water deficit susceptible and are affected almost at all stages of growth from seed germination to crop harvest and water deficiency is reported to severely affect the plant height, number of leaves plant^{-1} and fruit weight in tomato (Blum, 2011; Narusaka *et al*., 2003).

Polyethylene glycol (PEG) has been used as an osmoticum to induce water stress on plant tissues (Meneses *et al.,* 2011). The PEG molecules are too large to be absorbed by plant roots, thus increased PEG concentration in the surrounding medium causes outward movement of water from the plant cells (Mohammadkhani *et al.,* 2008). Thus plant cells undergo situations of water stress (Hamayun *et al.,* 2010). Under PEG induced water stress, resistant lines have been reported in tomato (Claussen *et al.,* 2005), with reduced shoot lengths in soybean (Sakthivelu *et al.,* 2008). Proline as an inert compatible osmolyte protects sub-cellular structures and macro-molecules under water stress conditions (Szabados & Savoure, 2009) and it is compatible osmo-protectant and osmolyte which accumulates largely under stress conditions (Seki *et al.*, 2007). Proline prevents molecular denaturation, scavenges reactive oxygen species and interacts with phospholipids (Kavikishor & Sreenivasulu, 2014). Amino acids involving proline, choline, glycinebetaine are the essential osmo-protectants against drought stress (Kavikishor *et al*., 2005). Declined water conditions cause reduced cell water potential and reduced chlorophyll contents (Ueda *et al.,* 2001; Kidokoro *et al*., 2009). Cell membrane rupturing is also the result of declined water conditions causing declined sustainability and reduced osmotic potential (Blokhina *et al.,* 2003).

Tomato is reported to carry limited genetic variability for drought tolerance (Kwon *et al.,* 2009). The best way to cope with the effects of drought stress involves the use of genetically drought tolerant genotypes for crossing with commercial cultivars for the development of tomato hybrids or pure lines for commercial cultivation under drought conditions (Pena & Hughes, 2007; Cazares *et al.,* 2011). Keeping in view the emerging issues of water scarcity and losses caused by drought, the current study was initiated with the aim to screen and identify tomato germplasm that could survive under water deficit regime. Tomato is not possible to cultivate under water stress areas and the screening of the germplasm is expected to identify the tomato lines suitable for cultivation under low water requirements and for developing tomato cultivars tolerant to water stress. The study reports the evaluation of tomato germplasm for drought tolerance and explains the phenomenon involved in the prevalence of drought based on PEG, proline contents and membrane stability index.

Materials and Methods

Screening of tomato genotypes against PEG induced water stress: The study was conducted at Seed Preservation Laboratory, Plant Genetic Resources Institute (PGRI), NARC (33° 33' N and 73° 06'E), Islamabad during 2011-2012. Sixty seven genotypes of tomato were obtained from the genebank, PGRI, NARC and are listed in the Table 1.

Table 1. Mean values of tomato genotypes regarding germination%, root length (cm), and shoot length (cm) and fresh weight (g) under control and stress conditions induced by PEG.

Accession	Source	Germination %	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Proline content	Chlorophyll content	Membrane stability index
004458	Pakistan	25.00 ± 6.34	2.88 ± 0.98	2.31 ± 0.78	0.11 ± 0.03	-	-	-
004463	Pakistan	48.33 ± 3.33	5.92 ± 0.99	3.14 ± 0.33	0.28 ± 0.02	-	-	-
004465	Pakistan	17.50 ± 4.67	6.59 ± 1.35	2.55 ± 0.31	0.13 ± 0.03	-	-	-
004551	Unknown	40.00 ± 9.53	6.73 ± 1.42	3.34 ± 0.80	0.26 ± 0.04	-	-	-
006231	Pakistan	77.50 ± 2.89	7.35 ± 0.54	4.23 ± 0.21	0.30 ± 0.05	-	-	-
006232	Pakistan	79.16 ± 6.22	8.14 ± 0.58	4.06 ± 0.17	0.29 ± 0.01	125 ± 6.27	1.07 ± 0.06	18.1 ± 3.44
006233	Pakistan	82.50 ± 5.77	8.13 ± 0.39	4.75 ± 0.18	0.40 ± 0.03	113 ± 9.91	1.18 ± 0.01	40.9 ± 1.30
006234	Pakistan	85.00 ± 3.84	7.61 ± 0.41	4.51 ± 0.22	0.30 ± 0.02	74.1 ± 1.46	0.73 ± 0.06	16.2 ± 1.17
10307	Pakistan	87.50 ± 3.84	7.21± 0.83	3.54 ± 0.27	0.30 ± 0.01	-	-	-
10482	Pakistan	92.50 ±2.50	6.87 ± 0.41	3.59 ± 0.29	0.29 ± 0.01	-	-	-
10573	North Korea	45.83 ± 3.87	4.11 ± 0.60	2.18 ± 0.19	0.18 ± 0.07	-	-	-
10574	North Korea	52.50 ± 5.47	6.28 ± 0.47	3.40 ± 0.21	0.18 ± 0.05	-	-	-
10584	North Korea	73.33 ± 5.84	6.98 ± 0.42	4.18 ± 0.43	0.30 ± 0.03	96.3± 6.96	0.96± 0.02	21.1± 5.80
10585	North Korea	53.33 ± 10.68	8.39 ± 1.20	4.75 ± 0.36	0.32 ± 0.03	-	-	-
10587	North Korea	75.83 ± 5.83	7.51 ± 0.47	4.72 ± 0.36	0.31 ± 0.01	79.8± 2.25	0.65± 0.01	14.2± 1.33
10588	North Korea	65.83 ± 6.00	6.73 ± 0.39	3.88 ± 0.26	0.30 ± 0.01	-	-	-
10589	North Korea	51.67 ± 8.18	6.33 ± 0.45	3.96 ± 0.31	0.31 ± 0.05	-	-	-
10592	North Korea	23.33 ± 3.33	5.97 ± 0.96	2.75 ± 0.79	0.16 ± 0.04	-	-	-
10974	India	78.33 ± 4.65	6.41 ± 0.36	3.10 ± 0.25	0.32 ± 0.03	-	-	-
17857	Unknown	20.83 ± 4.17	5.58 ± 0.56	3.65 ± 0.70	0.10 ± 0.02	-	-	-
17860	Pakistan	50.83 ± 4.65	5.16 ± 0.60	2.75 ± 0.19	0.22 ± 0.03	-	-	-
17876	Unknown	56.67 ± 7.96	6.45 ± 0.65	3.13 ± 0.27	0.27 ± 0.03	-	-	-
17882	China	49.16 ± 7.45	4.84 ± 0.29	2.31 ± 0.12	0.17 ± 0.04	-	-	-
17883	Taiwan	52.50 ± 5.77	4.17 ± 0.20	3.08 ± 0.17	0.18 ± 0.03	-	-	-
17889	Taiwan	56.66 ± 11.23	7.96 ± 0.77	3.36 ± 0.15	0.30 ± 0.04	71.0± 2.58	0.79± 0.10	28.6±3.89
17890	Taiwan	61.66 ± 6.53	5.77 ± 0.94	2.74 ± 0.18	0.23 ± 0.03	-	-	-
17902	Taiwan	76.66 ± 4.70	7.98 ± 0.27	3.45 ± 0.32	0.30 ± 0.02	118± 6.17	0.55± 0.01	29.7± 2.20
17903	Taiwan	80.83 ± 7.15	6.09 ± 0.36	2.77 ± 0.28	0.15 ± 0.03	-	-	-
17904	Taiwan	71.66 ± 3.65	7.13 ± 0.29	3.71 ± 0.30	0.26 ± 0.04	136± 1.09	0.54± 0.02	28.1± 1.63
19288	Pakistan	95.00 ± 3.04	7.33 ± 0.41	4.56 ± 0.23	0.34 ± 0.01	137± 1.70	0.89± 0.12	23.2± 3.57
19289	Pakistan	80.00 ± 3.94	7.73 ± 0.61	3.95 ± 0.34	0.39 ± 0.02	141± 5.11	0.95± 0.03	21.4± 3.10
19290	Pakistan	70.83 ± 6.04	7.77 ± 0.37	3.19 ± 0.32	0.31 ± 0.07	145± 2.49	1.11± 0.08	12.6± 0.78
19291	Pakistan	89.16 ± 3.11	6.22 ± 0.31	3.95 ± 0.25	0.20 ± 0.03	292± 71.24	0.86± 0.05	14.9± 3.04

Table 1. (Cont'd.).

Accession	Source	Germination %	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Proline content	Chlorophyll content	Membrane stability index
19292	Pakistan	75.00 ± 5.00	7.50 ± 0.61	3.19 ± 0.09	0.33 ± 0.03	-	-	-
19299	Pakistan	48.33 ± 5.90	3.20 ± 0.31	2.99 ± 0.25	0.17 ± 0.05	-	-	-
19887	El Salvador	56.67 ± 1.67	5.63 ± 0.34	2.72 ± 0.31	0.20 ± 0.03	-	-	-
19889	Unknown	55.00 ± 13.79	6.44 ± 0.37	3.21 ± 0.10	0.29 ± 0.04	-	-	-
19890	Czech	54.17 ± 6.52	5.00 ± 0.34	1.82 ± 0.17	0.32 ± 0.10	-	-	-
19891	Poland	36.67 ± 4.17	5.24 ± 0.54	2.78 ± 0.25	0.36 ± 0.11	-	-	-
19892	UK	70.83 ± 2.20	6.12 ± 0.66	2.60 ± 0.38	0.46 ± 0.12	-	-	-
19893	Nepal	77.50 ± 5.00	7.88 ± 0.78	5.14 ± 0.35	0.32 ± 0.04	111± 6.55	0.79± 0.02	39.6± 8.66
19894	Hungary	45.83 ± 12.3	5.22 ± 0.90	4.13 ± 0.82	0.20 ± 0.06	-	-	-
19895	Unknown	70.00 ± 3.94	4.28 ± 0.88	3.55 ± 0.55	0.17 ± 0.03	-	-	-
19896	Unknown	82.50 ± 5.21	5.13 ± 0.23	4.40 ± 0.30	0.13 ± 0.01	187± 91.60	1.01± 0.07	30.9± 5.33
19897	Unknown	63.33 ± 8.90	3.55 ± 0.20	3.75 ± 0.18	0.12 ± 0.01	-	-	-
19898	Unknown	61.67 ± 8.21	5.07 ± 0.30	3.71 ± 0.20	0.19 ± 0.02	-	-	-
19899	Unknown	24.17 ± 5.49	5.24 ± 0.57	4.70 ± 0.51	0.08 ± 0.04	-	-	-
19900	Ecuador	20.00 ± 10.47	2.75 ± 0.86	4.10 ± 1.71	0.09 ± 0.04	-	-	-
19901	Ecuador	29.17 ± 6.08	5.66 ± 0.76	3.51 ± 0.53	0.18 ± 0.03	-	-	-
19902	Peru	32.50 ± 7.64	5.90 ± 0.49	3.27 ± 0.44	0.22 ± 0.02	-	-	-
19903	Unknown	51.67 ± 5.50	3.63 ± 0.20	2.88 ± 0.34	0.24 ± 0.04	-	-	-
19904	Thailand	51.67 ± 6.84	5.34 ± 0.24	3.42 ± 0.17	0.36 ± 0.04	-	-	-
19909	USA	67.50 ± 2.50	5.07 ± 0.34	3.03 ± 0.22	0.30 ± 0.06	-	-	-
4526(3)	Unknown	49.17 ± 5.09	5.29 ± 0.77	2.66 ± 0.35	0.60 ± 0.22	-	-	-
Avinash-2	Nepal	72.50 ± 3.04	6.36 ± 0.47	3.56 ± 0.24	0.37 ± 0.02	76.7 ± 6.65	1.26 ± 0.03	43.9 ± 6.58
Feston	Italy	87.50 ± 2.50	7.53 ± 0.45	3.95 ± 0.17	0.41 ± 0.03	98.3 ± 4.04	1.23 ± 0.00	47.2 ± 8.55
Indian	Pakistan	77.50 ± 3.82	6.79 ± 0.51	3.51 ± 0.30	0.39 ± 0.02	-	-	-
LO2692	Pakistan	51.67 ± 8.46	4.76 ± 0.53	2.25 ± 0.22	0.14 ± 0.02	-	-	-
Nagina	Pakistan	73.33 ± 5.50	6.42 ± 0.54	2.71 ± 0.20	0.30 ± 0.01	128 ± 16.02	0.65 ± 0.01	40.7 ± 4.48
Punjab Chuhara	Pakistan	87.50 ± 3.84	8.31 ± 0.36	3.95 ± 0.17	0.41 ± 0.01	134.7 ± 5.72	0.51 ± 0.05	38.8 ± 2.26
Ratan	Pakistan	88.33 ± 3.84	7.07 ± 0.24	3.77 ± 0.17	0.38 ± 0.01	149.5 ± 19.95	1.07 ± 0.02	41.8 ± 3.21
Roma	Pakistan	64.17 ± 4.78	7.41 ± 0.38	3.61 ± 0.19	0.43 ± 0.03	-	-	-
T-4	Pakistan	85.00 ± 3.82	5.67 ± 0.16	3.21 ± 0.14	0.28 ± 0.05	89.6 ± 9.80	1.05 ± 0.03	50.57 ± 1.05
Tom Round	Pakistan	39.17 ± 4.41	6.05 ± 0.89	2.70 ± 0.27	0.24 ± 0.07	-	-	-
Walter	Japan	65.83 ± 6.31	5.43 ± 0.70	3.41 ± 0.49	0.33 ± 0.02	-	-	-
Money Maker	Pakistan	73.33 ± 7.27	5.81 ± 0.42	2.81 ± 0.18	0.32 ± 0.03	-	-	-
Kuri Hara	Pakistan	70.83 ± 5.24	4.50 ± 0.12	2.62 ± 0.11	0.26 ± 0.03	-	-	-

PEG assay: Initially PEG concentration was optimized through a series of experiments that included a range from 2% to 12%, and 4% be observed optimum for screening of tomato germplasm, and similar concentration has been used by (Kulkarni and Deshpande, 2007). The germplasm was investigated against induced water stress using PEG-6000 at 4%. Germination test was conducted according to ISTA rules using double sheets of paper towels (22 cm x 23 cm; Victory brand, Shinbashi Paper Company, Shizuoka, Japan) for seedling growth (ISTA, 1993). Fifty seeds of each tomato genotype were spread on paper towel in rows while the other sheet was kept aside, distilled water was applied to moisten the sheet until it was thoroughly damped for the control treatment, whereas 4% PEG-6000 solution was used for the treated genotypes. The second towel sheet was then carefully placed on the first paper towel, leaving the seeds sandwiched between the two towels. The two sheets with the seeds in-between were then rolled up and placed in an erect position in a plastic beaker that retained the left over moisture. The beaker was then covered with polythene bag and placed in an incubator under controlled conditions of light and temperature (25±1°C). After ten days period, towels were removed from incubator and unwrapped carefully so that fragile seedlings couldn't break and the data were recorded for germination percent, seedling vigour, root and shoot lengths (cm) and seedling fresh weight (g).

Biochemical analyses: Out of sixty seven tomato genotypes, twenty genotypes (006232, 006233, 006234, 10584, 10587, 17889, 17902, 17904, 19288, 19289, 19290, 19291, 19893, Avinash-2, Feston, Nagina, Punjab Chohara, Ratan and T-4) with better performance against PEG simulated osmotic stress were further analyzed for proline contents, chlorophyll contents and MSI against water stress induced at flowering stage. The plants evaluated for these characters were grown in pots and kept under controlled conditions in the greenhouse.

Water stress ınduction: Each genotype was transplanted into twenty pots, ten of these were kept as control, whereas remaining were induced for water stress by stopping irrigation after flower induction in them. All the plants were irrigated normally until flower induction stage. The day after flowering started, normal irrigation was continued for control plants while stopped in the remaining pots. When leaf curling/wilting started in stressed plants, irrigation was resumed prior to permanent wilting. Once the plants resumed normal growth, were again induced water stress and the process was repeated three times. After completion of stress induction phase sampling of control as well as treated plants was conducted for biochemical analyses.

Proline contents for both the control and stressed leaves were determined by the method explained by Bates *et al.* (1973) with modifications in which samples were kept suspended in sulfosalicylic acid over night instead of grinding them in sulfosalicylic acid (Liu *et al.*, 2013). From control plants, fully expanded fresh green leaves were sampled for proline estimation while from stressed genotypes sample of curled and drooped leaves was collected. From control plants, fresh leaves (unstressed) were selected, while from stressed genotypes curled leaves were selected to make a comparison. The samples were collected in air tight polythene bags and were carried by placing them in ice to avoid biochemical degradation. The leaf samples within each genotype were cut and mixed randomly from top, bottom, lateral and center of the branches. Ninhydrin reagent was prepared by warming 1.25g ninhydrin powder in 20ml phosphoric acid (13ml of phosphoric acid powder and 7ml of distilled water) and 20ml glacial acetic acid, agitated till it dissolved completely and stored at 4°C. The reagent remains stable for 24 hours. Leaf samples (0.1g) were added to 10ml of 3% Sulfosalicylic acid in the test tubes and kept overnight at room temperature. Two ml of the leaf filtrate with 2ml of phosphoric acid and 2ml of ninhydrin reagent were heated together in water bath at 100°C for 1hour, and then reaction was terminated in an ice bath. The reaction mixture was extracted by adding 4ml of toluene to all the test tubes, mixed thoroughly by stirring at vortex mixture for 5-10 seconds. The two separate layers carried toluene containing chromophore at top. This chromophore was collected and absorbance was read at 520nm in a spectrophotometer, using toluene as a blank. The proline value was determined by using the following formula:

$$\mu\text{m olesproline/g of fresh weight} = \frac{\text{Absorbance x (35) x (10)}}{\text{Wt. of sample taken (g)}}$$

For chlorophyll estimation, leaf samples from both control and stressed plants were collected in the similar way as collected for proline estimation. Approximately 0.1g of leaf sample was added in test tubes containing 10ml of 80% ethanol, and then agitated vigorously by stirring at vortex mixture for 5-10 seconds followed by heating for 3-5 minutes at 100°C in water bath in order to get chlorophyll extract thoroughly. Reading of the solution extract obtained was determined at 666nm using spectrophotometer (Arnon *et al*., 1949). Chlorophyll content was determined using the following formula:

$$\text{Chlorophyll Content (mg/fresh weight g)} = (\text{Absorbance} - 0.01) \times 1 / 92.6474 \times 10 / \text{F. Wt.(g)}$$

The MSI was measured using 0.1g of leaf sample in two sets of test tubes containing 10ml of distilled water. Test tubes of one set were heated in water bath at 40°C for 30 min and electrical conductivity of the water containing the sample was measured as C1. Test tubes of second set were heated in water bath at 100°C for 15 min and electrical conductivity of the water containing the sample was measured as C2 (Sairam *et al.*, 1994). Reading of MSI was determined by the following formula:

$$MSI = [1 - (C1/C2)] \times 100$$

Results and Discussion

Among 67 genotypes, twenty indicating preliminary tolerance to drought and grouping in various clusters were further analyzed for biochemical analysis, *i.e.,* proline, chlorophyll contents and membrane stability index (MSI). Germination ranged from 17.5% to 92.5% and was significant for genotype × treatment interaction that indicated the altered abilities of tomato genotypes to stress conditions which might be attributed to consequence of genetic differences (Des-Marais *et al.,* 2013).The maximum root length (8.39 cm) was exhibited by the genotype 10585, whereas reduced root length was produced by the genotype 19900 (2.75 cm) as the results presented in the Table 1. The osmotic stress induced by PEG indicated significant differences for fresh weight for genotypes, whereas treatments and genotype × treatment interaction were insignificant. Significant differences for all the traits indicated the genetic dissimilarities of genotypes sampled from the germplasm, however treatment did not affect the germination and fresh weight that indicated the extent of heritability for these traits (Table 2).

Biochemical Analyses: Tomato genotypes were significantly different for proline contents, and the maximum proline accumulation was observed in the genotype "19896", whereas the lowest was in the genotype "6234" (Fig. 1). The proline accumulation has the linearity to osmotic stress (Ghorbanli *et al.,* 2012). The declined proline values have also been reported in Al-gaimi cultivar of wheat (Akhkha *et al.,* 2011) and in tomato (Claussen *et al.,* 2005). Elevated proline content under drought stress is proposed to maintain plant existence and cell water level that has been explained by Ghorbanli *et al.* (2012), and thus preventing plants from damages caused by drought conditions (Pirzad *et al.,* 2011). Proline acting as an osmotic subsists water particulars, stores carbon and nitrogen after water stress recovery and stabilizes macromolecule, proteins and cell membranes in plant tissues (Farooq *et al.,* 2009). Kumar *et al.* (2011) proposed proline as an essential osmolyte under water stress situations where stressed plants withstand drought conditions due to activation of free proline amino acids. The increased proline contents were observed in our study and similar findings have been reported by Javed and Ikram *et al.,* 2008 and Yamada *et al.,* 2005 who reported increased proline under drought stress.

Tomato genotypes responded variably for chlorophyll contents under stressed conditions and most of the treated genotypes showed higher levels of chlorophyll as compared to control. Higher chlorophyll contents under stress conditions were found in the genotypes, 19290, 10584, Punjab Chuhara, 19288, 17904, 6232, 19289, 17902, Ratan, 6233, 19893, 19896, Feston, 10587, 6234 and Nagina (Fig. 1). The relative increase in chlorophyll content was 271.34% in 19290, whereas 107.02% was observed in Nagina (Table 3). Declined chlorophyll contents tend to reduce photosynthetic rates, rendering plants to damages under stress conditions (Herbinger *et al.,* 2002). Decreased chlorophyll under water stress generally occurs due to damage of chloroplasts caused by oxidative bursts or due to changed ratios of lipid protein complexes or elevated chlorophyllase activity which degrades chlorophyll and damages light harvesting machinery (Kaya *et al.,* 2006). In contrast plants showing increased chlorophyll values are considered to be drought tolerant (Dhanda *et al.,* 2004). Elevated chlorophyll contents have also been reported by Akhkha *et al.,* 2011 in Al-gaimi cultivar of wheat and sesame (Mensha *et al.,* 2006), respectively.

Tomato genotypes exhibited different behavior regarding MSI both for control and treated ones. The stressed genotypes showing higher values for MSI included Punjab Chuhara, Avinash-2, Ratan, 19893, 19291 and 6233, respectively (Fig. 1). Relative MSI values ranged between 107.61% to 169.75% in 6233 and Punjab Chuhara, respectively (Table 3). Under stress conditions, cell membrane rupturing is increased resulting in declined sustainability and reduced osmotic potential. Drought conditions also lead towards membrane cleavages decreased osmotic adjustments and cytoplasm content depositions (Blokhina *et al.,* 2003). Declined membrane sustainability indicates the extent of lipid peroxidation which results in large amounts of oxidative bursts under water deficit conditions, whereas contrary to this plants ability to maintain MSI with water retention indicates plant tolerance (Blackman *et al.,* 1995).

Cluster analysis

Control genotypes: Hierarchical tree for the genotypes under control indicated four groups at one third dissimilarity the same distance as for treated experiment (Fig. 2A). The Group-A consisted 28 genotypes, the Group-B consisted five genotypes, the Group-C of 15 genotypes, where eighteen genotypes were grouped together in the Group-D. Among twenty genotypes, accessions Feston and Avinash-2 were present at far off distance indicating highest dissimilarity with each other whereas closely related genotypes were Ratan and Punjab Chuhara; 19289 and 6233; 10587 and 6232. Group-B was the smallest one including 5 genotypes while 18 genotypes were present in Group-D. None of the genotypes from twenty tolerant accessions were present in Group-B and D. In Group-C, accessions T-4 and 10584 were tolerant ones present at a distance indicating dissimilarity with each other, whilst genotypes showing similar behavior were 19896, 19291 and 17904.

Table 2. Mean square values from ANOVA table for germination%, root length (cm), shoot length (cm) and fresh weight (g) of tomato genotypes grown under PEG induced water stress

S.O.V	Df	Germination %	Root length (cm)	Shoot length (cm)	Fresh weight (g)	Proline content	Chlorophyll content	Membrane stability index
Variety	66	2394.00**	741.68**	203.92**	0.059**	14698.0**	0.354**	876.57**
Treatment	1	149.316^{ns}	204.05**	4.30**	0.002^{ns}	2314.4**	1.008**	3050.21**
Variety × Treatment	66	206.76**	165.31**	1.05**	0.011^{ns}	23846.4**	0.127**	528.62**
Error	268	130.84	354.05	0.64	0.009	1278.4	0.004	21.43
C.V%		18.57%	18.90%	23.39%	34.51%	28.53%	7.41%	15.37%

**Highly significant difference at 0.01 probability level

^{ns}Non significant difference

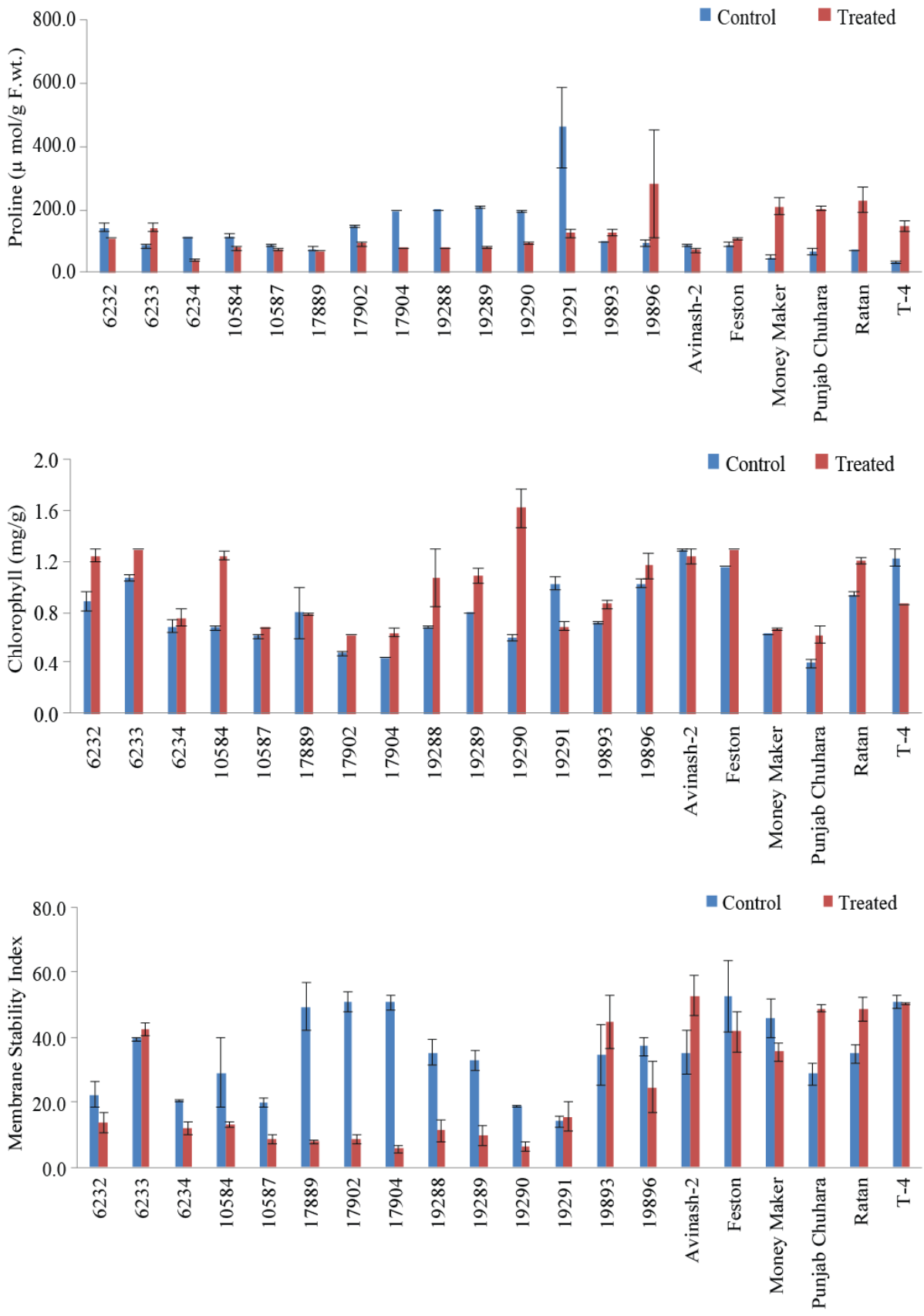

Fig. 1. Proline, chlorophyll and Membrane stability index of tomato genotypes under control and treated conditions.

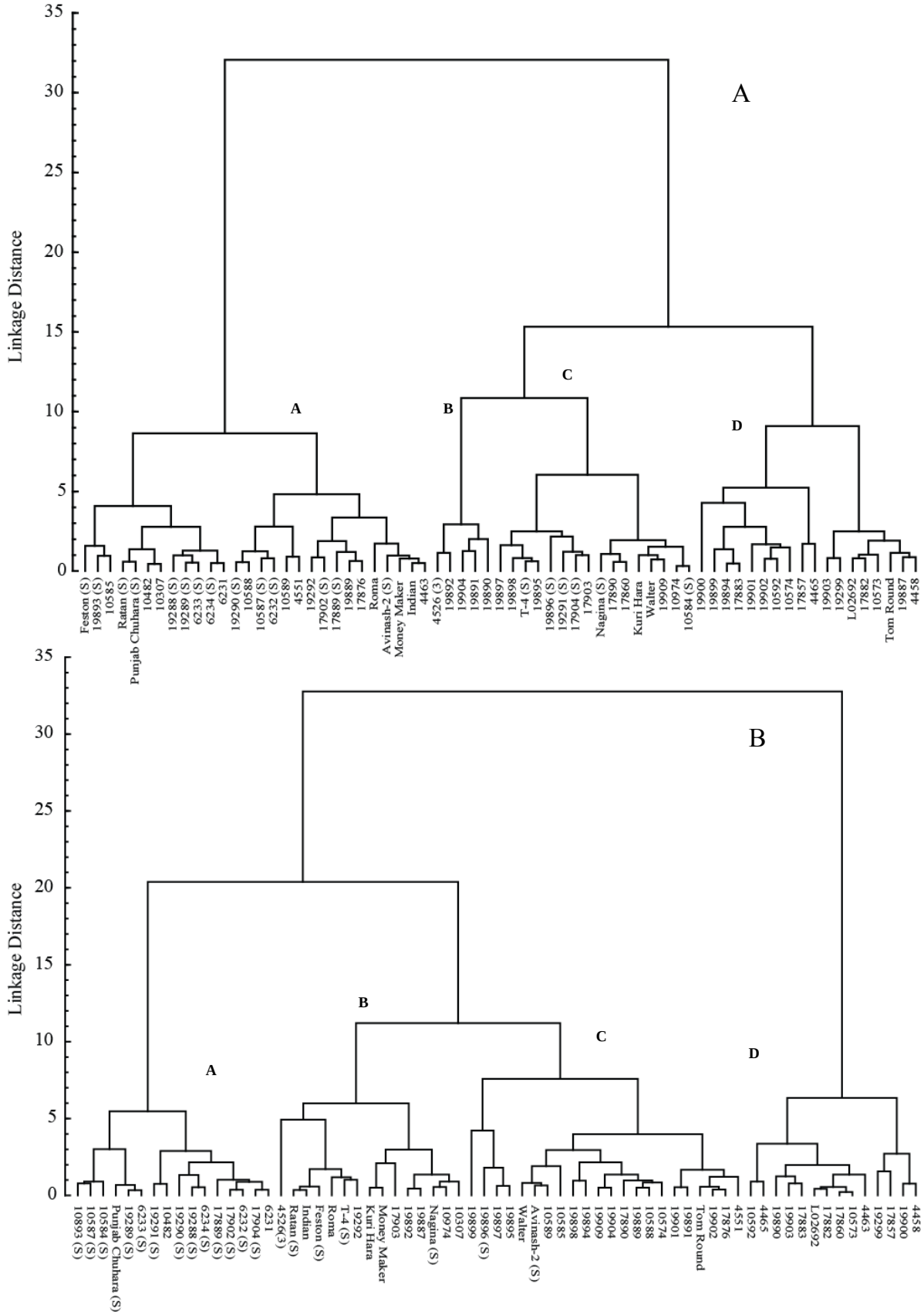

Fig. 2. Cluster analysis of tomato genotypes under (A) Control and (B) PEG conditions.

Table 3. Relative performance of tomato genotypes regarding proline, chlorophyll and MSI.

Genotypes	Relative proline content (%)	Relative chlorophyll content (%)	Relative membrane stability ındex (%)
6232	76.28	140.57	61.87
6233	164.39	121.02	107.61
6234	34.53	109.48	59.48
10584	65.80	182.10	45.18
10587	85.95	110.32	44.22
17889	89.31	97.72	15.79
17902	61.45	130.91	16.57
17904	39.45	144.79	11.02
19288	38.93	155.26	31.96
19289	38.09	135.86	29.58
19290	48.14	271.34	34.38
19291	26.83	67.18	109.97
19893	130.73	119.94	129.43
19896	559.84	113.38	65.95
Avinash-2	82.50	95.79	148.87
Feston	121.46	111.19	79.29
Money Maker	439.02	107.02	77.12
Punjab Chuhara	309.81	158.95	169.75
Ratan	327.00	127.19	138.31
T-4	468.89	70.02	98.66

Treated genotypes: Cluster analysis separated the genotypes into four major groups at the same distance as for control experiment (Fig. 2B). The Group-A consisted of 16 genotypes, out of which 14 were amongst the best selected genotypes with good performance under drought conditions induced by PEG. It depicted major variance attributing towards traits related to PEG and drought tolerance. Thus Group-A could be considered as best cluster containing tolerant genotypes. Group-B consisted of 15 genotypes, Group-C 22 genotypes and the Group-D 14 genotypes. Maximum dissimilarity existed between 19893 and Avinash-2 accessions from Group-A and Group-C, respectively. It was observed that the genotypes in Group-D were more likely grouped on the basis of sensitivity to drought based on PEG and thus suitable to grow under irrigated conditions, whereas genotypes each from Group-B and Group-C included 3 (Ratan, Feston and T-4) and 2 (19896 and Avinash-2) drought tolerant genotypes, respectively.

Grouping patterns have been observed under drought conditions induced by PEG in canola cultivars by Shahverdikandi *et al.* (2011) who reported one group consisting of tolerant lines than rest of the groups. However in potato crop grown under PEG simulated water stress, grouping pattern has been observed for tolerant, moderate and susceptible genotypes (Hassanpanah, 2010). Cluster analysis revealed drought tolerant and susceptible genotypes in many other crops including sunflower (Saensee *et al.,* 2012), sorghum (Ali *et al.,* 2011), wheat (Farshadfar *et al.,* 2013), Durum Wheat (Khayatnezhad *et al.,* 2011) and rice (Abarshahr *et al.,* 2011). The present study highlights the diversity in tomato for water deficit regimes and the tolerant genotypes are likely to incorporate in the development of pure lines org the hybrid tomato with combination of yield potential and drought tolerance.

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