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Physiological and biochemical alterations due to low temperature stress in papaya genotypes

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ABSTRACT

Papaya (*Carica papaya* L.) being a typical tropical plant, is highly sensitive to low temperature stress. The present experiment was conducted under completely controlled conditions of National Phytotron Facility, ICAR-IARI, New Delhi to investigate the effect of different low temperature regimes on antioxidant enzymes and other physiological and biochemical parameters in five papaya genotypes and one distant relative *i.e.*, cold tolerant genus *Vasconcellea cundinamarcensis*. The outcomes suggested a genotype-specific substantial increase of antioxidant enzymes activities, *viz*., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR) under the cold treatment regimes. The highest per cent increase of SOD, APX, GPX and GR activities were found in the tolerant genotype *V. cundinamarcensis*, while the highest increase in CAT activity was observed in P-7-9 at the 20˚/10˚C (day/night) temperature regime over the control. Low temperature regimes led to increase in membrane injury in papaya leaves possibly through the phase transition or oxidative damage of cell membrane due to ROS generation in the photosystem because of disruption in the photosynthetis process. The highest increase in the membrane injury index was noted in genotype Red Lady (79.93%). The photosynthetic rate was severely reduced under the low temperature regimes. The total sugars and total soluble proteins content in papaya leaves were observed to increase under the low temperature regimes may be due to cold induced osmotic stress.

Key words: Antioxidant enzymes, low temperature regimes, membrane injury, papaya, photosynthesis.

INTRODUCTION

Low temperature stress is one of the major factors affecting the growth and development of tropical plants like papaya (*Carica papaya* L.). Chilling and freezing injury ensue from low temperatures affect the plant in two ways, *i.e*., by transition of the cell membrane into solid gel and dehydration of cell due to ice nucleation in the intercellular spaces inducing osmotic stress. Photosynthesis is a highly synchronized process and is vulnerable to any change in environmental conditions, as it needs to balance the absorbed light energy of photosystems with the energy consumed by metabolic processes of the plant. Low temperature stress aggravates an imbalance between the source of energy and the metabolic process, thus generating the reactive oxygen species (ROS) leading to oxidative stress. Antioxidant enzymes (SOD, CAT, APX, GPX and GR) are compounds which can retard or overcome the process oxidative stress inhibiting the initiation or multiplication of oxidizing reactions (Ruelland *et al*., 11).

Although the distant relatives of cultivated papaya (*C. papaya* L.), *viz*., *Vasconcellea cundinamarcensis*, *V. pennata* and *V. pentagona* are resistant to frost but almost every commercial variety of papaya is highly sensitive to low temperature stress, which limits its successful cultivation in the sub-tropical areas. Reports showed that the optimum temperature range for the growth and development of papaya is 21º to 33ºC, while winter temperatures below 12-14ºC can hamper the growth and production considerably (Ram, 10). Irrespective of the above facts, neither the germplasm nor the physiology behind the cold stress tolerance in papaya has been studied in depth. The aim of the present research was to perceive the interactions between the antioxidant enzymes and low temperature regimes in papaya and to identify a low temperature tolerant papaya genotype.

MATERIALS AND METHODS

Plant material for the experiment included five *C. papaya* L. genotypes (Red Lady, Pusa Nanha, P-7-15, P-7-9 and P-9-5) and one cold tolerant genotype (*Vasconcellea cundinamarcensis*). Evaluation for low temperature stress was undertaken at the controlled environment conditions in the National Phytotron Facility, ICAR-IARI, New Delhi during

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2016-17. The seeds of above mentioned genotypes were sown in trays containing the growing medium comprising of perlite, vermiculite, cocopeat and vermicompost (1:1:1:1) and then transplanting was done 8 week after sowing, into plastic pots filled with same potting medium under the growth chamber. All other recommended standard operations were performed at proper growth stage of the plants. A temperature regime of 28/18ºC (day/night) along with a photoperiod of 12 h 30 min. (L/D) and relative humidity of 70 ± 5% during day and 85-90% during the night and irradiance of 700-800 µmol $m^{-2}s^{-1}$ at leaf level. After proper establishment of the transplanted seedlings, the temperature treatments were induced by lowering the temperature in the growing chamber by 2ºC per two day from 26/ 16ºC (day/ night) to 20/ 10ºC (day/ night) up to 8 days. In total, three replications comprising of 9 plants per replication for each genotype were maintained. In control (T_0), three plants for each genotype were maintained at 28/ 18ºC (day/ night) regime. The details of temperature treatments are portrayed in Table 1. The observations on leaf physiological and biochemical parameters were recorded after each low temperature treatment. Five matured leaves from three plants per each treatment were selected and their photosynthesis rate (µmol m^{-2} s^{-1}) was measured by using an infrared gas analyser, while leaf membrane injury index (MII) was estimated following the method of Blum and Ebercon (2).

Total soluble proteins content in the leaf tissue was estimated by the method given by Bradford (3), while total soluble sugars were estimated using anthrone reagent method (Sadasivam and Manickam, 12). Leaf anti-oxidant enzyme assays were conducted in stress exposed and control plants. The catalase (CAT, EC: 1.11.1.6) assay was conducted as per method of Aebi (1), superoxide dismutase (SOD, EC: 1.15.1.1) as per Dhindsa *et al*. (4), glutathione peroxidase (GPX, EC 1.11.1.9) as per Srivastava and Huystee (13), ascorbate peroxidase (APX, EC 1.11.1.11) as per method of Nakano and Asada (8) and glutathione reductase (GR, EC 1.6.4.2) activity was estimated by the method of Halliwell and Foyer (5).

Table 1. Details of controlled temperature regimes maintained under growth chamber.

Treatment No.	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

The statistical analysis of the data which comprised of five treatments including control (T_0) and three replications were analysed 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.

RESULTS AND DISCUSSION

The data presented clearly demonstrated that papaya plants had marked changes due to exposure to low temperature regimes with a uniform and substantial increase in the leaf dry weight, MII, leaf total soluble proteins, total soluble sugars content and activity of antioxidant enzymes, while decline in the leaf fresh weight and photosynthetic rate.

Amongst the genotypes, *V. cundinamarcensis* (872.13 mg) maintained the highest mean leaf FW, which was statistically at par with P-9-5 (854.80 mg) (Table 2). Amongst the G × T interaction, Red Lady × T_4 (228.00 mg) exhibited the lowest leaf FW, while the highest was in *V. cundinamarcensis* × T_0 (919.00 mg). The highest reduction in leaf FW within the genotypes from the T_0 to T_4 was observed in the genotype P-7-15 (15.48%), while the lowest was in Pusa Nanha (5.39%). Amongst the temperature regimes, the mean leaf DW was observed to be highest in T_4 (77.50 mg), which was 25.90% higher than the T_1 (61.56 mg) (Table 2). The genotype P-9-5 (129.60 mg) exhibited the highest leaf DW. Amongst all the possible G × T combinations, the highest leaf DW was in P-9-5 × T_3 (149.00 mg). Although the highest mean fresh weight was noted in *V. cundinamarcensis* (872.13 mg) followed by P-9-5 (854.80 mg) but the highest mean leaf dry weight was noted in P-9-5 (129.60 mg) followed by *V. cundinamarcensis* (104.93 mg), which may be due to higher moisture content in the fresh leaves of *V. cundinamarcensis* owing to its castor-like with more thickness.

Amongst the mean value of different treatments, T_4 plants had significantly higher total protein content (2.17 µg protein $µl^{-1}$) than all others and it was observed to be 35.51% higher than the T_0 (1.60 µg protein $µl^{-1}$) (Table 3). Amongst the possible G × T combinations, P-9-5 × T_4 (2.71 µg protein $µl^{-1}$) and P-9-5 × T_3 (2.51 µg protein $µl^{-1}$) had statistically similar and higher protein content. The highest increase from T_0 to T_4 was noted in *V. cundinamarcensis* (79.06%), while the lowest was in the genotype Pusa Nanha (8.78%). In this study, leaf FW was observed to decrease under the low temperature regimes, while leaf DW reflected the opposite trend. The relative water content was also observed to decrease under the low temperature stress (data not presented). The results suggested a significant increase in the protein

Table 2. Effect of different temperature regimes on leaf fresh and dry weights in papaya genotypes under controlled phytotron conditions.

Genotype	Leaf fresh weight (mg)						Leaf dry weight (mg)					
Temp. regime	T_0	T_1	T_2	T_3	T_4	Mean	T_0	T_1	T_2	T_3	T_4	Mean
Red Lady	251.67^{ijk}	251.00^{jk}	245.67^{jk}	232.67^{k}	228.00^{k}	241.80^{e}	25.00^{mno}	24.00^{no}	23.00^{o}	41.33^{lm}	40.00^{lmn}	30.67^{e}
Pusa Nanha	420.33^{e}	408.00^{ef}	407.33^{ef}	385.00^{efg}	397.67^{efg}	403.67^{c}	48.67^{lk}	47.00^{lk}	47.67^{lk}	52.33^{ljk}	50.00^{lk}	49.13^{d}
P-7-15	355.33^{efg}	350.33^{efg}	335.67^{hg}	315.00^{ih}	300.33^{ihj}	331.33^{d}	55.67^{lijk}	24.00^{no}	50.33^{lk}	47.33^{lk}	61.67^{hijk}	47.80^{d}
P-7-9	580.67^{d}	579.33^{d}	584.00^{d}	547.00^{d}	538.00^{d}	565.80^{b}	55.67^{lijk}	41.00^{lm}	68.67^{hij}	72.00^{hi}	72.33^{gh}	61.93^{c}
P-9-5	899.33^{a}	899.33^{a}	883.00^{ab}	813.33^{c}	779.00^{c}	854.80^{a}	130.00^{b}	108.00^{ed}	126.33^{bc}	149.00^{a}	134.67^{ab}	129.60^{a}
V. cund.	919.00^{a}	912.00^{a}	879.33^{ab}	827.67^{bc}	822.67^{bc}	872.13^{a}	93.33^{ef}	125.33^{bc}	111.00^{cd}	88.67^{fg}	106.33^{ed}	104.93^{b}
Mean	571.06^{a}	566.67^{a}	555.83^{a}	520.11^{b}	510.94^{b}		68.06^{bc}	61.56^{c}	71.17^{ab}	75.11^{a}	77.50^{a}	
LSD (P ≤ 0.05)												
Genotype (G)						28.46						7.36
Temp. regime (T)						25.98						6.72
G × T						63.63						16.45

Table 3. Influence of different temperature regimes on leaf total soluble proteins and total soluble sugars content in papaya genotypes grown under controlled phytotron conditions.

Genotype	Total soluble proteins (µg protein µl^{-1} enzyme extract)						Leaf total soluble sugars (mg g^{-1})					
Temp. regime	T_0	T_1	T_2	T_3	T_4	Mean	T_0	T_1	T_2	T_3	T_4	Mean
Red Lady	1.43^{jkl}	1.53^{ijk}	1.56^{ijk}	1.58^{ijk}	1.79^{ghi}	1.58^{cd}	29.54^{pq}	31.57^{op}	36.40^{mno}	39.57^{lmn}	42.97^{kl}	36.01^{e}
Pusa Nanha	2.20^{cde}	2.23^{bcd}	2.36^{bc}	2.37^{bc}	2.39^{bc}	2.31^{a}	36.50^{mno}	40.16^{lmn}	54.38^{fg}	74.70^{c}	135.58^{a}	68.26 ^{a}
P-7-15	1.82^{fghi}	2.28^{bcd}	2.30^{bcd}	2.34^{bc}	2.36^{bc}	2.22^{ab}	34.44^{nop}	40.72^{klm}	44.05^{ijkl}	46.37^{hijk}	66.53^{d}	46.42^{d}
P-7-9	1.40^{kl}	1.44^{jkl}	1.54^{ijk}	1.56^{ijk}	1.67^{hijk}	1.52^{d}	41.47^{klm}	43.95^{jkl}	49.25^{ghij}	57.22^{ef}	65.65^{d}	51.51^{c}
P-9-5	1.59^{ijk}	1.79^{ghi}	2.01^{defg}	2.51^{ab}	2.71^{a}	2.12^{b}	50.58^{gh}	56.47^{ef}	59.21^{ef}	62.09^{de}	75.98^{c}	60.87^{b}
V. cund.	1.17^{l}	1.63^{hijk}	1.70^{hij}	1.90^{efgh}	2.10^{cdef}	1.70^{c}	24.51^{q}	49.57^{ghij}	49.80^{ghi}	61.11^{de}	84.47^{b}	53.89^{c}
Mean	1.60^{d}	1.82^{c}	1.91^{c}	2.04^{b}	2.17^{a}		36.17^{e}	43.74^{d}	48.85^{c}	56.84^{b}	78.53^{a}	
LSD (P ≤ 0.05)												
Genotype (G)						0.13						2.57
Temp. regime (T)						0.12						2.35
G × T						0.30						5.76

Temperature regime: T_0 (control) = 28/18°C (day/ night); T_1 = 26/16°C (day/ night); T_2 = 24/14°C (day/ night); T_3 = 22/12°C (day/ night); T_4 = 20/10°C (day/ night)

content of low temperature treated plants, which may be due to decrease in leaf water content. Lee and Lee (7) also noted increase in the leaf protein content in cucumber under chilling stress (4ºC, 12 h) and also attributed the same reason of decrease in leaf relative water content for higher protein accumulation.

Although a little change in mean MII in T_1 (5.67%) was observed over the control but the mean MII of T_4 plants (0.44) was found to be 57.65% higher than the control plants (0.28) (Fig. 1A). Amongst the mean MII of genotypes, the lowest value was noted in P-7-15 (0.31) followed by *V. cundinamarcensis* (0.33). Amongst the possible G × T combinations, Red Lady × T_4 had the highest MII (0.57), while the lowest was observed in *V. cundinamarcensis* × T_0 (0.23) followed by P-7-9 × T_0 (0.25). The per cent increase in MII from T_0 to T_4 was highest in the genotype Red Lady (79.93%). In the present study, the ROS generation may have initiate membrane lipid peroxidation (data not shown), weaken membrane lipid unsaturation, trigger membrane protein polymerization, and resulted in an increase in membrane permeability, which was evident from the higher MII. The findings of the present study agree with the results of Pennycooke *et al*. (9), which showed that membrane is the primary site of chilling or freezing injury in plants.

It was reported that low temperatures affect different aspects of photosynthesis process. It reduces the activity of different enzymes involved in the Calvin cycle and ROS-scavenging systems resulting in ROS generation in PSI and PSII. The change in redox poise imposed by ROS accumulation, resulted in reduction of photosynthetic rate (Ruelland *et al*., 11). In the present study, a severe decline in the photosynthetic rate was observed in the papaya genotypes under the cold stress. The control (T_0) plants showed the highest (2.512 µmol m^{-2} s^{-1}) mean photosynthetic rate (*A*) value, while temperature treatment reduced it (Fig. 1B). Amongst the genotypes, P-7-15 exhibited the maximum mean *A* (2.05 µmol $m^{-2}s^{-1}$). Amongst G × T interactions, plants of Red Lady × T_0 expressed the highest *A* (3.073 µmol $m^{-2}s^{-1}$), while the lowest was expressed in Red Lady × T_4 (0.550 µmol $m^{-2}s^{-1}$). As compared to the control the highest per cent decrease in the *A* at T_4, was observed in Red Lady (82.10%), while lowest in P-7-9 (24.26%). Jeyakumar *et al*. (6) through their study on the physiological performance of papaya genotypes under abiotic stress conditions reported that leaf relative water content had significant influence on photosynthetic rate. In the present study, also both the leaf FW and photosynthetic rates were observed to decrease under the low temperature regimes.

The highest mean total sugars content in the leaves was observed in T_4 (78.53 mg g^{-1}), which is 117.10% higher than the control (36.17 mg g^{-1}) (Table 3). Amongst the six genotypes, Pusa Nanha exhibited the highest mean total sugars content (68.26 mg g^{-1}), while the lowest was noted in Red Lady (36.01 mg g^{-1}). Amongst all the possible combinations of G × T interactions, the highest total sugars content was maintained by the plants of Pusa Nanha × T_4 (135.58 mg g^{-1}). A dramatic increase (81.50%) in the total sugars content was observed in Pusa Nanha at the 20/10°C (day/ night) over 22/12°C (day/ night) temperature regimes. It was noted that the highest increase from T_0 to T_4 was in Pusa Nanha (271.47%) followed by *V. cundinamarcensis* (244.70%). The

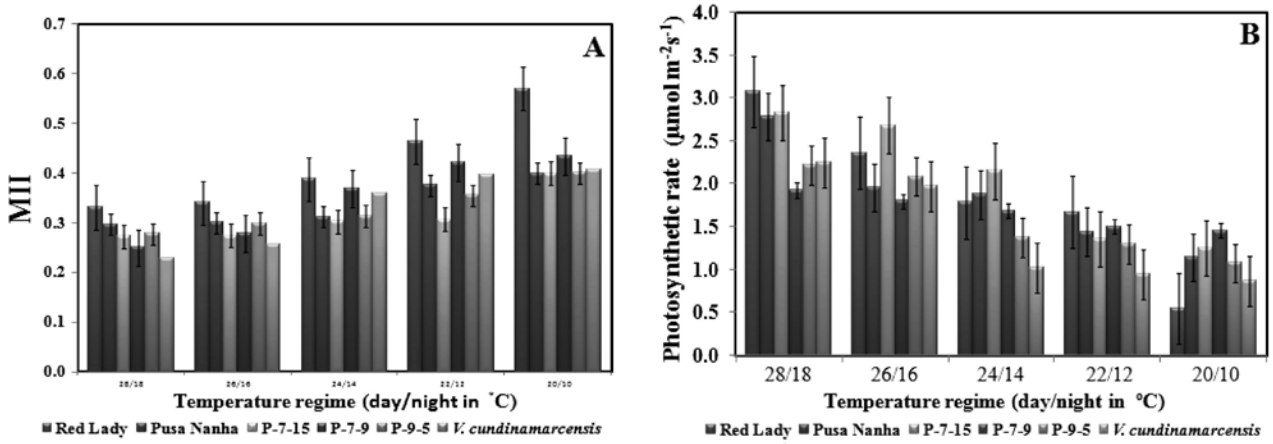

Fig. 1. Effect of different temperature regimes on membrane injury index (MII) and photosynthetic rate of papaya genotypes grown under controlled phytotron conditions; Temperature regime: T_0 (control) = 28/18°C (day/night); T_1 = 26/16°C (day/night); T_2 = 24/14°C (day/night); T_3 = 22/12°C (day/night); T_4 = 20/10°C (day/night), Vertical bars indicate ± SE mean.

increase of leaf total soluble sugars content may be due to the fact that sugars change the osmotic potential of the cell and consequently diminish the difference in water potential between the ice formed in the apoplastic space and the solution within the cell. As a result, the rate at which water is withdrawn from the cell is reduced and the cell membrane becomes more stable to resist the effect of the stress generated by the low temperature. Our results are in agreement to those of Stushnoff *et al*. (14) on Red Delicious apple, where correlation was obtained between cold hardiness in cortical tissues and buds with sorbitol, total sugars and raffinose family oligosaccharides (RFO).

In this study, plants of T_4 (4.08 µmoles H_2O_2 hydrolysed mg^{-1} TSP min^{-1}) was observed to have the highest mean CAT activity, which is 141.76% higher than the control plants (1.69 µmoles H_2O_2 hydrolysed mg^{-1} TSP min^{-1}) (Fig. 2A). Of the possible G × T interactions, highest increase in the activity of CAT was recorded in Red Lady × T_4 (5.36 µmoles H_2O_2 hydrolysed mg^{-1} TSP min^{-1}). Decreased temperature regimes from T_0 to T_4 caused the highest per cent increase in CAT activity in the genotype P-7-9 (207.40%), while it was lowest in *V. cundinamarcensis* (87.71%). The highest mean SOD activity was observed in the T_4 (8.27 unit mg^{-1} TSP min^{-1}) leaves, which was 47.13% higher than the control (5.26 unit mg^{-1} TSP min^{-1}) (Fig. 2B). Of the various interactions, the highest increase in SOD activity was recorded in *V. cundinamarcensis* × T_4 (9.38 unit mg^{-1} TSP min^{-1}), while the lowest activity was recorded in plants of P-7-15 × T_4 (4.98 unit mg^{-1} TSP min^{-1}). The highest per cent increase in SOD activity (from T_0 to T_4) was observed in *V. cundinamarcensis* (83.85%), while it was lowest in Red Lady (29.96%). Plants under T_4 (43.47 unit mg^{-1} TSP min^{-1}) were observed to have the highest mean GPX activity, which was 44.14% higher than the control plants (30.16 unit mg^{-1} TSP min^{-1}) (Fig. 2C). Amongst the mean GPX activity of genotypes, the highest value was noted in *V. cundinamarcensis* (42.83 unit mg^{-1} TSP min^{-1}), while amongst the possible G × T combinations, *V. cundinamarcensis* × T_4 had the highest GPX activity (54.54 unit mg^{-1} TSP min^{-1}). As compared to the control. The highest per cent increase in GPX activity in T_4, was observed in *V. cundinamarcensis* (74.99%). APX activity was found to be significantly increased under low temperature regimes. The highest mean APX activity was found in T_4 plants (4.41 unit mg^{-1} TSP), which was 237.88% higher than the control (1.31 unit mg^{-1} TSP) (Fig. 2D). The genotype *V. cundinamarcensis* was found to have the highest mean APX activity (3.91 unit mg^{-1} TSP), while lowest in Pusa Nanha (2.13 unit mg^{-1} TSP). Amongst the possible G × T combinations, *V. cundinamarcensis* × T_4 had the highest APX activity (6.18 unit mg^{-1} TSP). Temperature treatments also increased the APX activity within the genotypes from T_0 to T_4, which was highest in the genotype *V. cundinamarcensis* (295.52%). The highest mean GR activity was found in T_4 plants (4.76 unit mg^{-1} TSP), which was 126.90% higher than the control (2.10 unit mg^{-1} TSP) (Fig. 2E). The genotype *V. cundinamarcensis* was found to have the highest mean GR activity (4.08 unit mg^{-1} TSP), while amongst the possible G × T combinations, *V. cundinamarcensis* × T_4 had the highest GR activity (6.36 unit mg^{-1} TSP). The per cent increase in GR activity from T_0 to T_4 was the highest in genotype *V. cundinamarcensis* (200.11%).

An explanation for differences in the susceptibility of plants to chill-temperature photo-inhibition is that for chilling-tolerant plants the ROS produced in different cellular organelles is efficiently scavenged by the antioxidant enzymes, *viz*., CAT, SOD, APX, GR, GPX *etc.* Here also, an increase in activity of all the above antioxidant enzymes was noted. In the present study, the highest increase in activities of SOD, APX, GPX and GR was found in the tolerant genotype *V. cundinamarcensis*, while the highest increase in CAT was observed in P-7-9. Amongst the *C. papaya* genotypes, P-9-5 registered the highest enhancement in activities of APX, GPX and GR. The results of our study are in agreement with that of Wang and Li (15), Pennycooke *et al*. (9) and Lee and Lee (7). It was striking to note that, the cold tolerant genotype, *V. cundinamarcensis* registered the lowest increase in CAT at 20/ 10ºC (day/ night) regime over the control. Although the genotype Red Lady was observed to be susceptible to low temperature stress based on most of the other parameters but it registered the highest mean CAT activity. It may be due to the fact that CAT is produced in mitochondria and peroxisomes but absent in chloroplast, which is one of the important site of H_2O_2 generation under stress conditions. Compared to APX, it is also considered as a less efficient system of H_2O_2 scavenging due its higher Km value for H_2O_2 than APX.

In conclusion, exposure of papaya genotypes to low temperature stress can result in reduction of photosynthetic rate leading to higher antioxidant enzyme (SOD, CAT, APX, GPX and GR) activities to counteract the effect of reactive oxygen species generated in response to oxidative stress of low temperature regimes. The low temperature stress also affected the cell membrane, which is evident from higher membrane injury index. The concomitant increase in total soluble sugars and proteins content were noted in response to the osmotic stress induced due to reduction in the leaf fresh weight under the

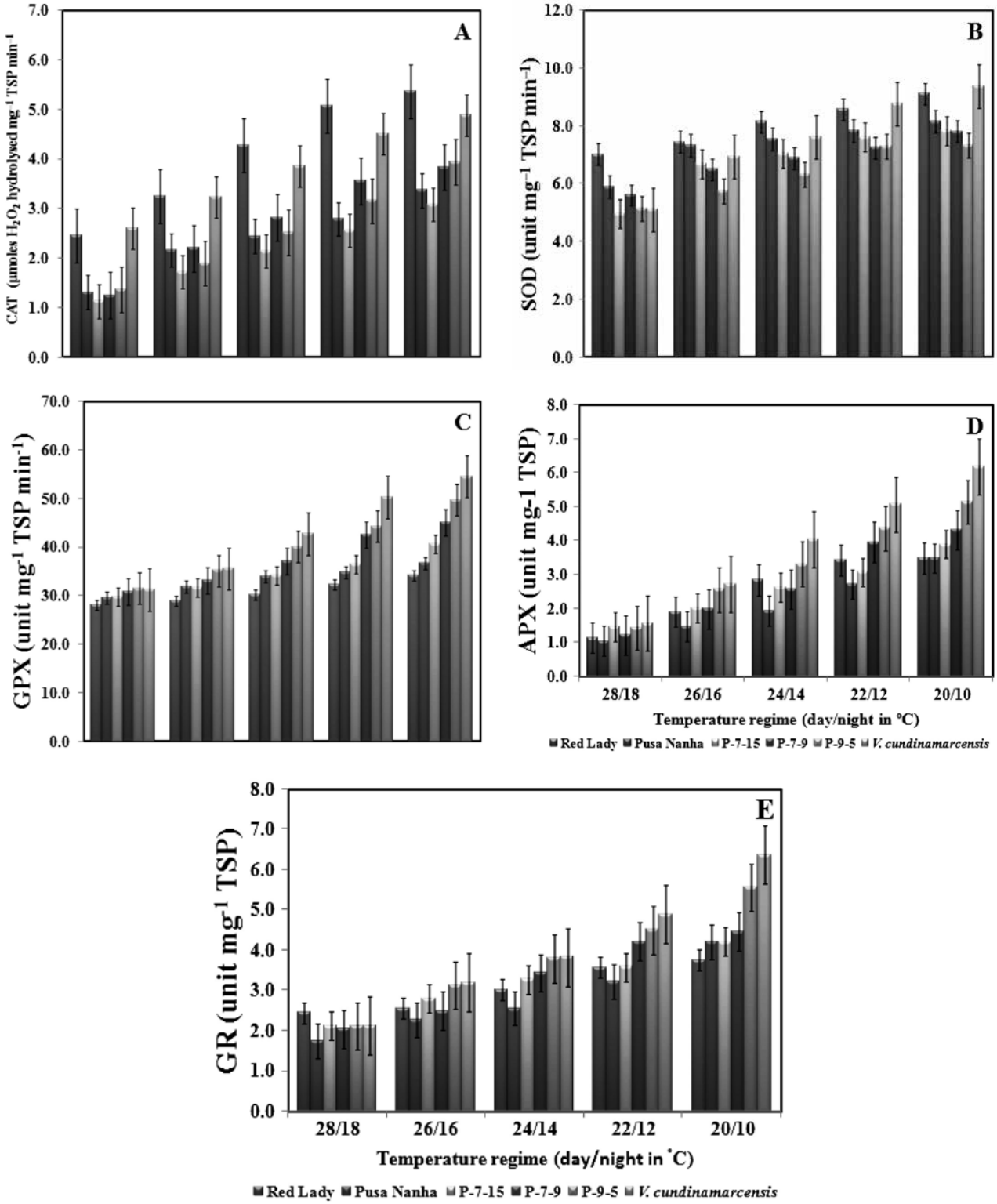

Fig. 2. Effect of different temperature regimes on antioxidant activities in papaya genotypes raised under controlled phytotron conditions; Temperature regime: T_0 (control) = 28/18°C (day/night); T_1 = 26/16°C (day/night); T_2 = 24/14°C (day/night); T_3 = 22/12°C (day/night); T_4 = 20/10°C (day/night); Vertical bars indicate ± SE mean.

low temperature regimes. Based on the results, the genotypes P-9-5 and P-7-9 can be regarded as tolerant to low temperature stress, compared to the tolerant genotype (*Vasconcellea cundinamarcensis*).

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