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Screening of tomato genotypes for vegetative and reproductive characters under low temperature regime

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ABSTRACT

Twenty five tomato genotypes were screened for low temperature tolerance under open field conditions during 2012-13 and further selected tolerant and susceptible genotypes were screened under control conditions (phytotron) for confirmation. When grown under field conditions during winter, the membrane stability index was highest (83.27%) in Pusa Sadabahar followed by Pusa Sheetal (81.17%), whereas, it was least in line H-88 (29.67%). The highest number of flowers per truss was observed in the wild species *Solanum pimpinellifolium* and *S. peruvianum* and these lines also had viable pollens, but the number of fruits per truss was negligible in the *S. peruvianum* lines. The cultivar Pusa Sadabahar recorded the highest number of fruits per truss followed by 120-1. *Solanum pimpinellifolium* and *S. peruvianum* lines, S-699 and DTR-2 showed considerably high shoot dry weight. The physiological parameters like membrane stability index, relative water content, tolerance index and shoot dry weight were reduced in all the genotypes under phytotron in response to low temperature (15/8 and 16/10°C) as compared to plants grown under optimum conditions (26/22°C). Pusa Sadabahar, Pusa Sheetal and DTR-2 showed tolerance to low temperature (15/8 and 16/10°C) with respect to the vegetative characters studied.

Key words: Low temperature tolerance, membrane stability index, relative water content, *Solanum lycopersicum*, tolerance index.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most consumed vegetables in the world and is the dietary source of vitamins, minerals and fiber, which are important for human nutrition and health. It is extensively grown throughout the world. However, its sensitivity to low temperatures limits the tomato's geographic distribution and the time of year for planting in open field cultivation. The cultivated tomato genotype displays limited growth and development at temperatures under 12°C (Hu *et al*., 8). Depending on the intensity and duration of exposure to the chilling temperatures, photosynthesis, respiration, membrane integrity, water relations and the hormone balance of the plants may be affected (Graham and Patterson, 7). In North Indian plains, the temperature goes below 10°C especially during December-January, which drastically affects the fruit set of tomatoes. Generally, the normal temperature for fruit set is from 15 to 25°C, depending upon the variety. Poor fruit set at low temperature is due to poor anther dehiscence, poor pollination and poor pollen viability. Most tomato cultivars are sensitive to chilling temperatures during all stages of plant development, including seed germination, early and late vegetative growth and reproduction (Scott and Jones, 13; Wolf *et al*., 16). However, there are relatively good existence of genetic variation within and between species of tomato, which could be utilized to improve chilling tolerance of tomato cultivars. Cold tolerant genotypes may offer substantial advantages for tomato production under sub-optimal temperatures in the field or in the greenhouse (Foolad and Lin, 4). Cold tolerant plants may grow more vigorously at initial stages and thus become established faster than cold sensitive plants. This may result in improved earliness, adaptability, water use, and yield of high-quality fruits when grown under low temperature regimes. The present study was carried out to evaluate the effect of low temperature regimes on some vegetative and reproductive behaviour of tolerant and susceptible tomato genotypes and develop screening method for low temperature tolerance.

MATERIALS AND METHODS

The experiments were conducted by screening tomato genotypes under two sets of conditions (open field conditions during winter and phytotron). The former experiment was carried out at the Vegetable Research Farm, IARI, New Delhi during 2012-13, where 25 genotypes including wild accessions of *Solanum pimpinellifolium* and *S. peruvianum* (Table 1) were evaluated. The tomato seeds were sown during October, 2012 and three-week-old tomato seedlings were transplanted in the field. Three replications for

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Table 1. Performance of tomato genotypes at low temperature regime under open field conditions.

Genotype	MSI (%)	Pollen viability (%)	No. of flowers/ truss	No. of fruits/ truss	Yield/plant (kg)	Shoot dry wt. (g)
TH-348	56.67^{defg}	56.27^{fg}	7.17^{cd}	1.73^{cd}	1.29^{fgh}	5.07^{defghij}
S-699	70.33^{abcd}	63.08^{def}	4.03^{ijk}	1.30^{cdef}	1.27^{ghi}	10.83^{bc}
Pusa 120	52.50^{fg}	15.68^{k}	3.60^{kl}	0.00^{k}	0.70^{lm}	3.15^{hij}
Pusa Rohini	51.10^{fgh}	16.48^{k}	4.85^{fgh}	0.20^{k}	0.90^{jkl}	1.52^{j}
Pusa Ruby	63.00^{cdef}	29.24^{j}	5.33^{f}	0.30^{jk}	1.30^{fgh}	4.12^{fghij}
DTR-1	47.50^{gh}	58.88^{efg}	4.75^{fghi}	0.40^{hijk}	1.10^{hijk}	2.50^{ij}
TH-348-11	45.50^{gh}	29.88^{ij}	4.31^{hijk}	0.35^{ijk}	0.85^{jkl}	1.77^{j}
120-1	69.50^{abcde}	58.33^{efg}	5.36^{f}	3.30^{ab}	1.65^{de}	9.33^{bcd}
C-10-19	56.00^{efg}	29.94^{ij}	3.90^{jk}	1.43^{cdef}	1.50^{defg}	3.83^{fghij}
H-88	29.67^{i}	10.26^{k}	2.95^{l}	0^{k}	0.95^{jkl}	2.83^{ij}
TH-348-5-1	45.50^{gh}	69.20^{cd}	4.78^{fgh}	1.25^{cdefg}	0.85^{jkl}	4.27^{efghij}
Pusa Uphar	50.83^{fgh}	48.64^{h}	4.53^{ghij}	0.50^{ghijk}	1.60^{def}	2.57^{ij}
DTR-3	58.17^{cdefg}	68.78^{cd}	7.23^{cd}	1.45^{cdef}	1.15^{hij}	9.43^{bcd}
TH-348-4-2	64.83^{bcdef}	30.41^{ij}	5.37^{f}	0.97^{efghij}	0.80^{kl}	7.67^{bcdefgh}
DTR-2	70.50^{abcd}	75.34^{bc}	6 .20^{e}	2.86^{b}	2.12^{bc}	9.99^{bc}
TH-348-SPS	37.50^{hi}	64.57^{de}	5.13^{fg}	1.63^{cde}	1.15^{hij}	3.57^{ghij}
Pusa Sheetal	81.17^{a}	81.48^{ab}	5.22^{fg}	1.95^{c}	2.18^{ab}	8.77^{bcde}
Pusa Gaurav	62.17^{cdef}	55.17^{gh}	6.60^{de}	1.10^{defghi}	1.35^{efgh}	7.82^{bcdefg}
Pusa Sadabahar	83.27^{a}	84.89^{a}	6.88^{cde}	3.70^{a}	2.45^{a}	4.90^{defghij}
Chiku	63.83^{bcdef}	26.57^{j}	5.35^{f}	0.70^{fghijk}	1.80^{cd}	6.37^{cdefghi}
H-24	57.33^{defg}	37.01^{i}	3.70^{k}	0^{k}	0.83^{jkl}	8.30^{bcdef}
S. pimpinellifolium-1	71.83^{abc}	85.66^{a}	6.38^{e}	2^{c}	0.91^{jkl}	20.77^{a}
S. pimpinellifolium-2	77.50^{ab}	86.84^{a}	7.60^{bc}	1.15^{defgh}	0.73^{l}	7.83^{bcdefg}
S. peruvianum-1	63.00^{cdef}	80.87^{ab}	8.23^{b}	0.20^{k}	0.40^{m}	11.31^{b}
S. peruvianum-2	53.00^{fg}	83.60^{a}	9.53^{a}	0^{k}	0.38^{m}	11.21^{b}
CD (P = 0.05)	14	7.46	0.73	0.76	0.33	4.57
CV (%)	14.39	8.43	8	40.44	16.47	41.02

*Means with the same letter(s) are not significantly different

each genotype were taken for the screening test in randomized block design (RBD). They were planted at a distance of 45 cm × 45 cm. All cultural practices recommended for successful cultivation of tomato crop were carried out. The data were recorded on five randomly selected plants in each genotype in each replication. The observations were recorded for six traits namely membrane stability index (MSI), pollen viability (PV), number of flowers per truss, number of fruits per truss, yield/plant and shoot dry weight. The membrane stability index was recorded after two weeks of cold stress, during December-January, when the minimum temperature dropped down below 10°C. Percentage of pollen viability was tested a day before anthesis. Flower buds were collected from five plants per genotype by removing pollens from the anthers using a needle. The pollen grains were inoculated on glass slide for determining the number of viable pollen grains through acetocarmine test. The first 5 flower truss per plant were tagged and allowed to develop until fruits were formed during the cold stress period to record the number of flowers and fruits per truss. Shoot dry weight was taken at the time of harvesting and oven-dried at 85°C for seven days.

The second experiment was carried out under control conditions (phytotron) during 2013 to confirm the tolerance level of selected seven tomato genotype from the previous experiment, with respect to their vegetative behaviour of tolerance to low temperature

at National Phytotron Facility, IARI, New Delhi. The genotypes included for the study were Pusa Sadabahar, Pusa Sheetal, DTR-2 and *Solanum habrochaites* LA1777 (cold tolerant) and Pusa Rohini, Pusa Uphar and Pusa 120 (susceptible). Plants were arranged in randomized complete block designs with three replications, each replicate had three plants. They were grown under three different temperature regimes, *viz*. 15/8, 16/10 and 26/22°C day/night temperature 12/12 h, respectively to evaluate the effect of low temperature on the vegetative aspects. Seeds of the seven selected genotypes were placed under germination papers and the germinated seedlings were carefully transplanted containing mixture of vermiculite, peat and sand (1:1:1). After three weeks the seedlings were transplanted having same medium and kept in the phytotron under control conditions of 26/22°C (normal) day/night temperatures. The plants were initially kept at normal conditions and each genotype were placed into chambers with the environmental conditions required for the treatment (15/8 & 16/10°C) by slowly decreasing the temperature at an interval of two days to avoid shock to the plants and same set of genotypes were allowed to grow at that required normal temperature.

The data on membrane stability index and relative water content were recorded after 2 weeks of cold stress from three plants in each treatment. The method suggested by Blum and Ebercon (1) was employed for the estimation of membrane injury index of leaf. The membrane stability index (MSI) was then calculated using formula (Sairam, 11). The method for determining relative water content suggested by Brass and Weatherley (2). Plant sample were oven-dried at 85°C for seven days, and shoot (leaf + stem) dry weight of individual plants determined. In all the treatments, plants were grown for five weeks before they were harvested. Tolerance index was also computed (Foolad and Lin, 5). The data was subjected to analysis of variance (ANOVA) using the generalized linear model (GLM) of SAS (Statistical Analysis System) software version 9.1 and significance level was determined at $P<0.05$.

RESULTS AND DISCUSSION

Temperature has a significant influence on many aspects of growth and development in tomato. The drastically reduced MSI was obtained in H-88 (29.67%), whereas Pusa Sadabahar (83.27%) showed a highly significant stability followed by Pusa Sheetal, the lines of *S. pimpinellifolium* and DTR-2. The temperature stress modifies composition and structure of cell membrane by weakening the hydrogen bonds and electrostatic interactions between the polar groups of proteins within the aqueous phase of the membrane. Thus, integral membrane proteins (which are associated with both hydrophilic and lipid regions of the membrane) tend to associate more strongly with the lipid phase. Disruption and damage to membranes alters their permeability, and results in loss of solute (electrolyte leakage). The consensus is that the electrolyte leakage reflects damage to cellular membranes (McDaniel, 9) and is therefore, an important factor in stress related studies.

The extent of low temperature and genetic variation is also related to flowering, pollen viability and number of fruits in the plants during cold stress period. The number of flowers per truss was found to be highest in *S. peruvianum*-2 (9.53) and the presence of least number of flowers per truss in H-88 (2.95). At low temperature regime, the viability of pollen grains varied among genotype. The decreasing level of viability at low temperature varied from 86.84 to 10.26% among all genotypes. *Solanum pimpinellifolium*-2 (86.84%) had the highest viable pollen followed by *S. pimpinellifolium*-1 (85.66%) and Pusa Sadabahar (84.89%). The least viability of pollens was observed in H-88 (10.26%). It is known that pollen development especially after meiosis is most affected at low temperature (Charles and Harris, 3). The highest number of fruits per truss was recorded in Pusa Sadabahar (3.7) followed by 120-1 (3.3), thus contributing to a higher yield per plant in Pusa Sadabahar (2.45 kg/plant). However, the wild accessions of *S. peruvianum*, Pusa 120, H-88 and H-24 failed to develop fruits during the cold stress period. In sensitive genotypes as the non coldset varieties included in the present study, the susceptibility of post meiotic phase of pollen development to low temperature probably leads to production of non-viable pollen, which fails to germinate on the stigmatic surfaces, further resulting in absence of fruit set. Slow growth of pollen tube leading to failure of fertilization at temperature below 10°C had also been reported by several workers (Charles and Harris, 3).

Growth under stress is a function of plant vigour and stress tolerance. The accessions of *S. pimpinellifollium* and *S. peruvianum* and few other genotypes like S-699, 120-1 and Pusa Sheetal showed a relatively high shoot dry weight, which may confer to bringing up tolerant genotypes, whereas, Pusa Rohini recorded the lowest shoot dry weight (1.52 g) among all the genotypes. Dry matter accumulation is a good indicator of chilling tolerance and can be associated with photosynthetic capacity (Oyanedel *et al*., 10). However, this factor may also be attributed to its growth habit (determinate and indeterminate types).

In the phytotron, plants grown under normal temperature possessed higher values for MSI as compared to the plants subjected to cold stress at 15/8 and 16/10°C. Pusa Sadabahar showed a high value of membrane stability index (62.33 and 65.33%) at both the low temperature regimes given in the experimental set followed by DTR-2, *S. habrochaites* LA1777 and Pusa Sheetal. When grown under the optimum temperature (26/22°C), it is observed that DTR-2 has the highest stability index as compared to other genotypes (Table 2) but the rate of reduction in its stability was high when subjected to the lower temperature regimes, whereas, Pusa Sadabahar recorded for a stable behaviour maintaining high MSI under both the cold stress conditions. Pusa 120 on the other hand recorded a very low stability index throughout the given temperatures for cold stress, which shows a poor tolerance level of this genotype to low temperatures. In all the genotypes, the relative water content (RWC) showed a decreasing trend with the decrease in day/night temperatures from 26/22 to 15/8°C. Relative water content was maximum in Pusa Sadabahar followed by Pusa Sheetal, *S. habrochaites* LA1777 and DTR-2. Pusa 120 showed the least tolerance to low temperature even on the basis of relative water content. Srivastava *et al*. (14) and Yadav *et al.* (15) also recorded higher thermostability (low membrane injury) and relatively higher water content in the leaves of heat tolerant tomato genotypes, which can be applied for the same to screen cold tolerant genotypes too.

The shoot dry weight was reduced in all the genotypes in response to cold stress. When grown under optimum condition, the highest shoot dry weight was recorded in DTR-2 with a weight of 8.31 g. The dry weight decreased along by 48.5 and 52.71% when subjected to cold stress at 15/8 and 16/10°C, respectively. Though Pusa Sadabahar exhibited higher shoot dry weight value than Pusa Sheetal at optimum temperatures, the shoot dry weight of Pusa Sheetal was 2.86 and 4.12 g when grown under the cold stress conditions, which was higher than the dry weight of Pusa Sadabahar under the same stress conditions. Pusa 120 grown under cold stress showed a significantly low shoot dry weight as compared to the plants grown in the normal growing conditions.

To differentiate between vigour and tolerance, plant growth must be evaluated under both stress (15/8 & 16/10°C) and non-stress conditions (26/22°C). Growth under stress as a percentage of growth under non-stress conditions (TI) is a reliable measure of stress tolerance (Shannon, 12; Forster *et al*., 6). The tolerance index (Fig. 1) gives a picture of DTR-2 with high level of tolerance in both the cold stress conditions and Pusa 120 exhibiting a very low tolerance index. Though Pusa Sheetal showed a higher value of tolerance index than DTR-2 at 16/10°C, but was not so when grown under 15/8°C. This may be attributed to its growth habit (determinate) as compared to the indeterminate type in DTR-2. Genes contributing to vigour might be different from genes conferring tolerance (Forster *et al*., 6). However, for developing tomatoes for efficient production under sub-optimal temperatures, genes for both plant vigour and cold tolerance are important, hence, combining genes conferring both traits may be useful.

Variation in temperature responses between currently cultivated tomato cultivars is limited,

Table 2. Effect of low temperature regimes under phytotron on membrane stability index, relative water content and shoot dry weight of tomato genotypes.

Genotype	MSI (%)			RWC (%)			Shoot dry weight (g)		
	15/8°C	16/10°C	26/22°C	15/8°C	16/10°C	26/22°C	15/8°C	16/10°C	26/22°C
Solanum habrochaites LA1777 (indeterminate)	48^{b}	53.67^{bc}	65.33^{c}	82.13^{ab}	84.45^{ab}	84.81^{ab}	1.15^{c}	2.42^{b}	7.04^{ab}
DTR-2 (indeterminate)	47.33 ^{b}	58.67^{ab}	82.67^{a}	80.50^{b}	83.42^{b}	86.52^{a}	4.03^{a}	4.38^{a}	8.31^{a}
Pusa Rohini (semi-determinate)	26^{cd}	42de	75.33^{b}	70.23^{c}	71.86^{c}	79.54^{c}	1.28^{c}	1.87^{bc}	4.20^{c}
Pusa Sadabahar (determinate)	62.33^{a}	65.33^{a}	79.67^{ab}	85.65^{a}	87.55^{a}	88.02^{a}	2.61^{b}	3.95a	7.49^{ab}
Pusa Uphar (indeterminate)	29.67 ^{c}	44.67^{cde}	80^{ab}	71.25^{c}	72.75^{c}	80.33^{bc}	1.24^{c}	1.67^{bc}	3.46^{c}
Pusa Sheetal (determinate)	45.67 ^{b}	52b^{cd}	78.33^{ab}	82.84^{ab}	84.83^{ab}	86.01^{a}	2.86^{b}	4.12^{a}	7.27^{ab}
Pusa 120 (semi-determinate)	20 ^{d}	37.33^{e}	57^{d}	67.83^{c}	70.92^{c}	80.19^{bc}	1.12^{c}	1.37^{c}	6.58^{b}
CD (P = 0.05)	9.37	10.98	6.11	4.34	3.98	5.27	0.71	0.81	1.29
CV (%)	13.22	12.22	4.64	3.16	2.81	3.54	19.46	16.04	11.45

*Means with the same letter(s) are not significantly different

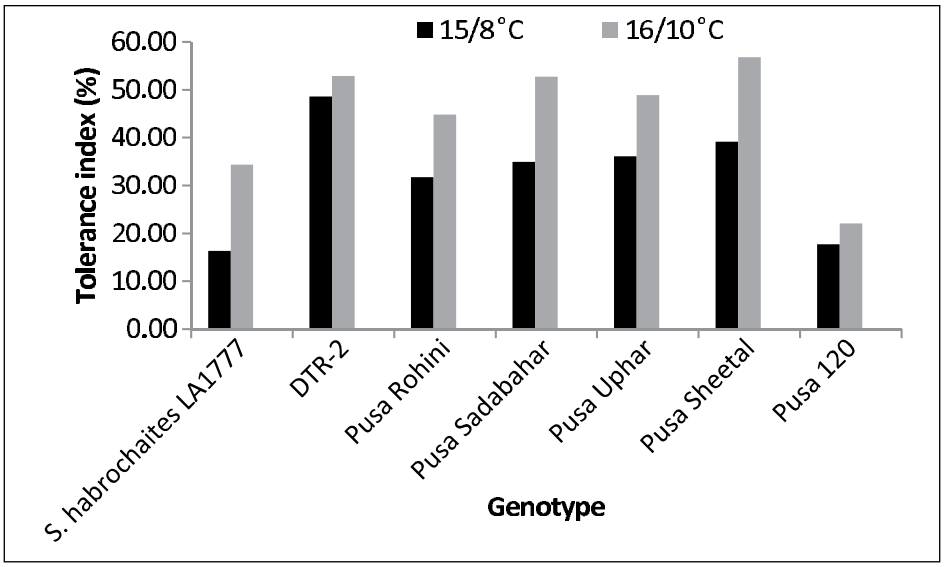

Fig. 1. Cold tolerance index in tomato genotypes.

which hampers breeding for equal economic levels of production at lower temperatures. Therefore, breeders must look for alternative sources of variation in the temperature response of tomato. The parameters such as membrane stability index, relative water content, pollen viability, shoot dry weight, tolerance index and number of flowers and fruits per truss could be used as a selection criterion for cold tolerant genotype with better fruit set. On the basis of selection through the aforementioned traits, *S. pimpinellifolium* lines, DTR-2, Pusa Sadabahar and Pusa Sheetal can be used as a potent source of cold tolerance genes for further tomato breeding programme.

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