Research Article

Screening of Tomato (*Lycopersicon esculentum* Mill.) Germplasm for Chilling Stress Tolerance during Two Growing Seasons

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Abstract | Forty-five tomato (*Lycopersicon esculentum* Mill.) lines were evaluated for chilling tolerance using morpho-biochemical parameters. Experiment was performed in polytunnel conditions (30±2 °C) as control and open field conditions (10±2 °C) as chilling stress. After transplanting tomato plants were divided into controlled and chilling stressed. Chilling stressed set was placed in open field to apply stress while control treatment was kept in polytunnel. All the genotypes showed significant variability in cold tolerance during two growing seasons. A significant reduction in plant height, setting %age and fruits per plant was observed in sensitive genotypes. Antioxidant enzyme essay showed enhanced production of Superoxide Dismutase and Catalase coupled with higher accumulation of proline in tolerant genotypes while their production was lower in sensitive genotypes during two growing seasons under stress. All the genotypes made two major clusters. Main cluster "I" was comprised of two genotypes. Main cluster "II" was comprised of 43 genotypes. Cluster "II" was divided into A and B. "B" contained 10 genotypes while "A" was 35 genotypes. Finally, forty-five genotypes of tomato were divided into seven clusters in such a way that all the genotypes within the cluster had smaller D^2 values among themselves than those belonging to different clusters. The tolerant genotypes made cluster with each other showing maximum similarity among their genetic makeup. Among all the parameters, electrolyte leakage seemed to be more authentic parameter for screening of genotypes because it shows direct effect of stress on cellular membranes. It is recommended that this method can be employed to shortlist genotypes on cost effective basis.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important crop of high economic value grown in tropical and sub-tropical areas of the world. It has antioxidant and anti-cancer properties due to the presence of lycopene in its fruit. Due to rise in population, demand for tomato is constantly increasing worldwide (Raiola *et al*., 2014). Being tropical plant, tomato is well-adapted to almost all climatic zones of the world; however, the environmental stresses are primary limitations of yield potential. Chilling

stress is a detrimental abiotic stress that affects fruit quality and yield of most of tropical and sub-tropical plants which exhibit distinct symptoms of damage when exposed to temperatures below zero. During the last three decades, climate models are predicting extreme changes in temperature ranging either very low or extremely high (Hatfield and Prueger, 2015). These unexpected spikes in temperature are putting agriculture and food security under threat. It is reported that sensitive plants exposed to chilling stress show adverse changes in physiological and biochemical pathways that may be associated to genetic makeup of a genotype and its ability to acclimatize in adverse conditions (Hussain *et al*., 2018). Genotypes behave differently when exposed to stress due to genetic variability and acclamation also depends on number of exposures plants exposed to stress. More number of exposures develops stress memory in plants that help them to cope stress epigenetically (Raison, 1974). Temperatures below 16.8°C have been shown to inhibit vegetative growth and reduce net photosynthesis, in addition, irregular fruit formation leading to poor yields (Kuden, 2020). In plant root tissues, cold temperatures may impair root elongation and cause shell breakdown, as well as changes in metabolic rates and water conduction. In addition, cooling stress usually leads to increased electrolyte leakage, which is associated with disturbance of membrane integrity (Herner, 1990). Numerous experimental evidence indicated that exposure to lower positive temperatures often induced structural changes in membrane lipids, which were associated with adverse symptoms seen in plant tissues subjected to oxidative stress (Kissoudis *et al*., 2015).

It is previously reported that Abdul-Baki (1990) worked on heat tolerance of selected tomato cultivars and germplasm lines. Some other researchers such as Shaheen *et al*. (2015) worked on Morpho-physiological evaluation of tomato genotypes under high temperature stress conditions. Similarly, Hasan *et al*. (2009) worked on the Screening of tomato (Lycopersicon esculentum) cultivars against cadmium through shotgun approach and Alsamir *et al*. (2017) research work was reported on Morpho-Physiological Traits Linked to High Temperature Stress Tolerance in Tomato. Furthermore, Seyie *et al*. (2015) also worked on Screening of tomato genotypes for vegetative and reproductive characters under low temperature regime.

After going through literature review it was observed that much work has been done in the area in one and another form but could not find the specific work for screening of tomato (*Lycopersicon esculentum* Mill.) germplasm for chilling stress tolerance using morpho-biochemical parameters.

Chilling stress is one of the most common environmental stresses affecting plant growth and agricultural production. Chilling stress severely affects the growth, development and productivity of tomato crop. For this purpose, this study was conducted to evaluate forty-five tomato (*Lycopersicon esculentum* Mill.) germplasm for chilling stress tolerance using morpho-biochemical parameters with the aim of improving tomato tolerance to low temperature.

Materials and Methods

Forty-five genotypes of tomato were collected from Plant Genetic Resources Institute (PGRI) and Vegetable Crops Research Program, National Agricultural Research Centre (NARC) Islamabad and screened for chilling stress during winter (December to March) 2018-19 and 2019 at NARC, Islamabad, Pakistan. Tomato germplasm with their descriptive code is presented in Table 1.

Table 1: *Germplasm of tomato and their coding.*

Germplasm	Code	Germplasm	Code
A17857	L1	A19297	L24
A17860	L2	A19842	L25
A17864	L3	A19844	L26
A17865	L4	A19852	L27
A17872	L5	A19853	L28
A17873	L6	A19855	L29
A17874	L7	A19856	L30
A17876	L8	A19860	L31
A17877	L9	A19873	L32
A17878	L10	A19887	L33
A17879	L11	A19888	L34
A17880	L12	A19889	L35
A17882	L13	A19890	L36
A17885	L14	A19891	L37
A17889	L15	A19892	L38
A17890	L16	A19893	L39
A17902	L17	A19897	L40
A19288	L18	A19898	L41
A19289	L19	A19899	L42
A19290	L20	A19900	L43
A19291	L21	A19903	L44
A19292	L22	A19904	L45
A19293	L23		

Physico-Chemical analysis of soil was carried out before sowing and at harvest stage. Based on morpho-physiological traits, genotypes were evaluated for chilling stress tolerance. The seeds of tomato were sterilized in 95% ethanol for 3-4 min, soaked in 10% Chlorox for 2-3 min and subsequently washed 3-4 times with the sterilized water. After drying seeds were germinated in peat trays and 30 days after germination, seedlings were transplanted in earthen pots. Experiment was laid in completely randomized design (CRD) replicated thrice. Two temperature levels i.e. T_o control in polytunnel where temperature was maintained at (30±2° C day temperature) and T_1 as chilling stress (open field at 10±2° C day temperature). Tomato plants in their vegetative stage, twenty days after transplanting were divided into two sets viz. controlled and chilling stressed. Chilling stressed set was placed in open field for 2 weeks to apply stress while control treatment set was continuously kept in polytunnel. After subsequent treatment, data were recorded for different morphological and biochemical parameters. The different morphological parameters are plant height, fruit %age, number of fruits, root of fresh weight (RFW), root of dry weight (RDW), shoot of fresh weight (SFW), shoot of dry weight (SDW) and plant spread. Similarly, the different biochemical parameters used in this research study are superoxide dismutase (SOD), catalase (CAT) and proline content.

Superoxide dismutase (SOD) was determined following the method described by Beauchamp and Fridovich (1976) and catalase activity was performed by using the method used by Kumar *et al.* (2010) with some modifications.

Electrolyte leakage shows direct effect of stress on cellular membranes as described by Jennings and Saltveit (1994). Leaf samples from each genotype were collected in separate test tubes having 10mL de-ionized water, kept for 30 minutes and Electrical Conductivity (EC) of the solution recorded with Electrical Conductivity meter. After recording initial Electrical Conductivity, tubes were autoclaved and EC2 was recorded. Electrolyte leakage was expressed as μmho g^{-1} (FW).

Proline was measured by following Cross *et al.* (2006) and Carillo *et al.* (2008) based on the following formula.

µmoles per gram tissue = [(µg proline/ml) x ml toluene)/115.5 µg/µmole] / [(g sample)/5]

Collected data were subjected to analysis of variance technique as outlined by Steel *et al.* (1997), least significant and mean comparison test between normal and stressed conditions to find the difference among different variables and their effects.

Results and Discussion

At the start of experiment, soil was analyzed for different characteristics and results are presented in Table 2.

Table 2: *Soil characteristics of the experimental field at NARC Islamabad.*

Soil variable	Critical value	Observed value
pH	5-7	6.8
CEC	25	42.1
Total N%	0.1	0.13
P-average (ppm)	25	12
K-cmol/kg	0.13-0.20	0.14
Ca (cmol/kg)	2	0.89
Mg (cmol/kg)	0.24	0.21
%OM	1.5	1.8
% sand		58.90
% clay		26.87
Textural class	Sandy loam	Sandy clay loam

Cation exchange capacity (CEC) and organic matter (OM) in the soil were found to be high in the soil used for experiment. Inorganic nutrient concentrations were in the range of critical limits while some of the nutrients were below the critical level. Significant variability for plant height, shoot of fresh and dry weight, root of fresh and dry weight, plant spread, and fruit percentage were found to be present among treatments and genotypes (Table 3) during successive years 2018. While plant height, shoot of fresh and dry weight, root of fresh and dry weight, plant spread, and fruit setting percentage were also recognized as significantly variable (Table 4) during season 2019 under normal and cold stress conditions. According to (Tables 5 and 6) results regarding plant height showed that genotypes L8, L14, L17, L20, L25, L26, L27, L28, L34 and L42 performed better under normal and stress treatments. While genotypes L2 and L21 were poor performer in normal as well as stress conditions in 2018-19 and 2019. Results about fruit set %age showed that genotypes L8, L14, L17, L20, L25, L26, L27, L28, L34 and L42 performed better under normal and stress treatments while genotypes L2

Table 3: *Mean squares of different traits in 45 tomato genotypes during 2018 under normal and cold stress.*

SOV	df	PH	SFW	SDW	RFW	RDW	PS	FS %age	F/P	SOD	CAT	Proline	Ion
Germplasm	44	62.1988**	67.56**	61.478**	16.681**	13.47**	65.388**	0.783*	35.8109**	0.02192*	0.00341*	0.05307*	28.90*
Treatments	1	2.1218**	2.3674ns	1.35647ns	1.908*	2.1934*	3.4687*	153.571**	0.7717*	0.00060*	1.111E-06ns	0.00318*	1362.91**
Error	44	30.3770**	56.4632	45.3785*	8.7913**	11.5467*	3.4767*	75.150**	4.6135*	0.00433*	9.498E-04ns	0.01030ns	1289.56*

SOV: sources of variations; df: degrees of freedom; PH: plant height; SFW: shoot of fresh weight; SDW: shoot of dry weight; RFW: root of fresh weight; RDW: root of dry weight; PS: plant spread; FS %age: fruit set percentage; F/P: fruit percentage; SOD: superoxide dismutase; CAT: catalase.

Table 4: *Mean squares of different traits in 45 tomato genotypes during 2019 under normal and cold stress.*

SOV	df	PH	SFW	SDW	RFW	RDW	PS	FS %age	F/P	SOD	CAT	Proline	Ion
Variety	44	63.6959**	62.06**	59.343**	14.691**	11.12**	56.393**	272.448**	35.8476*	0.02362*	0.00145ns	0.09930*	0.971*
Treatments	1	2.1218*	2.3674ns	1.35647ns	1.908*	2.1934*	2.267*	0.870ns	0.7211ns	0.00131ns	1.344E-06*	0.00177*	1498.90**
Error	44	28.879*	51.967*	39.3311*	7.343**	13.126*	6.4837*	256.842*	4.4441ns	0.003ns	4.4604ns	0.0107ns	1307.1*

** - P<0.05, ** - P<0.01 and n.s. - not significantly different at P=0.05. SOV: sources of variations; df: degrees of freedom; PH: plant height; SFW: shoot of fresh weight; SDW: shoot of dry weight; RFW: root of fresh weight; RDW: root of dry weight; PS: plant spread; FS %age: fruit set percentage; F/P: fruit percentage; SOD: superoxide dismutase; CAT: catalase.*

and L21 were poor performer in normal as well as stress conditions in 2018-19 and 2019. Although all genotypes showed decline in fruit setting percentage under chilling stress but mentioned genotypes showed small decrease compared with other tested genotypes. Results regarding Number of fruits showed that Genotypes L15, L19, L37 and L44 were poor performer during both seasons. Results regarding Superoxide Dismutase showed that Genotypes L15, L19, L37 and L44 were poor performer during both seasons. Genotypes L8, L14, L17, L20, L25, L26, L27, L28, L34 and L42 performed better under normal and stress treatments while genotypes L2 and L21 were poor performer in normal as well as stress conditions in 2018-19 and 2019. Although all genotypes showed increase in Superoxide Dismutase content under chilling stress but mentioned genotypes showed higher accumulation of Superoxide Dismutase content compared with other tested genotypes. Results regarding catalase showed that genotypes L8, L14, L17, L20, L25, L26, L27, L28, L34 and L42 performed better under normal and stress treatments while genotypes L2 and L21 were poor performer in normal as well as stress conditions in 2018-19 and 2019.

Results related to plant root and shoot traits i.e., root fresh weight, root dry weight, shoot fresh weight, shoot dry weight and plant spread showed that Genotypes L8, L14, L17, L20, L25, L26, L27, L28, L34 and L42 performed better under normal and stress treatments while genotypes L2 and L21 were poor performer in normal as well as stress conditions in 2018-19 and 2019 according to Tables 5 and 6.

Similarly, results regarding electrolyte leakage showed that it is more reliable parameter as compared to other parameters because it shows direct effect of stress on cellular membranes. The dendrogram obtained from the cluster analysis formed by Euclidean method grouped the forty-five tomato genotypes into two main clusters (Figure 1).

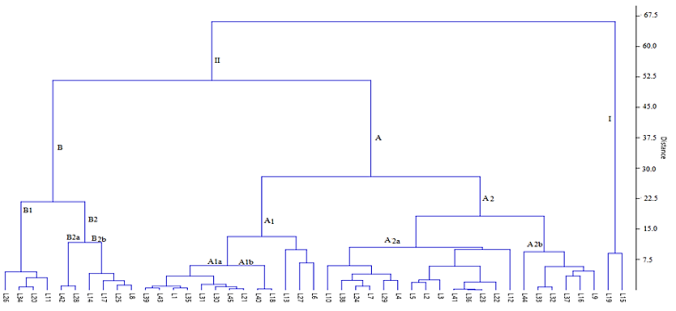

Figure 1: *Dendrogram of 45 tomato genotypes under two growing seasons in cold stress environments.*

Based on different morphological and biochemical parameters, 45 tomato genotypes were grouped according to dissimilarity matrices. All the genotypes made two major clusters (Iand11). Main cluster "I" was comprised of two genotypes L15 and L19. Main cluster "II" was comprised of 43 genotypes. Main cluster "II" was subdivided into two sub-clusters namely A and B. Sub-cluster "B" was comprised of L26, L34, L20, L11, L42, L28, L14, L17, L25 and L8. Sub-sub-clusterB1 comprised of L26, L34, L20 and L11. Sub-sub-cluster B2 contained L42, L28, L14, L17, L25 and L8. A1 contained L39, L43, L1,

Table 5: *Mean values of comparative analysis of Lycopersicon esculentum Mill. for the year 2018 under normal and stressed conditions.*

Germplasm and code no.	PH (cm)		FS (%)		NOF		RFW (g)		RDW (g)		SFW (g)		SDW (g)		PS		PC		SOD		CAT		EL
	N	S	N	S	N	S	N	s	N	S	N	S	N	S	N	S	N	S	N	S	N	S	2018
A17857 (L1)	20	12	64	49	21	12	15	9	6	3	95	75	41	30	10	5	0.45	0.5	0.35	0.36	0.2	0.3	140
A17860 (L2)	18	11	59	51	20	11	15	9	5.9	3	95	75	41	31	15	8	0.45	0.5	0.21	0.22	0.2	0.3	135
A17864 (L3)	12	10	59	51	22	14	16	10	6.9	3.5	95	75	42	31	20	10	0.3	0.48	0.34	0.35	0.2	0.3	145
A17865 (L4)	21	12	58	51	21	13	16	10	6.9	3.5	95	75	42	32	21	14	0.3	0.48	0.4	0.41	0.2	0.3	110
A17872 (L5)	19	10	59	49	22	14	17	11	8	4.1	95	75	43	33	15	9	0.45	0.5	0.43	0.44	0.2	0.3	105
A17873 (L6)	17	10	78	65	21	15	16	10	7	4	95	75	42	30	20	10	0.3	0.48	0.43	0.44	0.2	0.3	155
A17874 (L7)	18	17	55	40	22	15	17	10.5	8	4.1	97	75	43	32	18	8	0.3	0.48	0.4	0.4	0.2	0.3	100
A17876 (L8)	22.5	19	85	75	23.5	15.1	19	17	10	8.2	98	79	45	35	27	18	0.3	0.9	0.3	0.59	0.2	0.3	70
A17877 (L9)	18	10	50	40	21	14	16	9	6	3	94	75	42	31	12	4	0.4	0.5	0.3	0.41	0.21	0.3	100
A17878 (L10)	20	10	59	42	20	11	17	10	6.2	3.5	94	75	42	32	20	10	0.4	0.5	0.3	0.31	0.21	0.3	150
A17879 (L11)	20	10.5	78	69	22	15	17	10	6.2	4	94	75	42	32	18	9	0.4	0.5	0.3	0.31	0.21	0.35	150
A17880 (L12)	21	15	58	55	22	14	16	9	4.9	2.5	93	74	41	31	17	8	0.25	0.4	0.3	0.3	0.2	0.35	120
A17882 (L13)	22	15	65	63	22	14.2	12.5	7	3.9	0.2	92	73	40	30	19	10	0.4	0.56	0.25	0.61	0.21	0.35	160
A17885 (L14)	29	21	90	78	24	16	18	16	9.5	8	98	71	46	35	29	21	0.38	0.8	0.21	0.4	0.35	0.35	70
A17889 (L15)	20	18	38	25	20.5	12	17	11	7.9	5	97	75	45	33	20	10	0.4	0.6	0.21	0.4	0.2	0.3	140
A17890 (L16)	21	19	45	38	20	12	14	9	5.9	2.3	95	74	41	32	19	10	0.4	0.55	0.21	0.3	0.2	0.3	110
A17902 (L17)	29	25	89	79	24	17	19	17	10	8.5	98	78	48	36	28.5	20	0.4	1	0.5	0.6	0.2	0.3	70
A19288 (L18)	20	17	68	58	20	13	14	9	6	2	95	75	41	30	22	15	0.38	0.5	0.5	0.7	0.2	0.3	160
A19289 (L19)	19	22	30	20	20	12	13	8	4.5	1.5	95	70	41	30	20	11	0.4	0.6	0.41	0.42	0.2	0.3	170
A19290 (L20)	31	29.5	79	75	25	18	19	16.5	10	11	98	80	48	36	32	25	0.4	0.9	0.41	0.42	0.2	0.3	80
A19291 (L21)	20	13	75	69	21	12	14	8	4.1	1.5	95	75	40	30	18	10	0.38	0.6	0.4	0.6	0.35	0.35	170
A19292 (L22)	20	11	59	55	21	12	15	9	5.9	2.1	95	75	41	30	21	12	0.4	0.7	0.4	0.41	0.35	0.35	160
A19293 (L23)	19	12	59	55	21	12	15	9	5.9	2.1	95	74	41	30	16	8	0.5	0.75	0.39	0.4	0.21	0.35	120
A19297 (L24)	18	14	55	38	22	19	15	9	5.9	2.1	95	73	41	30	18	10	0.5	0.75	0.39	0.4	0.21	0.35	170
A19842 (L25)	31	30	83	79	25	18	18	18	10.5	10	99	80	48	37	32	23	0.4	0.85	0.4	0.41	0.21	0.38	80
A19844 (L26)	31.5	28	80	70	24.5	17	18	17	10	9.7	98	79	47	35	33	24	0.45	0.75	0.39	0.7	0.21	0.35	60
A19852 (L27)	31	29	70	65	25	18	20.5	19	11	10.2	99	80	48	38	31	23	0.52	0.7	0.39	0.69	0.21	0.3	80
A19853 (L28)	30.5	30	91	80	24.5	18	18	17	9.5	10	98	79	48	35	35	25	0.52	0.7	0.38	0.68	0.39	0.3	40
A19855 (L29)	20	12	58	42	20	12	14	12.5	4.5	4	97	70	41	30	18	10	0.35	0.52	0.38	0.68	0.35	0.22	90
A19856 (L30)	14	12	68	58	21	13	15	9	6	2.4	96	70	42	30	19	11	0.35	0.52	0.45	0.5	0.35	0.22	110
A19860 (L31)	20	50	68	58	23	15	15	9	6	2	96	70	42	30	22	15	0.51	0.51	0.45	0.5	0.35	0.22	120
A19873 (L32)	23	12	50	40	21	13	13.5	8	5	1.5	96	70	41	30	22	14	0.3	0.4	0.45	0.5	0.35	0.22	150
A19887 (L33)	20	12	50	40	19	12	15	14.5	5.5	4	96	75	41	31	21	14	0.4	0.52	0.45	0.5	0.35	0.3	145
A19888 (L34)	30.5	31	78	62	26	18	19.5	17	10.1	9	99	80	48	38	37	28	0.4	0.8	0.45	0.5	0.35	0.3	75
A19889 (L35)	27	12	62	58	21	12	14	8	4.1	1	97	75	40	30	21	13	0.3	0.5	0.45	0.7	0.35	0.3	175
A19890 (L36)	18	11	60	45	22.5	15	15	9	5.9	2.1	97	75	41	31	18	10	0.3	0.5	0.45	0.55	0.36	0.3	170
A19891 (L37)	17	10	45	35	22.5	15	15	10	7	4	97	75	42	32	18	9	0.3	0.5	0.3	0.4	0.22	0.3	140
A19892 (L38)	20	11	57	40	22.5	15	15	10	7	4	97	75	43	33	23	15	0.4	0.52	0.3	0.4	0.22	0.3	110
A19893 (L39)	23	11	70	60	19	12	18	12	9	5.8	98	78	45	31	25	17	0.4	0.52	0.3	0.39	0.22	0.3	110
A19897 (L40)	26	12	65	55	19	12	15	10	6.1	2.8	99	76	42	32	21	11	0.3	0.51	0.3	0.39	0.22	0.3	140
A19898 (L41)	19	10	60	50	21	14	13	7	4	0.5	96	75	40	31	21	11	0.4	0.52	0.3	0.39	0.22	0.3	145
A19899 (L42)	22	10.5	92	79	27	19.5	20.5	19	11.9	10.1	90	80	49	38	37	28	0.45	0.85	0.3	0.49	0.39	0.4	75
A19900 (L43)	19	10	70	55	21.5	14	18	12	9	6	98	78	46	36	20	11	0.4	0.66	0.35	0.39	0.2	0.2	160
A19903 (L44)	22	10.5	45	30	23	15	18	11	8	5	95	78	44	35	21.5	12	0.4	0.66	0.35	0.39	0.2	0.2	160
A19904 (L45)	33	22	68	45	21	14	18	12	9	6	98	78	45	35	18	10	0.45	0.7	0.35	0.39	0.2	0.21	170

Table 6: *Mean values of comparative analysis of Lycopersicon esculentum Mill. for the year 2019 under normal and stressed conditions.*

Germplasm and code no.	PH (cm)		FS (%)		NOF		RFW (g)		RDW (g)		SFW (g)		SDW (g)		PS		PC		SOD		CAT		EL
	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	N	S	2019
A17857 (L1)	18	10	70	58	20	12	16	10	7	4.5	95	75	46	35	11	3	0.23	0.62	0.39	0.4	0.28	0.28	140
A17860 (L2)	21	15	59	45	18	10	16	10	7	4.5	95	75	46	35	15	8	0.23	0.62	0.37	0.38	0.28	0.28	160
A17864 (L3)	22	19	58	42	20	12	17	11	8	5	95	75	48	38	20	10	0.2	0.6	0.37	0.38	0.35	0.35	150
A17865 (L4)	19	11	49	42	19	11	17	11	8	5	95	75	48	38	21	13	0.2	0.6	0.39	0.4	0.31	0.31	125
A17872 (L5)	17	10	50	45	20	12	18	12	8	6.2	95	75	49	39	17	9	0.21	0.62	0.37	0.38	0.31	0.31	135
A17873 (L6)	16	9	62	60	20	12	17	11	7	5	95	75	48	38	20	10	0.2	0.6	0.42	0.43	0.31	0.31	190
A17874 (L7)	20	11	58	40	22	12	18	13	9	5.5	95	75	48	38	19	9	0.2	0.6	0.39	0.4	0.3	0.3	110
A17876 (L8)	19.5	10	85	78	23	14	19	18	11	9.5	95	75	50	38	27	18	0.3	1	0.35	0.55	0.3	0.3	70
A17877 (L9)	28	20	50	40	20	12	18	11	7	4	90	70	47	35	12	3	0.3	0.69	0.3	0.4	0.3	0.3	100
A17878 (L10)	21	18	58	40	19	10	17	11	8	5	93	68	49	35	20	10	0.3	0.69	0.3	0.32	0.38	0.38	150
A17879 (L11)	22	19	78	65	21	14	17	11	8	5	89	69	49	35	19	9	0.35	0.7	0.29	0.3	0.35	0.35	160
A17880 (L12)	27	24	58	55	19	10	16	10	7	3.5	89	69	48	35	18	8	0.2	0.6	0.29	0.3	0.33	0.33	120
A17882 (L13)	20	15	62	61	19	10	14	9	5	2	95	75	47	33	19	10	0.38	0.75	0.29	0.3	0.32	0.32	160
A17885 (L14)	19	11	90	78	23	15	19	18	10	9	100	80	50	39	29	20	0.3	0.9	0.28	0.6	0.31	0.31	100
A17889 (L15)	30	28	35	20	20	11	18	13	9	6	98	82	49	38	20	10	0.35	0.7	0.22	0.38	0.3	0.3	175
A17890 (L16)	20	12	48	38	20	11	11	10	7	3	90	85	48	37	20	10	0.35	0.69	0.25	0.3	0.3	0.3	100
A17902 (L17)	19	11	88	78	23	14	19	18	11	9.5	10	85	50	40	29	20	0.35	1.1	0.45	0.59	0.35	0.35	80
A19288 (L18)	18	12	65	55	19	11	14	10	7	3.5	90	75	48	35	23	15	0.3	0.69	0.4	0.63	0.25	0.25	150
A19289 (L19)	21	12	30	18	18	10	14	9	6	2.8	90	75	48	35	20	11	0.35	0.7	0.4	0.42	0.25	0.25	190
A19290 (L20)	30.5	12	78	68	24	15	20	19	11	13	100	80	50	40	32	25	0.35	1	0.41	0.43	0.25	0.25	75
A19291 (L21)	30.5	29	68	55	19	11	15	9	6	3	90	76	45	35	18	9	0.3	0.7	0.4	0.6	0.25	0.25	160
A19292 (L22)	30.5	29	60	49	19	11	15	10	7	3.5	90	76	47	35	21	13	0.4	0.8	0.4	0.42	0.24	0.24	140
A19293 (L23)	30.5	29	60	49	19	11	16	10	7	3.5	91	77	47	35	17	8	0.42	0.81	0.38	0.39	0.24	0.24	100
A19297 (L24)	20	11	55	38	20	12	16	10	7	3	91	77	47	35	19	10	0.42	0.81	0.38	0.39	0.35	0.35	150
A19842 (L25)	14	11	88	75	24	15	21	19	12	10.5	100	82	50	40	32	23	0.35	1	0.38	0.39	0.35	0.34	75
A19844 (L26)	20	11	80	68	23	15	20	18	11	10	100	82	50	39	33	25	0.45	0.8	0.38	0.7	0.3	0.33	90
A19852 (L27)	22	11	75	60	24	16	22	19	13	12	100	82	50	40	31	22	0.55	0.9	0.38	0.69	0.3	0.33	85
A19853 (L28)	20	11	93	80	24	16	18	17	10	8.5	100	82	50	38	35	28	0.58	0.9	0.38	0.69	0.3	0.32	80
A19855 (L29)	30	29	56	41	18	11	15	14	6	4.5	95	75	48	35	20	10	0.38	0.7	0.38	0.69	0.3	0.29	110
A19856 (L30)	25	12	68	55	19	11	16	10	7	4	95	75	48	35	20	10	0.38	0.7	0.49	0.5	0.3	0.28	120
A19860 (L31)	17	11	68	55	21	13	16	10	7	4	95	75	48	35	22	15	0.58	0.65	0.49	0.5	0.35	0.25	140
A19873 (L32)	16	10	55	38	19	11	16	9	6	3	95	75	47	34	22	15	0.2	0.59	0.49	0.5	0.35	0.25	150
A19887 (L33)	20	12	55	38	18	10	16	14	6.5	5.5	95	75	47	34	22	15	0.35	0.7	0.49	0.5	0.28	0.26	150
A19888 (L34)	22	12	78	68	25	16	19	18	12	10	100	85	50	40	37	28	0.4	0.9	0.49	0.5	0.28	0.26	80
A19889 (L35)	25	12	68	58	19	11	14	10	6	3	95	75	45	33	21	13	0.3	0.7	0.45	0.63	0.28	0.26	170
A19890 (L36)	18	10	60	50	21	13	17	10	7	5	95	75	47	35	18	9	0.2	0.57	0.41	0.58	0.32	0.35	140
A19891 (L37)	22	11	50	38	21	13	18	11	8	5	95	75	48	37	18	9	0.2	0.57	0.3	0.39	0.27	0.34	180
A19892 (L38)	19	10	55	40	21	13	18	11	8	4.5	98	80	48	37	22	15	0.38	0.78	0.3	0.39	0.27	0.34	125
A19893 (L39)	21	11	70	68	18	10	19	13	9.5	7	98	80	49	39	25	17	0.4	0.8	0.3	0.35	0.27	0.34	100
A19897 (L40)	33	28	65	50	18	10	17	11	7	4.5	95	88	48	38	21	12	0.3	0.7	0.3	0.3	0.27	0.34	145
A19898 (L41)	19	10	60	45	20	20	14	7	5	2	90	80	45	35	21	12	0.38	0.75	0.3	0.39	0.25	0.33	150
A19899 (L42)	22	10.5	95	79	25	13	21	20	13	12	100	85	52	40	37	28	0.42	0.9	0.25	0.55	0.25	0.33	90
A19900 (L43)	19	10	70	58	20	15	19	14	10	7	98	83	49	39	20	12	0.38	0.75	0.22	0.35	0.22	0.32	160
A19903 (L44)	22	10.5	45	30	22	14	18	14	8	6	98	83	48	38	22	13	0.39	0.6	0.22	0.38	0.22	0.34	140
A19904 (L45)	33	22	68	55	20	12	19	14	10	7	98	83	48	38	18	9	0.41	0.6	0.22	0.38	0.22	0.34	150

L35, L31, L30, L45, L21, L40, L18, L13, L27 and L16. A2 contained L10, L38, L24, L7, L29, L4, L5, L2, L3, L41, L36, L23, L22, L12, L33, L32, L37, L16 and L9 (Figure 1). Cluster analysis identified the groups with better performing germplasm. During season 2018, L34 and in 2019, L20, L23, L29 under chilling stress were observed with maximum plant height. L42 during both seasons and under cold stress environment showed highest values for fruit setting percentage and number of fruits. L17 showed maximum proline content during both seasons and treatments. L26 showed highest values for superoxide dismutase under chilling stress in 2018 and 2019. L42 in 2018 and L45 in 2019 were observed as better performing genotypes under cold stress conditions for catalase. For root of fresh weight, L27 in 2018 and L42 in 2019 showed maximum values under stress. While L42 and L20 showed better results for roots of dry weight during both seasons. For shoot fresh and dry weight, L42 and for plant spread, L34 were recognized as better performing genotypes among others for both treatments and both seasons in general. The genotype L19 showed maximum values for electrolyte leakage under normal and cold stressed conditions during both seasons (2018 and 2019).

Cation exchange capacity (CEC) and organic matter (OM) were found to be high in the soil used for experiment. Inorganic nutrient concentrations were in the range of critical limits while some of the nutrients were below the critical level. Most of the soils in Pakistan are basic in nature and similar behavior was found in the analyzed samples. Addition of organic matter reduced pH to somewhat near neutral. Tomato grows well in soil of pH 6.0 or higher but can also tolerate pH 4.3 to 4.8. Nutrient availability and accessibility are dependent on soil pH and can directly affect root cells (Alam *et al*., 1999; Nicol *et al*., 2008). Soil texture was sandy clay loam. Tomato grows in all soils, but its performance improves at well textured soils. Its growth enhances in sandy loam soil. For plant characteristics, almost all genotypes showed decline in plant height under chilling stress, but tolerant genotypes showed small decrease compared with other tested genotypes. Mean performance of all genotypes was checked in both treatments during two successive years and it was found that all the genotypes showed variable response. Hence, it was concluded from the results that some genotypes have adapted to local environment and have developed some mechanisms to cope stress. This tolerance in some genotypes is due to their genetic makeup that enabled them to cope stress (Liu *et al*., 2012).

It was demonstrated that tomato plants can produce an adaptive response to cold, which includes comprehensive morphological and metabolic regulation, thereby improving tolerance to cold. More importantly, results showed a strong similarity between response and cold acclimation, which is the process by which plants in temperate regions improve their freezing tolerance after exposure (Venema *et al*., 2005). Tolerant plant might also contain traits that make them grow better at moderately low or suboptimal temperatures: Temperatures above chilling but below the current optimum for tomato growth (He *et al*., 2022).

Number of fruits is related to number of flowers produced, successfully pollinated, fertilized, and turned into fruits. Genotypes that showed tolerance to chilling stress beard more fruits that sensitive ones. The variation in cold tolerance that exists among related Lycopersicon species could potentially be useful for the development of cultivars with increased energy efficiency (Venema *et al*., 2005). Genotypes showed variable production of proline in response to chilling stress that may be used as an indicator of chilling tolerance. Proline accumulation is one of many plant adaptations to stresses (Kumar *et al*., 2000). It has also been widely advocated that proline accumulation can be used as a selective parameter of stress tolerance (Ramajulu, 2001). Plants are subjected to environmental stresses and high proline levels allow plants to maintain low water potential, allowing additional water to be taken from the environment, thus reducing the immediate impact of stress. It protects plants from damage from free radicals (Teixeira and Fidalgo, 2009), thus it may be used as selection criterion for selection under stress conditions. In any environmental stress, plant experiences oxidative stress that affects plant growth and development. In higher plants, superoxide dismutase (SOD) acts as an antioxidant enzyme and a scavenger from ROS. Plant cells contain many isoforms of SOD that differentiate into a mineral at the active site of the enzyme, in addition to their location in subcellular chambers including cytosol, mitochondria, peroxisomes, and chloroplasts (Aydin *et al*., 2014; Wang *et al*., 2017). The differences of CAT activity were significant between stressed and normal conditions, vegetative and reproductive stages,

as well as between quality and quantity traits. When compared with normal conditions, the mean CAT activity in the roots and leaves was threefold more in stressed conditions. In comparison with the vegetative stage, CAT activity in vegetative stage showed a 15% reduction in roots and a 17% reduction in leaves at the reproductive stage. Catalase (CAT) plays an important role in the plant defense mechanisms against oxidative stress and decomposes H_2O_2 to water and oxygen (Mohamad et al., 2011).

Although electrolyte leakage was observed in all genotypes under chilling stress but mentioned genotypes showed lesser electrolyte leakage compared with other tested genotypes, depicting tolerance to chilling stress while all other genotypes showed vulnerability to chilling stress. Findings of this study showed that stress exposed plants showed increased electrolyte leakage. Tolerant plants showed lesser electrolyte leakage compared with sensitive plants. This shows membrane integrity even plants were exposed to stress (Chen and Yu, 1994; Vladimirov *et al*., 1980).

The dendrogram obtained from the cluster analysis formed by Euclidean method grouped the forty-five tomato genotypes into two main clusters. Based on different morphological and biochemical parameters, 45 tomato genotypes were grouped according to dissimilarity matrices. All the genotypes made two major clusters (Iand 11). Main cluster "I" was comprised of two genotypes. Main cluster "II" was comprised of 43. The tolerant genotypes made cluster with each other showing maximum similarity among their genetic makeup. The standardized mean Euclidian distances between pairs of genotypes are widely used as a measure of dissimilarity. Finally, forty-five genotypes of tomato were divided into six clusters in such a way that all the genotypes within the cluster had smaller D2 values among themselves than those belonging to different clusters. Cluster analysis has been widely used for description of genetic diversity and grouping based on similar characteristics (Golestani and Pakniat, 2007; Golabadi *et al*., 2006; Shahi *et al*., 2009; Souri *et al*., 2005).

Conclusions and Recommendations

It is concluded that sensitive plants exposed to chilling stress show adverse changes in physiological and biochemical pathways compared with tolerant plants that may be associated to genetic makeup of a genotype and its ability to acclimatize in adverse conditions. On the basis of results, it is also concluded that Electrolyte leakage proved to be a more reliable parameter as compared to other parameters in differentiating chill tolerant genotypes of tomato under normal and chilling stress conditions because it shows direct effect of stress on cellular membranes. It is recommended that this method can potentially be used to short list larger sets of germplasm for chilling stress tolerance.

Novelty Statement

The research presents a brief idea of tomato germplasms screening for chilling stress tolerance which will give new direction to future research.

Author's Contribution

Kokab Nazim: Performed experiments, collected data, did statistical analysis, and wrote the manuscript. **Asghari Bano and Ghulam Jellani:** This work is done under supervision and as co-supervisor helped in collection of data in field, helped in writing paper and made correction in paper.

Conflict of interest

The authors have declared no conflict of interests.

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