Indian Journal of Experimental Biology
Vol. 60, August 2022, pp. 615-627
DOI: 10.56042/ijeb.v60i08.47088

Physiochemical response of papaya genotypes exposed to low temperature regimes

Maurya NK[1], Goswami AK[1]*, Goswami S[2], Singh SK[1], Prakash J[1], Chinnusamy V[3], Talukdar A[4], Behera TK[5] & Pradhan S[1]

[1]Division of Fruits and Horticultural Technology; [2]Division of Biochemistry; [3]Division of Plant Physiology;
[4]Division of Genetics; [5]Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India

Received 03 March 2021; *revised 21 March 2022*

Susceptibility to low temperature stress is the major threat to papaya cultivation. Here, we studied a low temperature stress tolerance in papaya plant. We have investigated the effect of different low temperature regimes, 28°/18°C (day/night) to 16°/06°C (day/night) with a gradual decrease of 2°C on every two days and one set with direct exposure to the low temperature of 18°/08°C (day/night), called the acclimatized plant, in five diverse papaya genotypes (Pusa Nanha, Red Lady P-7-2, P-7-9, and P-7-14) and cold tolerant wild relative of cultivated papaya genotype (*Vasconcellea cundinamarcensis* V.M. Badillo) under controlled regulated conditions. It was observed that there were significant variations in the physiological and biochemical parameters like photosynthetic gas exchange parameters, chlorophyll content, fluorescence parameters, relative water content (RWC), membrane stability index (MSI), total sugars content, total soluble proteins content, lipid peroxidation, and proline accumulation in leaf tissues. Maximum stomatal conductance, chlorophyll fluorescence**,** RWC, MSI, total sugars, total soluble proteins, proline and lowest MDA contents were observed in *Vasconcellea cundinamarcensis* followed by inbred P-7-9 as compared to other genotypes under low temperature stress. Among all the papaya genotypes, P-7-9 showed more adaptability to low temperature stress and it further give new insights for developing low temperature tolerant papaya genotypes, especially under changing climate situations.

Keywords: Abiotic stress, *Carica papaya*, Cold stress, Mountain Papaya, Photosynthetic rate, Proline content, Stomatal conductance, *Vasconcellea cundinamarcensis*

Low-temperature stress (LTS) has a detrimental effect on the optimum growth and development of plants. It has adverse effects on a range of physiological and biochemical activities in the plant system, depending on the severity and duration of exposure to cold induced stress[1]. Exposure of plants to cold tress results in changes in multiple physiological and biochemical processes including alternations of membrane fluidity, enzyme activities and metabolism homeostasis[2]. Electrolyte leakage (EL) estimates tissue damages and durability by comparing the conductivity of leaked solutes from chilling injured and uninjured tissues[3].

Papaya (*Carica papaya* L.), is one of the important cultivated fruit species within the family Caricaceae and is widely cultivated for consumption as fresh fruit and made into drinks, jams, candies, dried, and crystallized slices[4]. Papaya is a herbaceous crop of tropical and subtropical regions. It is a rich source of vitamin A and has a good amount of calcium. In India, it is cultivated in an area of about 0.144 million ha with a production of 5.95 million tonnes having productivity of 41.32 MT/ha[5]. Commercial papaya cultivation is restricted to tropical and sub-tropical areas as it requires a warm and humid climate without risk of frost. The optimum temperature for papaya is reported by *Nagy et al.*[6] to be 21 to 33°C[6]. Low temperature adversely affects plant growth and fruit yield. Both foliage and fruit get damaged near 0°C or sub-zero temperatures[7]. Plant species show a difference in tolerance levels and vary widely in their ability to survive under low temperature but it is not much clarity as to how some species tolerate low temperature injury better, while others succumb[5]. Modification of protective molecules which include amino acids such as proline and maintenance of photosynthetic efficiency, are among these defense strategies which is widely reported in many plant species[8,9]. Plants growth at low temperatures also leads to oxidative stress through increasing reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion, and hydroxyl radicals[10,11]. The accumulation of ROS causes peroxidation of lipids and oxidation of proteins within cells, resulting in

*Correspondence:
Ph.: +91 11 25843214; +91 7428928799; Fax: +91 11 25846420
E-Mail: amit.tkg@gmail.com

inhibition to plant growth[12,13]. Therefore, to prevent the oxidative injury induced by ROS, plants have evolved a complex antioxidant system including enzymatic antioxidants and non-enzymatic antioxidants such as proline[14,15,].

Cold acclimation is a complex process involving many physiological and biochemical changes including a significant decrease in tissue hydration during the cold hardening process[16,17]. Many plants develop freezing tolerance upon continued exposure to low non-freezing temperatures, a phenomenon known as cold acclimation. The key function of cold acclimation is to stabilize the cell membrane against freezing injury. Cold acclimation prevents expansion-induced lysis and the formation of hexagonal II phase lipids in the rye and other plants[18].

Several physiological and biochemical changes occur in plants in response to low temperatures, including changes in gene expression[1]. A better understanding of genotypic responses to specific environmental factors will contribute to their efficient utilization in papaya development breeding programmes. Very limited efforts have been made to understand the physiological and biochemical changes occurring in papaya under low-temperature stress. An earlier study showed the effects of low temperature on cold sensitive herbaceous species[19]; however, the papaya crop is still not much explored for its response or adaptation to low temperature under controlled conditions. Irrespective of the above facts, neither the germplasm nor the physiology behind the cold stress tolerance in papaya has been studied in depth. Hence, in this study, we have made an attempt to perceive the interactions between the physio-biochemical parameters and low temperature regimes as well as to identify a low temperature tolerant papaya genotype.

Materials and Methods

Plant materials and treatment

The experiments were conducted at the National Phytotron Facility, ICAR-Indian Agricultural Research Institute, New Delhi during 2017-2018. Plant material included five *Carica papaya* L. genotypes (Pusa Nanha, Red Lady, P-7-2, P-7-9, and P-7-14) and one cold tolerant wild genotype (*Vasconcellea cundinamarcensis* V.M. Badillo), commonly called the Mountain papaya. The seeds of papaya genotypes were sown in the seed trays filled with the growing media comprising of perlite, vermiculite, coco-peat and vermicompost (1:1:1:1) and transplanting of seedlings was done 8 weeks after sowing, into plastic pots filled with the above potting medium under the temperature controlled glasshouse. The transplanted plants were irrigated at three-day intervals with tap water to maintain proper moisture conditions and all other recommended standard operations were performed at the proper stage. After the proper establishment of the transplanted seedlings, the temperature treatments were induced by sequentially lowering the temperature of the growth chamber by 2°C per two days interval from 28°/18°C (day/night) to 16°/06°C (day/night) (Table 1). The control plants (T_0) for each genotype were maintained at 28°/18°C (day/night) regime. All other environmental parameters were maintained at the optimum level of other factors (photoperiod of 12 h 30 min.; relative humidity (RH) of 70 ± 5% (day) and 85-90% (night); irradiance of 700-800 µmol $m^{-2}s^{-1}$ at leaf level) in the controlled glasshouse for control and growth chambers for low temperature treatments. A set of plants from each genotype were directly exposed to an 18°/08°C (day/night) temperature regime for one week. These plants were designated as acclimatized plants and were also considered as one treatment during statistical analysis. Three replications comprising of 9 plants per replication for each genotype were maintained for both control and treatments.

Measurement of photosynthetic gas exchange parameters

The leaf gas exchange traits such as internal CO_2 concentration (*Ci*), transpiration rate (*E*), stomatal conductance (g_s), and photosynthetic rate (*A*) were measured on four matured leaves for each replication using LCi-SD UltraCompact Photosynthesis System (ADC BioScientific Ltd., Global House, Hoddesdon, UK) after induction of treatment. ParametersE and g_s were expressed in mol $m^{-2}s^{-1}$, while *A* and *Ci* were expressed in µmol $m^{-2}s^{-1}$and µmol CO_2 mol^{-1}, respectively. Fully expanded leaves at the apex were clamped to the leaf chamber and the observations

Table 1 —Details of controlled temperature regimes maintained under growth chambers

Treatment	Day temp. (°C)	Night temp. (°C)
T_0 (control)	28±0.1	18±0.1
T_1	26±0.1	16±0.1
T_2	24±0.1	14±0.1
T_3	22±0.1	12±0.1
T_4	20±0.1	10±0.1
T_5	18±0.1	08±0.1
T_6	16±0.1	06±0.1
Acclimatization	18±0.1	08±0.1

were recorded when RH and *Ci* reached a stable value. The reading was taken during the forenoon between 9.00 to 11.00 A.M. uniformly in all the replicates.

Measurement of chlorophyll content and fluorescence parameters

Chlorophyll fluorescence parameter Fv/Fm, which is the ratio of variable to maximum fluorescence after dark-adaptation, represents maximum quantum yield of PSII. The parameter has begun to be used for detecting stress in plants. F_M was measured. The quantum efficiency of photosystem II was calculated using the following leaf chlorophyll content was recorded with the help of a chlorophyll meter (SPAD-502 PLUS, Konica Minolta Optics, INC) and expressed in terms of SPAD units. Chlorophyll changes measured in this experiment as one of the indicators of papaya plants responses to low temperature. Leaf chlorophyll fluorescence was estimated by the method given by Jammohammadi *et al.*[20]. Chlorophyll fluorescence parameters, such as minimum chlorophyll fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m), steady-state fluorescence yield (F_s), minimum fluorescence of the light-adapted state (F_0), and maximal fluorescence yield of the light-adapted state (F_m) were measured. All measurements were taken three times. Under 800 μmol m^{-2} s^{-1} light, the leaves of each treated plant reached a steady state after photochemistry, F_s was measured; then under saturated pulsed light (12,000 μmol m^{-2} s^{-1}), F_m was measured. The action light was closed and the far red light was turned on immediately; F_0 was measured after 2 s. After that, dark treatment was carried out for 30 min. with a dark adaptation clip; hence, F_0 and formula of chlorophyll fluorescence ratio:

$$\text{Chlorophyll fluorescence ratio} = \frac{(F_m - F_o)}{F_m} = \frac{F_v}{F_m}$$

Determination of relative water content

The relative water content in recently matured leaves was determined, following the method suggested by Barrs & Weatherley[21]. To reduce the chances of water loss from leaves, the samples were kept in a polythene bag and sealed properly. The bags were then placed in a thermo-cooler having ice flaks (10° to 15°C) and brought to the laboratory as soon as possible. Collected leaves were immediately cleaned with sterile distilled water and made into 8 mm discs with a stainless-steel cork borer. Ten such discs were selected and their fresh weight was measured and then floated over sterile double-distilled water in closed Petri-plates for 4 to 6 h. These discs were then surface dried by placing them in between two sheets of filter paper (Whatman No. 1). The saturated (turgid) weight of these discs was recorded. These samples were then dried in a hot air-oven at 70°C for 2 to 3 days until constant weight. Finally, the dry weight of the samples was recorded. The relative water content was estimated using the following formula:

$$\text{RWC (\%)} = \frac{(\text{Fresh weight} - \text{Oven dry weight})}{(\text{Turgid weight} - \text{Oven dry weight})} \times 100$$

Measurement of membrane stability index

Membrane stability index (MSI) was calculated by taking the electrical conductivity of leaf leachates in double-distilled water at 40° and 100°C by following the method of Sairam[22]. Mature leaf was cut into small pieces and taken (0.5 g) in test tubes having 10 mL of double-distilled water in two sets. One set was kept at 40°C for 30 min. and another set at 100°C in a boiling water bath for 15 min. and their respective electric conductivity's C_1 and C_2 were measured by a conductivity meter. The MSI was calculated using the following formula:

$$\text{MSI (\%)} = 1 - \left[\left(\frac{C1}{C2}\right) \times 100\right]$$

Determination of total sugars content

The total sugars content of the leaf tissue was determined using the Anthrone reagent method described by Sadasivam & Manickam[23]. In brief, fresh leaf samples (0.2 g) were homogenized in 80% ethanol (v/v) in a boiling water bath for 1 h, the supernatant was filtered through filter paper (Whatman No. 1) and (repeated twice). The collected supernatant was boiled with double distilled water (ddw) and make up the volume to 50 mL with ddw. One ml of the sugar sample was added with 4 mL of freshly prepared Anthrone reagent. The mixture was heated on a boiling water bath for 8 min. followed by cooling. The optical density of the cooled green to the dark green colour solution was taken at 630 nm. The concentration of total sugars was calculated by plotting the unknown OD values onto the graph plotted using glucose as a standard and the result was expressed as mg g^{-1} sample.

Determination of total soluble proteins

Fresh samples (1 g) of leaves were crushed into a fine powder using liquid nitrogen and transferred into the extraction buffer (Tris-HCl 100 mM, pH 6.8). The

homogenate was centrifuged at 14,000 rpm for 20 min. at 4°C and the supernatant was used for estimation of the total soluble proteins[24] using; BSA as the standard graph preparation.

Estimation of lipid peroxidation and proline accumulation

Lipid peroxidation is the oxidative degradation of lipid by reactive oxygen species (ROS). The lipid peroxidation was calculated as malondialdehyde (MDA) content using thiobarbituric acid (TBA) following the method of Heath & Packer[25]. The proline content in matured leaves in each treatment was estimated by a rapid colorimetric method[26].

Statistical analysis

The statistical analysis of the data comprised of eight treatments including control (T_0) and acclimatized. Three replications were analyzed in factorial completely randomized block design using statistical analysis system software, SAS package (9.3 SAS Institute, Inc, and USA) followed by t-test (LSD). P values ≤0.05 were considered as significant. Relationships amongst different physiological and biochemical parameters were computed using Pearson's simple correlation at $P \leq 0.01$ and $P \leq 0.05$.

Results

Low temperature stress is a major environmental factor that affects papaya growth and development and influences their productivity. The data presented in the present investigation clearly demonstrated that papaya plants had marked changes in their physiological and biochemical parameters such as photosynthetic gas exchange parameters, chlorophyll content, fluorescence parameters, relative water content (RWC), membrane stability index (MSI), total sugars, total soluble proteins, lipid peroxidation and proline accumulation due to exposure to low temperature regimes.

Physiological parameters associated with photosynthesis

The leaf internal CO_2 concentration (*Ci*) was found to be reduced significantly under the decreasing temperature regimes from T_0 to T_6 (Fig. 1A). The highest *Ci* was observed in the control T_0 (mean of T_0 of all the genotypes: 603.22 µmol CO_2 mol^{-1}), while the lowest was observed in the T_6 (mean of T_6 of all the genotypes: 296.94 µmol CO_2 mol^{-1}). The acclimatized plants (301.17 µmol CO_2 mol^{-1}) registered a statistically significant lower *Ci* value as compared to the control plants. Amongst the genotype × treatment (G × T) interactions, P-7-9 × T_0 (623.66 µmol CO_2 mol^{-1}) registered the highest *Ci* value, while the lowest *Ci* was observed in Red Lady × Accli. (268.66 µmol CO_2 mol^{-1}).

Temperature treated plants showed lower photosynthetic rate (A) value than the control plants (Fig. 1B) and the lowest was observed in T_6 (mean of T_6 of all the genotypes 0.69 µmol $m^{-2}s^{-1}$), while the control (T_0) plants showed the highest (mean of T_6 of all the genotypes 2.91 µmol m^{-2} s^{-1}) photosynthetic rate. Amongst the G × T interaction treatments, Pusa Nanha × T_0 (3.12 µmol m^{-2} s^{-1}) registered the highest

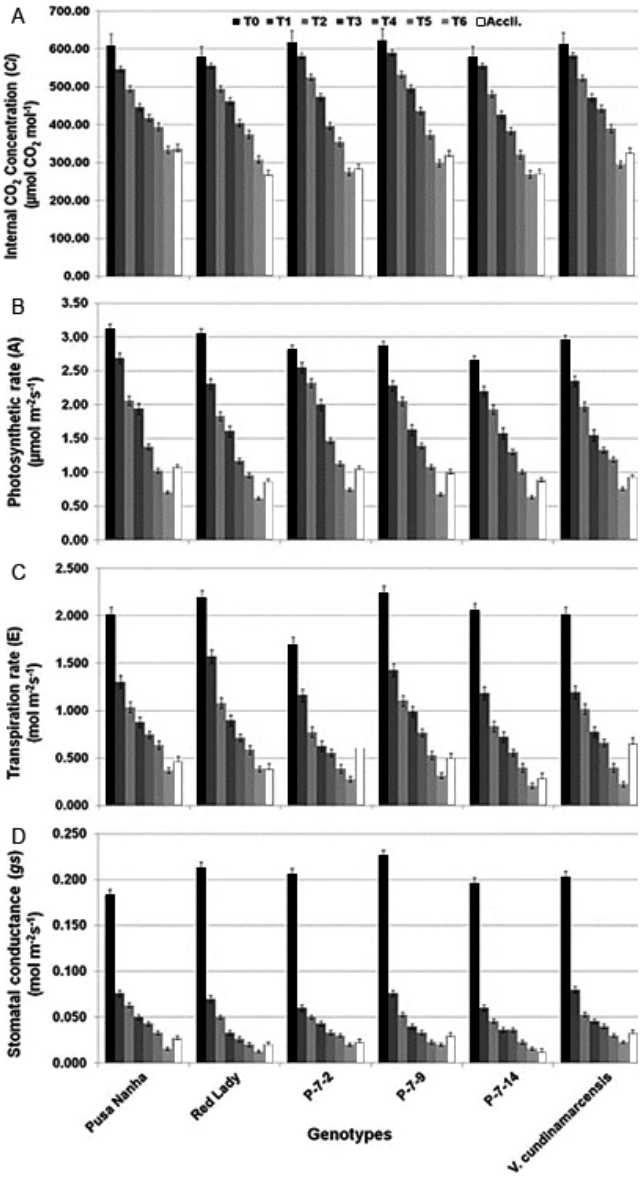

Fig 1 — Effect of different temperature regimes on leaf gas exchange parameters of papaya genotypes grown under controlled phytotron conditions. (A) Internal CO2 concentration; (B) Photosynthesis rate; (C) Transpiration rate; and (D) Stomatal conductance. [Vertical bars indicate ± SE mean]

A content, while the lowest *A* was observed in Red Lady × T_6 (0.62 μmol m^{-2} s^{-1}), which was statistically at par with P-7-14 × T_6 (0.64 μmol m^{-2} s^{-1}), followed by P-7-9 ×T_6 (0.68 μmol m^{-2} s^{-1}).

The control (T_0) plants were found to have the highest (2.03 mol m^{-2} s^{-1}) transpiration rate (E), which was statistically significant compared to all other treatments (Fig. 1C), while the lowest value (0.30 mol m^{-2} s^{-1}) was observed in T_6. The acclimatized plants (0.48 mol m^{-2} s^{-1}) registered a statistically significant lower E content as compared to the control plants. Amongst the genotypes, P-7-9 maintained significantly higher E (0.98 mol m^{-2} s^{-1}) followed by Red Lady (0.83 mol m^{-2} s^{-1}), while it was minimum in P-7-2 (0.76 mol m^{-2} s^{-1}).

The control (T_0) plants were found to have the highest stomatal conductance (g_s), (0.205 mol m^{-2} s^{-1}) followed by T_1 (0.185 mol m^{-2} s^{-1}), while it was registered to be lowest in T_6 (0.018 mol m^{-2} s^{-1}). The acclimatized plants (0.024 mol m^{-2} s^{-1}) registered statistically significant lower g_s value as compared to the control plants (Fig. 1D). Amongst the genotypes, *V. cundinamarcensis* (0.062 mol m^{-2} s^{-1}) exhibited the highest g_s value followed by P-7-9 (0.063 mol m^{-2} s^{-1}). Genotype P-7-14 was observed to have the lowest g_s value (0.053 mol $m^{-2}s^{-1}$). Amongst the G × T interactions, P-7-9 × T_0 (0.226 mol m^{-2} s^{-1}) registered the highest g_s value, the lowest g_s was observed in Red Lady × T_6 (0.013 mol m^{-2} s^{-1}).

Effect of low temperature stress on chlorophyll content and fluorescence

The control (T_0) plants were found to have the highest chlorophyll content (49.27 SPAD value), while it was lowest in T_6 plants (41.80 SPAD value). The acclimatized plants (43.25 SPAD value) registered a statistically significant lower value as compared to the control plants (Table 2). Amongst the six genotypes, P-7-9 exhibited the highest chlorophyll content (48.15 SPAD value) and in G × T interactions, P-7-9 × T_0 (registered the highest chlorophyll value 51.90 SPAD value), while the lowest chlorophyll content was observed in P-7-2 × T_6 (39.30 SPAD value). All genotypes range between 5.57- 8.43 (Table 3).

Low temperature exposed plants expressed significantly lower F_v/F_m ratio than the control (Table 4) and the lowest ratio (0.307) was observed in T_6 followed by T_5 (0.384), while the control (T_0) plants showed the highest (0.822) F_v/F_m ratio. The acclimatized plants (0.307) registered statistically significant lower content as compared to the control plants. All the genotypes exhibited statistically different F_v/F_m ratio values. Amongst the six genotypes, *V. cundinamarcensis* exhibited the highest F_v/F_m ratio (0.651) followed by P-7-9 (0.542) and the lowest F_v/F_m was observed in Red Lady (0.454) followed by P-7-2 (0.461).

In chlorophyll fluorescence, range of variation of values of Pusa Nanha, Red Lady, P-7-2, P-7-9,

Table 2 — Influence of different temperature regimes on total chlorophyll content (SPAD index) of papaya genotypes grown under phytotron conditions. [G, Genotypes; T, Treatments; G × T, Genotypes × Treatments]

Genotype	T_0 28/18	T_1 26/16	T_2 24/14	T_3 22/12	T_4 20/10	T_5 18/08	T_6 16/06	Accl. 18/08	Mean
Pusa Nanha	49.53^{bac}	49.06^{bdec}	48.06^{fhdecg}	$47.86^{fhdeicg}$	$46.43^{fhkmjeilg}$	$45.53^{ohkmjnil}$	43.10^{opstrq}	43.73^{opsnrq}	46.66^{b}
Red Lady	$47.90^{fhdeicg}$	$47.26^{fhkdjeicg}$	$46.66^{fhkdjeilg}$	$45.90^{hkmjnilg}$	$44.76^{opkmnlq}$	$44.16^{opmnrlq}$	42.33^{ustrq}	42.90^{opstrq}	45.23^{c}
P-7-2	$47.73^{fhdjeicg}$	$45.20^{opkmjnl}$	$44.23^{opmnrlq}$	41.73^{ustrv}	40.96^{utv}	40.13^{uv}	39.30^{v}	39.53^{v}	42.35^{d}
P-7-9	51.90^{a}	50.96^{ba}	49.26^{bdac}	48.36^{fbdecg}	$47.63^{fhdjeicg}$	$46.50^{fhkmjeilg}$	$44.06^{opmsnrlq}$	$46.56^{fhkmjeilg}$	48.15^{a}
P-7-14	49.76^{bac}	$47.60^{fhdjeicg}$	$46.40^{fhkmjilg}$	$45.40^{opkmjnil}$	$44.30^{opmnrlq}$	$44.30^{opmnrlq}$	41.46^{ustv}	$43.93^{opmsnrq}$	45.95^{cb}
V. cund.	48.83^{fbdec}	48.13^{fhdecg}	$47.36^{fhkdjeicg}$	$46.13^{hkmjnilg}$	$45.10^{opkmjnl}$	43.63^{opsnrq}	40.56^{utv}	42.86^{pstrq}	45.32^{c}
Mean	49.27^{a}	48.24^{ba}	47.20^{b}	46.06^{c}	45.05^{dc}	44.04^{de}	41.80^{f}	43.25^{e}	
LSD ($P \leq 0.05$)									
Genotype (G)									0.94
Temp. (T)									1.08
G × T									2.65

Table 3 — Range value of amongst various physiological and biochemical parameters as per the genotypes

Genotype	Total chlorophyll	Chlorophyll fluorescence	Relative water content	Membrane stability index	Total sugar content	Membrane lipid peroxidation	Leaf total proteins content	Proline content
Pusa Nanha	6.43	0.622	17.24	26.65	26.15	14.74	1.01	0.22
Red Lady	5.57	0.533	18.97	31.45	24.45	19.93	0.55	0.23
P-7-2	8.43	0.566	16.10	19.98	30.30	15.21	0.73	0.24
P-7-9	7.84	0.556	15.70	17.71	23.11	20.80	0.80	0.26
P-7-14	8.30	0.452	15.31	28.22	16.54	17.37	1.04	0.25
V. cund.	8.27	0.36	12.35	29.28	17.88	15.60	0.55	0.31

Table 4 — Influence of different temperature regimes on chlorophyll fluorescence (F_v/F_m) of papaya genotypes grown under phytotron conditions. [G, Genotypes; T, Treatments; G × T, Genotypes × Treatments]

	T_0	T_1	T_2	T_3	T_4	T_5	T_6	Accl.	
Genotype	28/18	26/16	24/14	22/12	20/10	18/08	16/06	18/08	Mean
Pusa Nanha	0.876^{ba}	0.724^{e}	0.672^{f}	0.581^{ih}	0.428^{l}	0.344^{o}	0.254^{qr}	0.326^{o}	0.526^{c}
Red Lady	0.759^{d}	0.633^{g}	0.536^{j}	0.499^{k}	0.378^{n}	0.325^{o}	0.226^{s}	0.272^{qp}	0.454^{e}
P-7-2	0.797^{c}	0.676^{f}	0.558^{ij}	0.433^{l}	0.397^{n}	0.330^{o}	0.231^{sr}	0.267^{q}	0.461^{ed}
P-7-9	0.895^{a}	0.730^{e}	0.628^{g}	0.543^{j}	0.476^{k}	0.398^{mn}	0.339^{o}	0.329^{o}	0.542^{b}
P-7-14	0.749^{ed}	0.688^{f}	0.595^{h}	0.487^{k}	0.395^{n}	0.329^{o}	0.297^{p}	0.225^{s}	0.471^{d}
V. cund.	0.853^{b}	0.798^{c}	0.734^{ed}	0.689^{f}	0.637^{g}	0.578^{ih}	0.493^{k}	0.425^{ml}	0.651^{a}
Mean	0.822^{a}	0.708^{b}	0.621^{c}	0.539^{d}	0.452^{e}	0.384^{f}	0.307^{g}	0.307^{g}	
LSD ($P \leq 0.05$)									
Genotype (G)									0.009
Temp. (T)									0.011
G × T									0.028

Table 5 —Influence of different temperature regimes on relative water content (%) of papaya genotypes grown under phytotron conditions. [G, Genotypes; T, Treatments; G × T, Genotypes × Treatments]

	T_0	T_1	T_2	T_3	T_4	T_5	T_6	Accl.	
Genotype	28/18	26/16	24/14	22/12	20/10	18/08	16/06	18/08	Mean
Pusa Nanha	92.01^{a}	91.46^{ba}	88.61^{ehdgcf}	83.77^{knmol}	80.14^{qsurt}	77.41^{vyuxzw}	74.77^{az}	77.34^{vyxzw}	83.19^{b}
Red Lady	91.06^{bdac}	90.64^{ebdac}	86.75^{hjgi}	82.56^{qnpmo}	78.77^{vsutw}	75.83^{yxz}	72.09^{a}	75.84^{yxz}	81.69^{c}
P-7-2	90.86^{ebdac}	89.78^{ebdacf}	87.67^{hgif}	85.23^{kjmil}	81.30^{qspro}	78.10^{vsutw}	74.76^{az}	78.80^{vsutw}	83.31^{b}
P-7-9	92.36^{a}	91.17^{bac}	88.35^{ehdgf}	85.91^{khjil}	83.33^{npmol}	80.62^{qsprt}	76.66^{yxzw}	79.69^{vsurt}	84.76^{a}
P-7-14	90.40^{ebdacf}	88.72^{ebdgcf}	86.38^{khjgi}	83.32^{npmol}	80.74^{qsprt}	77.57^{vyuxw}	75.09^{yz}	74.48^{az}	82.09^{cb}
V. cund.	91.67^{a}	90.45^{ebdacf}	88.08^{ehgf}	86.04^{khjgi}	84.26^{knjml}	82.05^{qnpro}	79.32^{vsurtw}	80.62^{qsprt}	85.31^{a}
Mean	91.40^{a}	90.37^{a}	87.64^{b}	84.47^{c}	81.42^{d}	78.60^{e}	75.45^{f}	77.80^{e}	
LSD ($P \leq 0.05$)									
Genotype (G)									0.99
Temp. (T)									1.14
G × T									2.79

P-7-14 and *V. cundinamarcensis* were 0.622, 0.533, 0.566, 0.556, 0.452 and 0.36, respectively (Table 3).

Effect of low temperature stress on relative water content

The interaction effects of low temperature stress treatment and papaya genotype were found significant for leaf relative water content (RWC) (Table 5). Temperature treated plants showed lower RWC value than the control plants and the lowest was observed in T_6 (mean of RWC of all the genotypes in T_6 (75.45%), while the control (T_0) plants showed the highest (91.40%) followed by T_1 (90.37%). The acclimatized plants (78.80%) registered a statistically significant lower content as compared to the control plants. Amongst the six genotypes, *V. cundinamarcensis* exhibited the highest RWC (85.31%), which is statistically similar to the RWC of P-7-9 (84.76%). The lowest RWC was noted in Red Lady (81.69%), followed by P-7-14 (82.09%). Amongst the G × T interactions, P-7-9 × T_0 (92.36%) registered the highest content, while the lowest value was observed in Red Lady × T_6 (72.09%). In relative water content variations in the range were between 12.35-18.97 (Table 3).

Effect of low temperature stress on membrane stability index

Low temperature exposed plants showed the lower MSI value than the control plants (Table 6) and accordingly the lowest (48.67%) was observed in T_6, while the control (T_0) plants showed the highest (74.22%) membrane stability. Amongst the six genotypes, *V. cundinamarcensis* exhibited the highest MSI (65.14%), which was statistically similar to P-7-9 (64.52%). The lowest MSI was noted in Red Lady (56.94%). Amongst the G × T interactions, *V. cundinamarcensis* × T_0 (78.02%) registered the highest MSI followed by P-7-14 × T_0 (75.25%). The lowest MSI was observed in Red Lady × T_6 (40.13%). The data in (Table 3) indicates that the overall MSI ranged from 17.71-31.45.

Effect of low temperature stress on total soluble sugar

Temperature treated plants showed higher total sugars content in the leaves than the control plants (Table 7) and the highest value (58.36 mg g^{-1}) was observed in T_6 followed by T_5 (54.56 mg g^{-1}), while the control (T_0) plants showed the lowest total sugars content (42.32 mg g^{-1}). Amongst the six genotypes, *V. cundinamarcensis* exhibited the highest total sugars content (67.21 mg g^{-1}) followed by P-7-14 (60.43 mg g^{-1}) and P-7-9 (56.19 mg g^{-1}).The lowest total sugars

Table 6 — Influence of different temperature regimes on membrane stability index (%) of papaya genotypes grown under phytotron conditions. [G, Genotypes; T, Treatments; G × T, Genotypes × Treatments]

Genotype	T_0 28/18	T_1 26/16	T_2 24/14	T_3 22/12	T_4 20/10	T_5 18/08	T_6 16/06	Accl. 18/08	Mean
Pusa Nanha	74.10^{bdac}	$71.81^{fbdehcg}$	68.83^{fijhkg}	65.25^{onmlkp}	59.92^{strq}	54.45^{wxvu}	47.45^{ba}	49.90^{byaz}	61.46^{b}
Red Lady	$71.58^{fbdehcg}$	68.34^{ijlhkg}	65.77^{onmlk}	61.94^{onrqp}	56.77^{wstvu}	50.11^{xyaz}	40.13^{c}	40.86^{c}	56.94^{c}
P-7-2	72.89^{fbdec}	$70.73^{fidjehg}$	67.71^{imjlhk}	64.26^{onmlqp}	61.27^{osrqp}	57.25^{stru}	52.91^{wxyvz}	50.55^{xyaz}	62.19^{b}
P-7-9	73.46^{bdec}	72.58^{fbdecg}	$70.79^{fidjehcg}$	66.45^{nmjlk}	63.83^{onmlqp}	60.86^{srqp}	55.75^{wtvu}	52.46^{wxyz}	64.52^{a}
P-7-14	75.25^{bac}	73.74^{bdac}	$70.97^{fidehcg}$	66.8^{imjlk}	61.05^{srqp}	54.02^{wxyvu}	47.03^{ba}	45.54^{b}	61.80^{b}
V. cund.	78.02^{a}	$75.90b^{a}$	72.74^{fbdecg}	68.99^{fijhkg}	63.70^{onmqp}	57.20^{stuv}	48.74^{baz}	55.86^{wtvu}	65.14^{a}
Mean	74.10^{bdac}	$71.81^{fbdehcg}$	68.83^{fijhkg}	65.25^{onmlkp}	59.92^{strq}	54.45^{wxvu}	47.45^{ba}	49.90^{byaz}	
LSD ($P \leq 0.05$)									
Genotype (G)									1.59
Temp. (T)									1.85
G × T									4.52

Table 7 — Influence of different temperature regimes on total sugar content (mg g^{-1}FW) of papaya genotypes grown under phytotron conditions. [G, Genotypes; T, Treatments; G × T, Genotypes × Treatments]

Genotype	T_0 28/18	T_1 26/16	T_2 24/14	T_3 22/12	T_4 20/10	T_5 18/08	T_6 16/06	Accl. 18/08	Mean
Pusa Nanha	27.51^{y}	30.72^{x}	31.73^{xw}	32.71^{w}	37.03^{ut}	37.15^{ut}	41.29^{sr}	53.66^{kj}	36.47^{f}
Red Lady	33.14^{w}	35.10^{v}	35.59^{uv}	38.53^{t}	42.63^{r}	45.33^{q}	48.25^{op}	57.59^{i}	42.02^{e}
P-7-2	36.59^{uv}	40.92^{s}	41.39^{sr}	42.49^{sr}	48.14^{op}	49.18^{on}	55.05^{j}	66.89^{d}	47.58^{d}
P-7-9	46.98^{p}	50.29^{mn}	51.32^{ml}	52.25^{kl}	57.05^{i}	60.09^{gh}	61.50^{gf}	70.09^{cb}	56.19^{c}
P-7-14	52.03^{l}	54.76^{j}	56.90^{i}	59.34^{h}	62.28^{f}	64.72^{e}	68.57^{c}	64.83^{e}	60.43^{b}
V. cund.	57.65^{i}	61.42^{gf}	64.68^{e}	66.65^{d}	68.68^{c}	70.89^{b}	75.53^{a}	72.18^{cb}	67.21^{a}
Mean	42.32^{h}	45.53^{g}	46.93^{f}	48.66^{e}	52.63^{d}	54.56^{c}	58.36^{b}	64.20^{a}	
LSD ($P \leq 0.05$)									
Genotype (G)									0.57
Temp. (T)									0.66
G × T									1.61

content was observed in Pusa Nanha (36.47 mg g^{-1}). About G × T interactions, *V. cundinamarcensis* × T_6 (75.53 mg g^{-1}) registered the highest total sugars content followed by P-7-14 (68.57 mg g^{-1}) and then P-7-9 (61.6 mg g^{-1}). The data in (Table 3) indicates that the overall total soluble sugar ranged from 16.54-30.30.

Effect of low temperature stress on lipid peroxidation

The levels of lipid peroxidation in papaya leaves were expressed as malondialdehyde (MDA) content (Table 8). Temperature treated plants showed a higher value of lipid peroxidation in the leaves than the control plants and the highest (54.08 µmol g^{-1} FW) was observed in T_6, which was statistically different from all other genotypes. While the control (T_0) plants showed the lowest value (36.81 µmol g^{-1} FW). Amongst the six genotypes, P-7-9 exhibited the highest MDA content (71.19 µmol g^{-1}FW), which was statistically different from all other genotypes. The lowest MDA content was noted in *V. cundinamarcensis* (27.54 µmol g^{-1}FW), followed by Pusa Nanha (31.13 µmol g^{-1}FW). In MDA, range of variation of values of Pusa Nanha, Red Lady, P-7-2, P-7-9, P-7-14 and *V. cundinamarcensis* were 14.74, 19.93, 15.21, 20.80, 17.37, and 15.60 respectively (Table 3).

Effect of low temperature stress on protein content and proline concentration

In treatment regime T_6 the plants had significantly higher protein content (2.35 µg protein μL^{-1}) followed by T_5 (2.27 µg protein μL^{-1}). The control plants (T_0) had the lowest protein content (1.66 µg protein μL^{-1}). The acclimatized plants (1.94 µg protein μL^{-1}) registered statistically significant higher total soluble proteins content as compared to the control plants (Table 9). The genotype *V. cundinamarcensis* exhibited the highest soluble proteins content (2.33 µg protein μL^{-1}), which was statistically different from all other genotypes. While the lowest value of total soluble proteins content was observed in Red Lady (1.69 µg protein μL^{-1}). Amongst the G × T interactions, the maximum total soluble proteins content was observed in the *V. cundinamarcensis* × T_6 (2.48 µg protein μL^{-1}), followed by P-7-9 (2.45 µg protein μL^{-1}) and P-7-2 (2.42 µg protein μL^{-1}). The lowest total soluble proteins content was observed in Pusa Nanha × T_0 (1.40 µg protein μL^{-1}). In total soluble proteins, range of variation of values of Pusa

Table 8 — Influence of different temperature regimes on membrane lipid peroxidation (µmol g^{-1} FW) of papaya genotypes grown under phytotron conditions. [G, Genotypes; T, Treatments; G × T, Genotypes × Treatments]

	T_0	T_1	T_2	T_3	T_4	T_5	T_6	Accl.	
Genotype	28/18	26/16	24/14	22/12	20/10	18/08	16/06	18/08	Mean
Pusa Nanha	24.51^{d}	25.36^{cd}	25.90^{cb}	30.52^{y}	33.65^{w}	35.50^{v}	39.25^{t}	34.38^{w}	31.13^{e}
Red Lady	45.78^{p}	47.54^{o}	53.37^{m}	60.62^{i}	63.79^{h}	64.76^{g}	65.71^{f}	56.84^{k}	57.30^{b}
P-7-2	29.60^{z}	30.73^{y}	32.47^{x}	37.68^{u}	38.25^{u}	43.64^{r}	44.81^{q}	38.51^{ut}	36.96^{d}
P-7-9	59.54^{j}	64.01^{hg}	68.79^{e}	71.50^{d}	75.05^{c}	78.53^{b}	80.34^{a}	71.78^{d}	71.19^{a}
P-7-14	41.40^{s}	45.87^{p}	48.28^{o}	50.71^{n}	54.67^{l}	55.97^{k}	58.77^{j}	50.53^{n}	50.77^{c}
V. cund.	20.02^{g}	21.68^{f}	23.45^{e}	26.48^{b}	28.40^{a}	31.08^{y}	35.62^{v}	33.67^{w}	27.54^{f}
Mean	36.81^{h}	39.19^{g}	42.04^{f}	46.25^{e}	48.97^{c}	51.58^{b}	54.08^{a}	47.62^{d}	
LSD ($P \leq 0.05$)									
Genotype (G)									0.31
Temp. (T)									0.35
G × T									0.87

Table 9 — Influence of different temperature regimes on leaf total proteins content (µg protein µl $^{-1}$ enzyme extract) of papaya genotypes grown under phytotron conditions. [G, Genotypes; T, Treatments; G × T, Genotypes × Treatments]

	T_0	T_1	T_2	T_3	T_4	T_5	T_6	Accl.	
Genotype	28/18	26/16	24/14	22/12	20/10	18/08	16/06	18/08	Mean
Pusa Nanha	1.40^{s}	1.55^{orsqp}	$1.75^{olnrsqmp}$	$1.92^{olkinhjgm}$	$2.13^{ekidhjgcf}$	2.34^{ebdac}	2.41^{bdac}	$1.91^{olkinhjgm}$	1.93^{cd}
Red Lady	1.52^{rsqp}	1.44^{rs}	1.52^{rsqp}	1.6^{onrsqp}	$1.76^{olknrsqmp}$	$1.81^{olkinrjqmp}$	$1.94l^{kinhjgmf}$	$1.99^{elkihjgmf}$	1.69^{e}
P-7-2	$1.69^{onrsqmp}$	$1.87^{olkinhjmp}$	$2.05^{elkidhjgmf}$	$2.11^{elkidhjgcf}$	$2.18^{ebidhagcf}$	2.31^{ebdacf}	2.42^{bdac}	$1.83^{olkinhjqmp}$	2.06^{cb}
P-7-9	1.47^{rsq}	1.53^{rsqp}	$1.77^{olknrsqmp}$	$1.86^{olkinhjmp}$	$1.91^{olkinhjgm}$	$2.16^{ebidhjgcf}$	$2.27^{ebdagcf}$	$2.2^{ebdhagcf}$	1.89^{d}
P-7-14	1.51^{rsqp}	$1.71^{onrsqmp}$	$1.91^{olkinhjgm}$	$2.16^{ebidhjgcf}$	2.35^{ebdac}	2.52^{ba}	2.55^{a}	$1.8o^{lknrjqmp}$	2.06^{b}
V. cund.	2.42^{bdac}	2.36^{ebdac}	2.39^{bdac}	2.33^{ebdac}	$2.28^{ebdagcf}$	2.43^{bac}	2.48^{bac}	$1.93^{olkinhjgm}$	2.33^{a}
Mean	1.66^{d}	1.74^{d}	1.89^{c}	1.99^{cb}	2.10^{b}	2.27^{a}	2.35^{a}	1.94^{c}	
LSD ($P \leq 0.05$)									
Genotype (G)									0.13
Temp. (T)									0.15
G × T									0.38

Table 10 — Effect of different temperature regimes on proline content (µM proline/g^{-1}FW) of papaya genotypes grown under phytotron conditions. [G, Genotypes; T, Treatments; G × T, Genotypes × Treatments]

	T_0	T_1	T_2	T_3	T_4	T_5	T_6	Accl.	
Genotype	28/18	26/16	24/14	22/12	20/10	18/08	16/06	18/08	Mean
Pusa Nanha	0.12^{u}	0.14^{ust}	0.18^{op}	0.20^{on}	0.23^{lm}	0.27^{ihj}	0.34^{fe}	$0.23l^{m}$	0.21^{f}
Red Lady	0.13^{ut}	0.16^{rsq}	0.19^{o}	0.23^{lm}	0.26^{ikj}	0.31^{g}	0.36^{de}	$0.25l^{kj}$	0.24^{e}
P-7-2	0.15^{rs}	0.18^{opq}	0.22^{nm}	0.27^{ihj}	0.31^{g}	0.34^{fe}	0.39^{c}	0.24^{lkm}	0.26^{c}
P-7-9	0.16^{rpq}	0.19^{o}	0.24^{lkm}	0.28^{h}	0.33^{fg}	0.38^{dc}	0.42^{b}	0.35^{fe}	0.29^{b}
P-7-14	0.13^{ut}	0.16^{rsq}	0.19^{o}	0.24^{lkm}	0.28^{h}	0.33^{fg}	0.38^{dc}	0.28^{h}	0.25^{d}
V. cund.	0.15^{rst}	0.19^{o}	0.24^{lkm}	0.28^{h}	0.35^{fe}	0.41^{b}	0.46^{a}	0.38^{dc}	0.31^{a}
Mean	0.14^{g}	0.17^{f}	0.21^{e}	0.25^{d}	0.29^{c}	0.34^{b}	0.39^{a}	0.29^{c}	
LSD ($P \leq 0.05$)									
Genotype (G)									0.008
Temp. (T)									0.009
G × T									0.022

Nanha, Red Lady, P-7-2, P-7-9, P-7-14 and *V. cundinamarcensis* were 1.01, 0.55, 0.73, 0.80, 1.04 and 0.55, respectively (Table 3).

With the decreasing temperature regimes, the concentration of proline in the leaves was found to be increasing (Table 10) and the T_6 plants recorded the maximum proline content (0.39 µM. g^{-1} FW), while it was minimum in control (0.14 µM. g^{-1} FW). The acclimatized plants (0.29 µM. g^{-1} FW) registered statistically significant higher total soluble proteins content as compared to the control plants. The genotype *V. cundinamarcensis* exhibited the highest proline content (0.31 µM. g^{-1} FW) followed by P-7-9 (0.29 µM g^{-1} FW), while the lowest value of total proline content was observed in Pusa Nanha (0.21 µM. g^{-1} FW). In total proline content, range of variation of values of Pusa Nanha, Red Lady, P-7-2, P-7-9, P-7-14 and *V. cundinamarcensis* were 0.22, 0.23, 0.24, 0.26, 0.25 and 0.31, respectively (Table 3).

Correlation and regression in inter combination parameters

The correlation study indicated significant correlations amongst all the possible inter combinations of parameters except for total sugars

with total soluble proteins content (Table 11). A significant positive correlation was found for MSI with RWC, chlorophyll content with RWC and MSI, chlorophyll fluorescence with RWC, MSI, and chlorophyll content. However, negative correlations were observed amongst MDA with RWC, MSI, chlorophyll content and chlorophyll fluorescence. Proline content was observed to be negatively correlated with all other parameters except MDA content.

The regression analysis between membrane stability index (%) and different parameters at the 16/06°C temperature regime revealed a higher R2 value for total proteins (0.8279) followed by RWC (0.7908) and proline (0.4876) (Fig. 2 E, A & F). chlorophyll fluorescence was found had the lowest (0.539) R2

Table 11 — Correlation coefficient of amongst various physiological and biochemical parameters as per the genotype means of each low temperature regime

	RWC	MSI	Chl. content	Chl. fluor.	MDA	Proline	Total Sugar	TSP
RWC	1.000							
MSI	0.981**	1.000						
Chl. content	0.992**	0.981**	1.000					
Chl. fluor.	0.985**	0.972**	0.983**	1.000				
MDA	–0.960**	–0.890**	–0.951**	–0.937**	1.000			
Proline	–0.969**	–0.915**	–0.967**	–0.939**	0.993**	1.000		
Total Sugar	–0.918**	–0.966**	–0.919**	–0.944**	0.782*	0.802*	1.000	
TSP	–0.885**	–0.792*	–0.878**	–0.843**	0.972**	0.970**	0.639^{NS}	1.000

[RWC, Relative Water Content; MSI, Membrane Stability Index; Chl. content, Chlorophyll content; Chl. fluor., Chlorophyll fluorescence; MDA, Malondialdehyde content; TSP, Total Soluble protein content. **Significant at $P \leq 01\%$, *Significant at $P \leq 05\%$ and ^{NS} Non Significant]

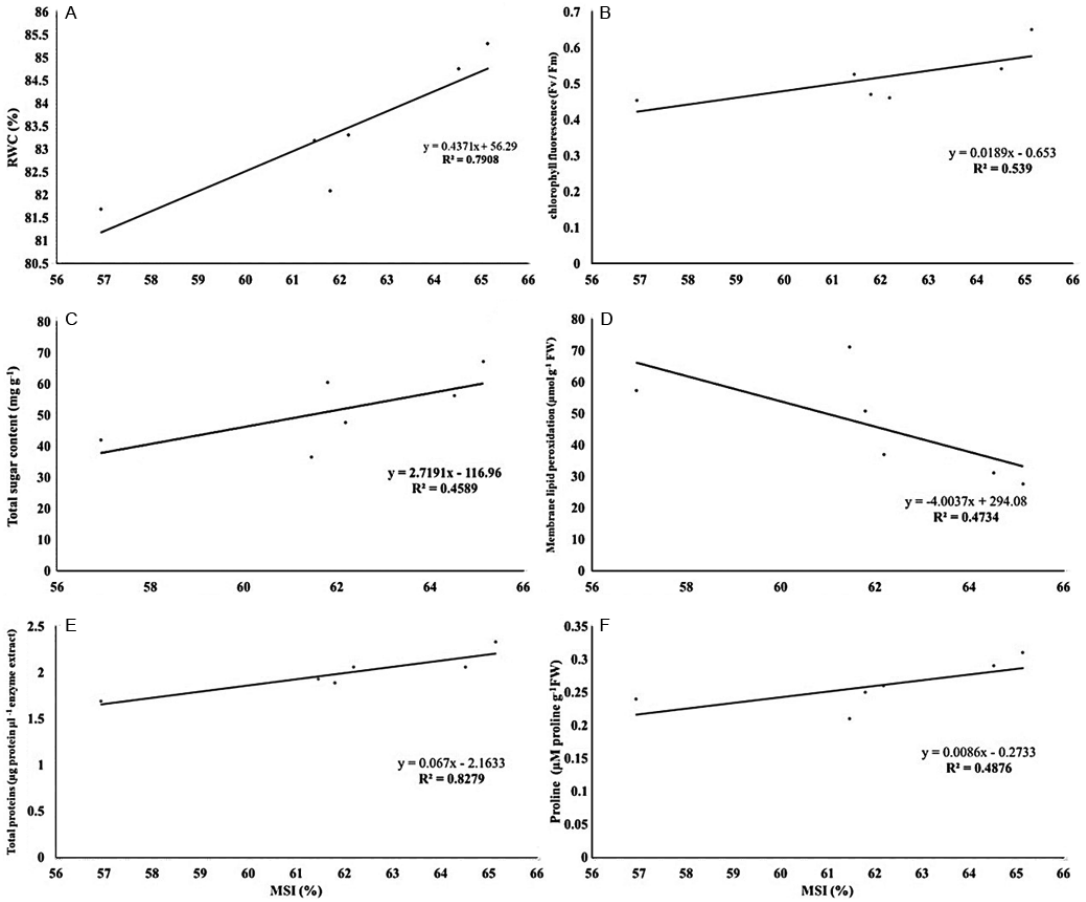

Fig 2 — Regression analysis of physiological and biochemical parameters with relation to MSI (%) as affected by low temperature regime of 16/06°C (day/night) in papaya genotypes. (A) RWC; (B) Chlorophyll fluorescence; (C) Total sugar content; (D) MDA content; (E) Total proteins; and (F) Proline. [X-axis corresponds to MSI (%), while Y-axis to physiological and biochemical parameters at 16/06°C (day/night) temperature regime]

value followed by total sugar content (0.4589) and membrane lipid peroxidation (0.4734) (Fig. 2 B, C & D). A positive value was found for the coefficient of 'x' variable in all the regression equations.

Discussion

Effect of low temperature stress on photosynthetic gas exchange parameters

Low temperature stress altered the normal rates of photosynthesis and other gas exchange attributes in crop plants. Various reports state that low temperature stress results in low photosynthetic rates in crop plants which also attribute to poor conductance of CO_2 in stomatal and mesophyll cells, impaired chloroplastic development, restricted metabolite transport, decreased quantum efficiency, and the quantum yield for CO_2 assimilation[27,28]. In the present study, the highest reduction in leaf internal CO_2 concentration due to low temperature stress (from 28°day/18°C night to16° day/06°C night) was recorded in P-7-2 (55.37 %), while it was lowest in Pusa Nanha (45.13 %). In the acclimatized plants, the same genotype P-7-2 (53.91 %) registered the highest reduction as compared to the control plants, while it was lowest in Pusa Nanha (44.75 %).

Grau & Halloy[29] have reported that after 4 days of exposure to low temperature regime (15° day/ 5°C night) photosynthesis rate was significantly reduced in the papaya genotypes and it was observed up to 15 % of control (25° day/15°C night). The photosynthetic rate varied significantly among the genotypes. Campostrini *et al.*[30] has reported maximum photosynthetic rates (25 μmolm^{-2} s^{-1}) for papaya variety 'Baixinho de Santa Amalia' and 20 μmol m^{-2} s^{-1} for varieties 'Sunrise Solo72/12','Sunrise Solo TJ' and 'Know-You'. Earlier Pradhan *et al.*[31] also reported in a comparative study between various papaya genotypes, the cold tolerant wild relative *V. cundinamarcensis* exhibited a lower transpiration rate as compared to other susceptible *Carica papaya* genotypes.

Reduction in stomatal conductance was observed under low temperature regimes and there was a 25-30 % decrease in g_s observed values as compared to the control. A similar reduction in the stomatal conductance in *Coffea arabica* L. under the low temperature regimes during winter has also been reported by Barros *et al.*[32]. Sudden changes in photosynthetic photon flux density influenced the interaction of photosynthetic rate and stomatal conductance in 'Red Lady' papaya leaves and demonstrated that the photosynthetic response of papaya is strongly linked to environmental conditions through stomatal behaviour[33].

Effect of low temperature stress on chlorophyll content and fluorescence

Low temperature regimes tended to decrease the leaf chlorophyll contents in all the papaya genotypes, however, the effect was most pronounced in P-7-2 (17.66 %) and least pronounced in Red Lady (11.62 %) compared to control plants. Glaszmann *et al.*[34] who reported the inhibition of chlorophyll accumulation in leaves of rice under low temperature stress have also found that the cold tolerant lines had higher chlorophyll, accumulation under cold stress than cold sensitive ones.

The value of *Fv/Fm* is reported to be close to 0.80 in healthy leaves, independently of the plant species[35]. *Fv/Fm* is an indicator of the maximum photochemical efficiency of PSII and a lower *Fv/Fm* value indicates the damage of the PSII reaction centre, a phenomenon called photoinhibition often observed in plants under stress condition. Earlier, Shi *et al.*[36] reported a 29.4 % loss of the maximum photochemical efficiency of PSII after freezing treatment in tea cultivars. Various reports state chlorophyll fluorescence imaging could be an alternative method for the determination of freezing tolerance in *Arabidopsis*[37] and soybean[38]. The result showed that the control plants of all genotypes showed the value of the *Fv/Fm* ratio nearer to 0.80, which was observed to be highly reduced at the temperature regime of 16° (day) and 06°C (night) except in *V. cundinamarcensis* (0.493). Within the papaya genotypes, low temperature stress treatment decreased the *Fv/Fm* ratio. Though it was highest in Pusa Nanha (71.00 %) and lowest in *V. cundinamarcensis* (42.20 %), respectively.

Effect of low temperature stress on relative water content and membrane stability index (MSI)

Low temperature treatment reduced the leaf relative water content, which and the highest reduction was in Red Lady (20.83 %), while the lowest was noted in *V. cundinamarcensis* (13.47 %). Barros *et al.*[32] reported a decline in leaf water potential in coffee with a lowering of temperatures, and Jeyakumar *et al.*[39] have reported the physiological performance of papaya genotypes under abiotic stress, where leaf RWC had a significant influence on photosynthesis. In the present study, similar trends

were observed, wherein the highest reduction in both photosynthetic rate and leaf RWC was observed in Red Lady.

Several states report significant depression in the membrane stability index (MSI) under cold stress as in wheat and rice[40]. Earlier Steponkus *et al*.[41] also reported the membrane systems of the cell are the primary site of freezing injury in plants. In the present study too, it was found that low temperature treated plants showed the lower membrane stability index than the non-stressed control plants. The lowest (48.66 %) was observed in 16°C day/06°C night temperature regime, while the highest (74.21 %) was in control plant sunder 28°C day/18°C night temperature regime.

Effect of low temperature stress on total soluble sugar

The total sugars content was observed to increase dramatically within the papaya genotypes under the low temperature regimes. In bud sand cortical tissues of Chardonnay and Riesling grape (*Vitis vinifera*), high levels of glucose, stachyose, fructose, and raffinose were highly correlated with cold hardines[42]. Stushnoff *et al*.[43] found a positive correlation between cold hardiness in cortical tissues in Red Delicious apple with sorbitol, total sugars, and raffinose. Fernandez *et al*.[44] reported that *Burkholderia phytofirmans* acclimated grapevine to cold stress by modulating carbohydrate metabolism. The concentration of most of the sugars, *i.e*., sucrose, fructose, mannose, raffinose, galactinol, glucose, and maltose were elevated during the exposure of grapevines to low temperature in the *B. phytofirmans* inoculated plants as compared to the controls. The result showed that the highest mean total sugars level was noted in *V. cundinamarcensis* (67.21 mg g^{-1}) followed by in P-7-14 (60.43 mg g^{-1}).

Effect of low temperature stress on MDA

The MDA content was observed to increase within the papaya genotypes due to exposure to decreasing temperature regimes. Earlier, Alonso *et al*.[2] also reported a significant increase in malondialdehyde content in roots of coffee seedlings exposed to 10°C for 6 days as compared to control (25°C). Free radical-induced peroxidation of membrane lipids might lead to membrane dysfunction through modification in fluidity and increased ion permeability, thus reducing the membrane stability index. There was an increase in the malondialdehyde level due to low temperature induction, which reflects lipid peroxidation leading to membrane damage and photo-oxidation. The rise in malondialdehyde content measured in the low temperature treated plants indicated an increased rate of oxidation of membrane lipids, which presumably lead to membrane injury. Xu *et al*.[45] have reported increased MDA concentration in strawberry on exposure to chilling stress. Amongst the six genotypes, P-7-9 exhibited the highest (71.19 µmol g^{-1}FW) malondialdehyde accumulation, while the lowest (27.54 µmol g^{-1}FW) was noted in *V. cundinamarcensis*. The lower malondialdehyde content and higher membrane stability index in *V. cundinamarcensis* indicated of stable cell membrane under the low temperature regimes.

Effect of low temperature stress on protein content and proline concentration

The total soluble proteins content was observed to increase within the papaya genotypes under the decreasing temperature regimes. In response to the low temperature stress, the plants synthesize some of the most hydrophilic proteins. Amongst these proteins dehydrins belonging to group II of the LEA proteins, are most abundant during cold acclimatization and participated in the stabilization of membranes against freeze-induced injury through sequestering functions[46]. It was noted that the highest increase in total soluble proteins content from 28º day/18ºC night to 16 day/06ºC night temperature was noted in genotype *V. cundinamarcensis* (72.14 %), while the lowest was in Pusa Nanha (2.47 %). Lee & Lee[47] have also reported a significant increase in the leaf protein content of cucumber during the period of chilling stress (4°C) for 12 h as compared to the control (25°C) and there was a significant increase in protein content in the chilling stressed-plants, which appeared to be due to the decrease in relative water content. Our present results are in confirmation with those of our earlier findings[48], where we had observed a higher accumulation of total soluble proteins in papaya leaves on exposure to low temperature stress.

The proline content was observed to increase within the papaya genotypes under the decreasing temperature regimes. Proline which is an amino acid is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. Kaplan *et al*.[49] reported a doubling of proline content in *Arabidopsis*, under cold stress. Various reports confirm the role of proline not only as an osmolytes but it also contributes to sub-cellular structures stabilization (*e.g*., membranes and

proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions[50,51]. Drew *et al*.[52] have reported that *C. papaya* × *V. cundinamarcensis* hybrids grew slowly in the hot summer months in a subtropical climate but more vigorously in the winter months. This behaviour may be due to the adaption of *V. cundinamarcensis* to the cool climates of its natural Andean habitat. Molecular study of revealed the presence of cold-inducible sequences in *V. cundinamarcensis* genome, which is similar to that in *Arabidopsis* explain the cold tolerant in *V. cundinamarcensis* as reported by Dhekney *et al*.[53]. The result showed that the leaf proline content was maximum in genotype *V. cundinamarcensis* (0.31 µM g^{-1} FW), followed by genotype P-7-9 (0.29 µM g^{-1} FW) on exposure low temperature exposure.

Conclusion

The present study has shown that exposure of selected papaya genotypes to low temperature stress significantly changed various leaf physiological and biochemical parameters. Amongst the six genotypes studied, advance line P-7-9 was found to be cold tolerant and comparable to the wild relative cold tolerant *Vasconcellea cundinamarcensis*. Amongst the various physiological and biochemical parameters studied, chlorophyll fluorescence, malondialdehyde content, and membrane stability index were observed to be highly significant and can be effectively used to screen diverse papaya genotypes for their cold tolerance.

Conflict of Interest

Authors declare no competing interests.

References

1 Alberdi M & Corcuera LJ, Cold acclimation in plants. A review. *Phytochemistry*, 30 (1991) 3177.

2 Alonso A, Queiroz CS & Magalhaes AC, Chilling stress leads to increased cell membrane rigidity in roots of coffee (*Coffea arabica* L.) seedlings. *Biochim Biophys Acta*, 1323 (1997) 75.

3 McKay HM, Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant vitality following cold storage. *Can J For Res*, 22 (1992) 1371.

4 Villegas VN, *Carica papaya* L. In: Edible Fruits and Nuts, Vol. 2, (Eds.Verheij EWM & Coronel RE; -Wageningen University, Netherlands), 1997

5 Anonymous, National Horticulture Board, Gurugram, Ministry of Agriculture and Farmers Welfare, 2021.

6 Nagy S, *Tropical and subtropical fruits: composition, properties and uses*. (AVI Publishing Co., Inc, Westport, Connecticut, USA), 1980.

7 Yadava UL, Burris JA & McCrary D, Papaya: a potential annual crop under middle Georgia conditions. In: *Advances in New Crops.* (Proceedings of the first national symposium 'New crops: research, development, economics', Indianapolis, Indiana, USA, 23-26 October 1988, Timber Press), 1990, pp. 364.

8 Rooy SS. B, Salekdeh GH, Ghabooli M, Gholami M & Karimi R, Cold-induced physiological and biochemical responses of three grapevine cultivars differing in cold tolerance. *Acta Physiol Plant*, 39 (2017) 264.

9 Janmohammadi M, Sabaghnia N & Mahfoozi S, Frost tolerance and metabolite changes of rye (*Secale cereale*) during the cold hardening and overwintering. *Acta Physiol Plant*, 40 (2018) 1.

10 Tasgin E, Atici O, Nalbantoglu B & Popova LP, Effects of salicylic acid and cold treatments on protein levels and on the activities of antioxidant enzymes in the apoplast of winter wheat leaves. *Phytochemistry*, 67 (2006) 710.

11 Hu Z, Fan J, Xie Y, Amombo E, Liu A, Gitau MM & Fu J, Comparative photosynthetic and metabolic analyses reveal mechanism of improved cold stress tolerance in bermudagrass by exogenous melatonin. *Plant Physiol Biochem*, 100 (2016) 94.

12 Cao YY, Yang MT, Chen SY, Zhou ZQ, Li X, Wang XJ & Bai JG, Exogenous sucrose influences antioxidant enzyme activities and reduces lipid peroxidation in water-stressed cucumber leaves. *Biol Plant*, 59 (2015) 147.

13 Nahar K, Hasanuzzaman M, Alam MM & Fujita M, Roles of exogenous glutathione in antioxidant defense system and methylglyoxal detoxification during salt stress in mung bean. *Biol Plant*, 59 (2015) 745.

14 Erdal S, Genisel M, Turk H, Dumlupinar R & Demir Y, Modulation of alternative oxidase to enhance tolerance against cold stress of chickpea by chemical treatments. *J Plant Physiol*, 175 (2015) 95.

15 Ghaderian SM, Ghasemi R, Heidari H & Vazirifar S, Effects of Ni on superoxide dismutase and glutathione reductase activities and thiol groups: a comparative study between *Alyssum* hyper accumulator and non-accumulator species. *Aust J Bot*, 63 (2015) 65

16 Levitt J, Responses of Plants to Environmental Stress. In:*Chilling, Freezing, and High Temperature Stresses*, Vol. 1, (Academic Press, New York), 1980.

17 Steponkus PL, Role of the plasma membrane in freezing injury and cold acclimation. *Annu Rev Plant Physiol*, 35 (1984) 543.

18 Steponkus PL, A contrast of the cryostability of the plasma membrane of winter rye and spring oat-two species that widely differ in their freezing tolerance and plasma membrane lipid composition. *Adv Low Temp Biol*, 3 (1993) 211.

19 Nahar K, Hasanuzzaman M, Alam MM & Fujita M, Roles of exogenous glutathione in antioxidant defense system and methylglyoxal detoxification during salt stress in mung bean. *Biol Plant*, 59 (2015) 745.

20 Janmohammadi M, Sabaghnia N & Mahfoozi S, Frost tolerance and metabolite changes of rye (*Secale cereale*) during the cold hardening and overwintering. *Acta Physiol Plant*, 40 (2018) 1.

21 Barrs HD & Weatherley PE, A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust J Boil Sci*, 15 (1962) 413.

22 Sairam RK, Effect of moisture-stress on physiological activities of two contrasting wheat genotypes. *Indian J Exp Biol*, 32 (1994) 594.

23 Sadasivam S & Manickam A, *Biochemical Methods.* New Age Int. Publisher, New Delhi, 1992, 11.

24 Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*, 72 (1976) 248.

25 Heath RL & Packer L, Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys*, 125 (1968) 189.

26 Bates LS, Waldren RP &Teare ID, Rapid determination of free proline for water-stress studies. *Plant Soil*, 39 (1973) 205.

27 Hussain HA, Hussain S, Khaliq A, Ashraf U, Anjum SA, Men S & Wang L, Chilling and drought stresses in crop plants: implications, cross talk, and potential management opportunities. *Front Plant Sci*, 9 (2018) 393.

28 Sowinski P, Rudzinska-Langwald A, Adamczyk J, Kubica I & Fronk J, Recovery of maize seedling growth, development and photosynthetic efficiency after initial growth at low temperature. *J Plant Physiol*, 162 (2005) 67.

29 Grau A & Halloy S, Effect of chilling on CO_2 gas-exchange in *Carica papaya* L and *Carica quercifolia* (A. St. Hil.) solms. *J Plant Physiol*, 150 (1997) 475.

30 Campostrini E & Yamanishi OK, Influence of mechanical root restriction on gas-exchange of four papaya genotypes. *Revista Brasileira de Fisiologia Vegetal*, 13 (2001) 129.

31 Pradhan S, Goswami AK, Singh SK, Jai P, Suneha G, Chinnusamy V & Sharma VK, Growth, nutrient acquisition and physiological responses of papaya (*Carica papaya*) plants to controlled low temperature stress. *Indian J Agric Sci*, 88 (2018) 726.

32 Barros RS, da Se Mota JW, Da Matta FM & Maestri M, Decline of vegetative growth in *Coffea arabica* L. in relation to leaf temperature, water potential and stomatal conductance. *Field Crops Res*, 54 (1997) 65.

33 Clemente HS & Marler TE, Drought stress influences gas-exchange responses of papaya leaves to rapid changes in irradiance. *J Am Soc Hort Sci*, 121 (1996) 292.

34 Glaszmann JC, Kaw RN & Khush GS, Genetic divergence among cold tolerant rices (*Oryza sativa* L.). *Euphytica*, 45 (1990) 95.

35 Maxwell K & Johnson GN, Chlorophyll fluorescence—a practical guide. *J Exp Bot*, 51 (2000) 659.

36 Shi YL, Cai ZY, Li D, Lu JL, Ye JH, Liang YR & Zheng XQ, Effect of Freezing on Photosystem II and Assessment of Freezing Tolerance of Tea Cultivar. *Plants*, 8 (2019) 434.

37 Ehlert B & Hincha DK, Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in *Arabidopsis* leaves. *Plant Methods*, 4 (2008) 12.

38 Tambussi EA, Bartoli CG, Guiamet JJ, Beltrano J & Araus JL, Oxidative stress and photodamage at low temperatures in soybean (*Glycine max* L. Merr.) leaves. *Plant Sci*, 167 (2004) 19.

39 Jeyakumar P, Kavino M, Kumar N & Soorianathasundaram K, Physiological performance of papaya cultivars under abiotic stress conditions. In: 1 *International Symposium on Papaya*, 740 (2005) 209.

40 Verma RK, Santosh Kumar VV, Yadav SK, Pushkar S, Rao MV & Chinnusamy V, Overexpression of ABA Receptor *PYL10* gene confers drought and cold tolerance to indica rice. *Front Plant Sci*, 10 (2019) 1488.

41 Steponkus PL, Role of the plasma membrane in freezing injury and cold acclimation. *Annu Rev Plant Physiol*, 35 (1984) 543.

42 Hamman RA, Dami IE, Walsh TM & Stushnoff C, Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines. *Am J Enol Vitic*, 47 (1996) 31.

43 Stushnoff C, Remmele RL, Essensee V & McNeil M, Low temperature induced biochemical mechanisms: implications for cold acclimation and de-acclimation. In: *Interacting Stresses on Plants in a Changing Climate; NATO ASI Series I: Global Environment Change*, Vol. 16. (Eds. Jackson MB & Black CR; Berlin: Springer, Berlin, Heidelberg), 1993, 647

44 Fernandez O, Theocharis A, Bordiec S, Feil R, Jacquens L, Clement C & Barka EA, Burkholderia phytofirmans PsJN acclimates grapevine to cold by modulating carbohydrate metabolism. *Mol Plant-Microbe Interact*, 25 (2012) 496.

45 Xu C, Wang MT, Yang ZQ & Zheng QT, Low temperature and low irradiation induced irreversible damage of strawberry seedlings. *Photosynthetica*, 58 (2020) 156.

46 Sun X, Rikkerink EH, Jones WT & Uversky VN, Multifarious roles of intrinsic disorder in proteins illustrate its broad impact on plant biology. *Plant Cell*, 25 (2013) 38.

47 Lee DH & Lee CB, Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. *Plant Sci*, 159 (2000) 75.

48 Pradhan S, Goswami AK, Singh SK, Prakash J, Goswami S, Chinnusamy V & Kumar A, Physiological and biochemical alterations due to low temperature stress in papaya genotypes. *Indian J Hort*, 74 (2017) 491.

49 Kaplan F, Kopka J, Sung DY, Zhao W, Popp M, Porat R & Guy CL, Transcript and metabolite profiling during cold acclimation of *Arabidopsis* reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. *Plant J*, 50 (2007) 967.

50 Hu Z, Fan J, Xie Y, Amombo E, Liu A, Gitau MM & Fu J, Comparative photosynthetic and metabolic analyses reveal mechanism of improved cold stress tolerance in bermudagrass by exogenous melatonin. *Plant Physiol Biochem*, 100 (2016) 94.

51 Kishor PK, Sangam S, Amrutha RN, Laxmi PS, Naidu KR, Rao KS & Sreenivasulu N, Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Curr Sci*, 88 (2005) 424.

52 Drew RA, Magdalita PM & OBrien CM, Development of *Carica* interspecific hybrids. In: *International Symposium on Biotechnology of Tropical and Subtropical Species Part 2*, 461 (1997) 285.

53 Dhekney SA, Litz RE, Moraga D & Yadav A, Is it possible to induce cold tolerance in papaya through genetic transformation? In: *International Symposium on Biotechnology of Temperate Fruit Crops and Tropical Species*, 738 (2005) 159.