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EFFECT OF LOW TEMPERATURE STRESS AND ACCLIMATION ON GROWTH AND PHOTOSYNTHETIC PARAMETERS IN BITTER GOURD SEEDLINGS

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ABSTRACT

This study investigated the impact of low temperature and acclimation on growth and photosynthetic traits in two bitter gourd genotypes: Punjab-14 and PAUBG-56. Fifteen-day-old seedlings were exposed to 5°C for seven days, either directly or following acclimation (a gradual reduction from 20°C to 5°C). Recovery was assessed at 25°C over seven days, with samples collected on the 1st, 3rd, and 7th days post-stress, and on the 14th day post-recovery. Acclimation resulted in 100% survival for both genotypes, compared to a 10.7% higher survival rate in non-acclimated PAUBG-56 seedlings versus Punjab-14. Acclimated Punjab-14 seedlings showed significant increases in root (16.45%) and shoot length (73.62%), whereas PAUBG-56 exhibited increases of 69.81% and 36.43%, respectively. Non-acclimated seedlings experienced reductions in these parameters. Photosynthetic pigments showed varied responses: total chlorophyll increased by 1.82% on the first day post-stress, while chlorophyll a, b, and carotenoids decreased by up to 20% in Punjab-14. PAUBG-56 showed less pronounced reductions. Acclimated seedlings had smaller decreases and better recovery. The Fv/Fm ratio decreased by 12.50% in both genotypes. Electrolyte leakage increased significantly in non-acclimated Punjab-14, peaking at 93.80% by the 3rd day, while PAUBG-56 reached 67.94% by the 7th day, with recovery values nearing controls. Acclimated seedlings had lower electrolyte leakage. Correlation analysis revealed significant positive correlations between total chlorophyll content and chlorophyll a (0.887), chlorophyll b (0.484), carotenoids (0.614), and the Fv/Fm ratio (0.491), and a negative correlation with electrolyte leakage (-0.498). In conclusion, acclimation improves the physiological performance of bitter gourd seedlings under low temperature stress, enhancing growth, photosynthetic efficiency, and membrane stability.

Keywords: Morpho-physiological parameters, Photosynthetic parameters, low-temperature stress

Bitter gourd (*Momordica charantia* L.), commonly known as *karela*, bitter melon, or balsam pear (Joseph and Jini, 2013), is a flowering vine belonging to the genus *Momordica* within the Cucurbitaceae family. This climbing herb prospers in tropical, subtropical, and temperate regions. Its cultivation has expanded globally due to the significant medicinal and dietary value of both its unripe and ripe fruits (Alam *et al.*, 2015). In India, bitter gourd was grown over 97,000 hectares, producing 1,137,000 metric tonnes during the 2017-18 period (Anonymous 2020). Typically an annual crop, bitter gourd can also be cultivated as a perennial in regions with mild, frost-free winters. It thrives in tropical conditions, ranging from lowland areas to altitudes of up to 1,000 meters (Behera *et al.*, 2010). This vegetable is a staple in parts of Asia, East Africa, the Caribbean, and South America, where it is valued both as a food source and for its medicinal properties. Given its tropical and subtropical origins, *Momordica charantia* lacks genetic adaptation for chilling tolerance.

Low temperature (LT) or cold stress significantly impacts plant growth and crop productivity, often leading to considerable crop losses. Chilling stress, characterized by injury without ice crystal formation within plant tissues, differs from freezing stress, which involves ice formation inside the tissues. Plants exhibit varying tolerances to chilling (0-15°C) and freezing (<0°C) temperatures, collectively termed low temperature or cold stress (Singh *et al.*, 2017). Typically, plants originating from tropical and subtropical regions are more sensitive to LT, whereas temperate plants show varying degrees of chilling tolerance (Sanghera *et al.*, 2011). Low-temperature stress can adversely affect the survival rate of crop plants and disrupt multiple physiological processes, including cell division, photosynthesis, plant growth, development, and metabolism. This, in turn, can reduce crop yields, particularly in tropical and subtropical regions (Lukatkin *et al.*, 2012; Li *et al.*, 2014). While the exact mechanisms by which chilling stress affects physiological and metabolic processes in higher plants remain incompletely understood, it is evident that photosynthesis is often the first process to be inhibited by low temperatures (Janmohammadi *et al.*, 2014). The reduction in photosynthesis is not solely due to photo-

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inhibitory damage but often involves efficient regulatory mechanisms designed to protect photosystem I (PSI) and photosystem II (PSII) from damage (D'Ambrosio *et al.*, 2006). Chilling temperatures can restrict photosynthesis through several mechanisms, including stomatal closure, inhibition of thylakoid electron transport, disruption of carbohydrate metabolism, and impediments in phloem loading (Huang and Guo, 2005). To assess the impact of chilling stress on photosystem inhibition, chlorophyll pigments and the maximum photochemical efficiency of PSII (Fv/Fm), as measured by chlorophyll fluorescence, are commonly used indicators.

In numerous plant species, exposure to low, non-freezing, and non-injurious temperatures can induce the acquisition of freezing tolerance (Janmohammadi *et al.*, 2015). This process, known as acclimation, involves physiological and biochemical changes that enable plants to better withstand subsequent cold stress, particularly during critical stages such as germination and early seedling growth (Dhingra, 2015; Gong *et al.*, 2011). Cold priming or acclimation triggers a range of physiological and biochemical responses, including membrane stabilization, enhanced detoxification of reactive oxygen species (ROS) and methylglyoxal (MG), activation of cold-sensitive protein kinases, and increased synthesis of nitric oxide (NO) and various hormones. Additionally, it leads to the accumulation of antioxidants, heat shock proteins (HSPs), cold-regulated proteins (CORs), and dehydrins (Hossain *et al.*, 2017). These alterations collectively enhance the plant's ability to protect itself from freezing-induced injury. For example, Gong *et al.* (2011) demonstrated that in maize seedlings, cold acclimation resulted in higher survival rates and reduced electrolyte leakage compared to non-acclimated seedlings, highlighting the effectiveness of cold acclimation in improving plant resilience to low temperatures.

Given the medicinal significance of bitter gourd, the present study aimed to investigate the effects of low temperature and acclimation treatments on its growth and morpho-physiological traits. The objective was to elucidate the fundamental mechanisms underlying bitter gourd's response to cold stress and to develop effective strategies to enhance its resilience to such conditions.

MATERIALS AND METHODS

Plant material

The plant material for this study comprised two bitter gourd genotypes: Punjab-14 and PAUBG-56. Seeds were obtained from the Department of Vegetable Science, Punjab Agricultural University (PAU), Ludhiana, Punjab, India. The seeds were sown in plastic trays filled with a growing medium consisting of coco peat, vermiculite, and perlite in a 3:1:1 ratio, respectively, and were cultivated in a greenhouse. Each tray accommodated 50 seedlings, which were watered every alternate day with tap water. Fifteen days after germination (DAG), the seedlings were subjected to two treatment conditions: (i) direct exposure to 5°C for seven days, and (ii) acclimation followed by low-temperature stress. The control plants were maintained at 25°C. All experimental conditions were conducted in a biological incubator with an 8-hour light and 16-hour dark cycle, and controlled humidity at 40%. For the acclimation treatment, the temperature was gradually decreased from 20°C to 5°C at a rate of 2°C per hour. The seedlings of both genotypes were acclimated to 5°C and held at this temperature for seven days. Following the cold exposure, the seedlings were transferred to 25°C for a seven-day recovery period. Leaf samples from directly exposed seedlings were collected on the 1st, 3rd, and 7th days after stress (DAS) and on the 7th day after recovery (DAR). For acclimated seedlings, leaf samples were collected on the 7th DAS and the 14th DAR. All samples were stored at -20°C until further analysis.

Growth parameters

The survival percentage of seedlings was assessed at the end of the seven-day recovery period. The survival percentage was calculated using the following formula:

Survival percentage = (number of seedlings survived after stress/total number of seedlings) × 100

Root length (RL) and shoot length (SL) of the seedlings were measured in centimeters (cm) as the average values obtained from five randomly selected seedlings. Additionally, the ratio of root length to shoot length (RL/SL) was calculated after the seven-day low-temperature (LT) stress period.

Estimation of electrolyte leakage

Electrolyte leakage was measured following the method outlined by Liu and Huang (2000). Leaf samples (0.25 g) from the seedlings were rinsed with deionized water and then immersed in 25 ml of deionized water for 24 hours, or alternatively, shaken in a shaker for 5 hours. The initial conductivity of the solution was recorded using a conductivity meter. Subsequently, the samples were placed in a boiling water bath for 30 minutes. After boiling, the conductivity of the solution was measured again.

The electrolyte leakage percentage was calculated as:

$$\text{Electrolyte leakage (\%)} = \frac{\text{Conductivity before boiling}}{\text{Conductivity after boiling}} \times 100$$

Estimation of chlorophyll pigments and F_v/F_m ratio

Chlorophyll and carotenoid pigments were extracted using dimethylsulphoxide (DMSO) according to the method described by Barnes *et al.* (1992). The quantification of these pigments was performed using the equations provided by Lichtenthaler (1987). The concentrations of total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids were calculated using the following formulas:

$$\text{Total chlorophyll} = \frac{20.2 \times A_{645} + 8.02 \times A_{665} \times V}{a \times 1000 \times W}$$

$$\text{Chlorophyll a} = \frac{12.47 \times A_{663} - 3.62 \times A_{645} \times V}{1000 \times W}$$

$$\text{Chlorophyll b} = \frac{25.06 \times A_{645} - 6.5 \times A_{663} \times V}{1000 \times W}$$

$$\text{Carotenoids} = \frac{1000 \times A_{480} - 1.29 \times \text{chlorophyll a} - 53.78 \times \text{chlorophyll b} \times V}{220 \times 1000 \times W}$$

where:

W: Fresh weight of sample in grams

V: Volume of the extract

a: Path length of light in the cell (1 cm)

A_{480}, $A_{645,}$ and A_{663} are absorbances of the sample at 480, 645 nm, and 663 nm respectively.

Chlorophyll and carotenoid contents are expressed as mg/g FW tissue.

Chlorophyll fluorescence (Fv/Fm) values were recorded from intact leaves that had been dark-adapted for 30 minutes prior to measurement. The readings were taken using a Pulse Amplitude Modulation (PAM) Chlorophyll Fluorescence meter.

Statistical analysis

In the experiment, two treatments (temperature and days of sampling) were applied, and each parameter was assessed across three biological replicates. Statistical analysis of variance (ANOVA) was conducted to determine significant differences between parameters and genotypes using a factorial Completely Randomized Design (CRD) with CPCS1 software. Pearson's correlation analysis was performed to evaluate the relationships among traits under low temperature conditions, also using CPCS1 software. Duncan's post hoc test ($p \leq 0.05$) was employed to identify significant differences among samples for each parameter. In the bar graphs, different letters (e.g., a, b, c) indicate significant differences among genotypes, with letters representing groups that are significantly different at the $p \leq 0.05$ level according to Duncan's multiple range test. Standard errors were calculated and are presented in the bar graphs to illustrate variability.

RESULTS AND DISCUSSION

Low temperature significantly influenced the mortality percentage of bitter gourd seedlings. After seven days of recovery from stress, a notable difference in survival percentage between the two genotypes, Punjab-14 and PAUBG-56, was observed (Fig. 1). Seedlings of PAUBG-56 demonstrated a 75% survival rate, which was 10.7% higher compared to Punjab-14 under direct exposure to 5°C. Punjab-14 seedlings exhibited a 64% survival rate, indicating that they also maintained substantial growth despite the low-temperature exposure. Cold acclimation markedly improved the survival percentage of bitter gourd seedlings subjected to 5°C. Following acclimation, both Punjab-14 and PAUBG-56 achieved a 100% survival rate. Low-temperature stress impacts both survival percentage and mortality rate in plants, with tolerant species showing higher survival rates under cold stress (Kim *et al.*, 2012; Morsy *et al.*, 2007). The higher survival rate observed in PAUBG-56 suggests superior cold tolerance compared to Punjab-14. Furthermore, acclimation enhanced the survival capability of both genotypes, likely due to improved cold tolerance mechanisms developed during exposure to non-lethal temperatures (Gong *et al.*, 2011; Kargiotidou *et al.*, 2010).

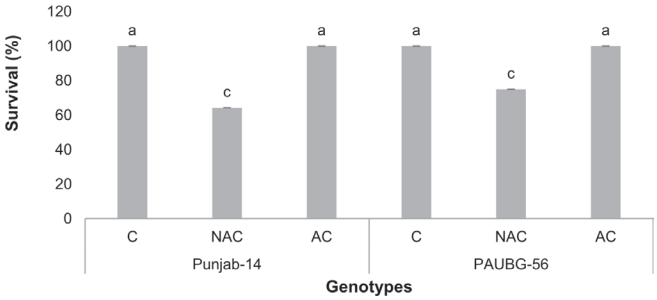

Fig. 1. Effect of low temperature (5°C) and cold acclimation on the survival percentage of bitter gourd seedlings. C: Control, NAC: Non-acclimated at 5°C, AC: acclimated at 5°C. Values represent the mean from three seedlings (CD at *p≤0.05*)

The growth of seedlings was assessed by measuring changes in root and shoot length. Acclimated seedlings under low-temperature stress did not exhibit negative growth. PAUBG-56 showed a 69.81% increase in root length, a 36.43% increase in shoot length, and a 24.76% increase in the root length to shoot length (RL/SL) ratio compared to the control group. In contrast, Punjab-14 showed a 16.45% increase in root length, a 73.62% increase in shoot length, and a 32.86% decrease in the RL/SL ratio. This disparity may be attributed to a greater allocation of energy towards shoot growth

rather than root development. Direct exposure to 5°C led to a 48.48% decrease in root length for Punjab-14 and a 70.58% decrease for PAUBG-56 compared to the control group (Fig. 2). The decrease in shoot length was less pronounced in PAUBG-56 (39.27%) compared to Punjab-14 (69.94%), indicating that cold stress adversely affects seedling growth. The RL/SL ratio increased by 71.73% in Punjab-14 seedlings, while it decreased by 51.43% in PAUBG-56 compared to the control. The increased root and shoot length and RL/SL ratio in acclimated PAUBG-56 seedlings suggest that this variety maintains relatively normal growth even under cold stress.

Several studies have documented that low temperature (LT) stress adversely affects seedling growth parameters, including germination and seedling development. It can lead to chlorosis, wilting of leaves, reduced leaf expansion, and tissue necrosis (Solanke and Sharma, 2008; Ruelland and Zachoeski, 2010; Nahar *et al.*, 2012). Specifically, low temperature stress has been shown to decrease shoot length (Fu *et al*., 2017) while increasing root length (Jan *et al.*, 2015), which results in a higher root-to-shoot ratio (Razmi *et al.*, 2013). An elevated root-to-shoot ratio under low temperatures may indicate water deficit stress associated with cold stress.

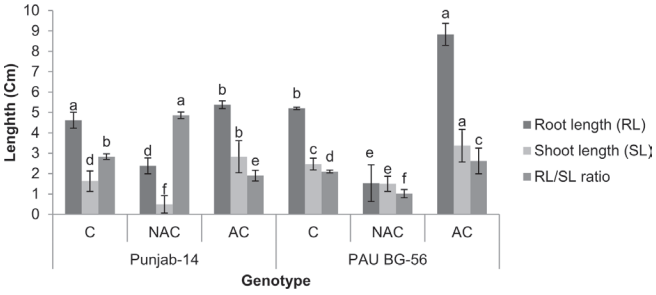

Fig. 2. Effect of low-temperature stress (5°C) on root length (RL) (cm), shoot length (SL) (cm), and RL/SL ratio in different bitter gourd genotype seedlings. C: Control, NAC: non-acclimated at 5°C, AC: Acclimated at 5°C. Values represent the mean from three seedlings (CD at *p≤0.05*)

Analysis of chlorophyll data revealed that exposure to low temperature led to a gradual decline in chlorophyll a (Fig. 3a), chlorophyll b (Fig. 3b), and total chlorophyll content in PAUBG-56 over the seven days of stress. Although initial levels of these pigments were higher in Punjab-14, the genotype still experienced reductions. Punjab-14 exhibited decreases in chlorophyll a of 25%, 41.86%, and 36.67% at the 3rd, 7th days after stress (DAS), and 14th day after recovery (DAR), respectively. In contrast, PAUBG-56 showed decreases of 11.32%, 38.46%, 82.35%, and 41.73% at the 1st, 3rd, 7th DAS, and 14th DAR, respectively. Chlorophyll b (Fig. 3b) also decreased under low-temperature stress, with reductions of 23.08% and 50% at the 1st and 3rd DAS in Punjab-14. By the 7th DAS and after recovery, chlorophyll b levels approached near control values. On the other hand, PAUBG-56 showed decreases of 60%, 26.67%, 6.67%, and 20% at the 1st, 3rd, 7th DAS, and 14th DAR, respectively. Acclimated seedlings of Punjab-14 maintained chlorophyll a (Fig. 4a) at levels similar to the control, while PAUBG-56 showed a slight 1.89% increase compared to the control. However, under stress at 5°C, chlorophyll a decreased by 22.22% in Punjab-14 and 13.67% in PAUBG-56. Chlorophyll b (Fig. 4b) remained higher in both genotypes, with Punjab-14 showing increases of 15.38% and 50% over control values, and PAUBG-56 showing increases of 8% and 6.67%, respectively. Total chlorophyll content decreased by 4.25% at the 1st DAS in Punjab-14 but remained near normal from the 3rd to 14th DAR. In contrast, PAUBG-56 showed increases of 1.82%, 0.91%, 2.0%, and 2.50% at the 1st, 3rd, 7th DAS, and 14th DAR, respectively. Acclimated seedlings of Punjab-14 and PAUBG-56 exhibited minimal reductions in total chlorophyll under stress (8.33% and 6.25%, respectively) compared to control values (Fig. 4d). After a seven-day recovery period, total chlorophyll content increased by 50% in Punjab-14 and 6% in PAUBG-56 seedlings. The chlorophyll a/b ratio (Fig. 4c) was higher at the 1st DAS, with increases of 14.28% in Punjab-14 and 80% in PAUBG-56 compared to controls. This ratio decreased during the exposure period but showed better recovery in PAUBG-56 (-6.98%) compared to Punjab-14 (-33.33%). At the 7th DAS, the chlorophyll a/b ratio increased by 30.50% and 39.50% in acclimated Punjab-14 and PAUBG-56 seedlings, respectively, compared to controls. By the 14th DAR, the ratio increased further by 37% and 43% in the acclimated seedlings of Punjab-14 and PAUBG-56, respectively, compared to the control.

Reduction in photosynthesis due to low temperature is a well-documented response in cold-sensitive plants. It has been observed that low-temperature stress typically leads to a decrease in chlorophyll a and total chlorophyll content, while changes in chlorophyll b content are often not significant (Esra *et al.*, 2010; Bertamini *et al.*, 2007). However, in the case of bitter gourd genotypes, a decrease in chlorophyll b was also observed under stress conditions. Low-temperature stress can increase chlorophyllase enzyme activity, which inhibits the synthesis of total chlorophyll and thereby reduces the overall content of photosynthetic pigments (Zonouri *et al*., 2014). Despite this, plants must retain sufficient chlorophyll levels to sustain photosynthesis to some extent under stress (Yang *et al.*, 2009). Acclimation plays a crucial role in mitigating these effects by helping plants maintain a higher chlorophyll a/b ratio and minimizing the reduction in total chlorophyll content (Yadegari *et al.*, 2007).

Carotenoids, known for their antioxidant properties, are crucial for plant defense against unfavorable and stressful conditions. In this study, control plants of PAUBG-56 exhibited higher carotenoid content compared to Punjab-14 (Fig. 3e). Low-temperature stress at 5°C led to a gradual decrease in carotenoid content from the 1st to the 7th day after stress (DAS) and the 14th day after recovery (DAR) in both Punjab-14 and PAUBG-56 seedlings. The reduction was more pronounced in Punjab-14, with decreases of 27.51%, 27.81%, 23%, and 46.74% at the 1st, 3rd, 7th DAS, and 14th DAR, respectively. In contrast, PAUBG-56 experienced smaller decreases of 16.17%, 4.11%, 1.38%, and 0.41% at the same intervals. Following recovery, carotenoid content in Punjab-14 remained lower, with a 46.74% reduction, while PAUBG-56 exhibited carotenoid levels close to those of control seedlings. Acclimated seedlings of Punjab-14 and PAUBG-56 showed smaller reductions in carotenoid content, with decreases of 8.33% and 6.25%, respectively, compared to controls (Fig. 4e). After recovery, carotenoid content increased by 7.14% in Punjab-14 and 10% in PAUBG-56. The maximum quantum efficiency of photosystem II (Fv/Fm ratio) remained unchanged in control plants of both genotypes but decreased by 12.50% in both Punjab-14 and PAUBG-56 under low-temperature stress. This reduction persisted throughout the exposure period, indicating that low temperatures adversely affected photosystem II (PS II). In acclimated seedlings, the Fv/Fm ratio decreased by 12.50% in Punjab-14, but remained closer to control levels in PAUBG-56 (Fig. 3f and 4f). Thus, PAUBG-56 demonstrated better resilience to low-temperature stress compared to Punjab-14.

Carotenoids, while not directly involved in photosynthesis, function as crucial accessory pigments that play a significant role in protecting photosystems from damage. They accumulate under low-temperature conditions and act as natural antioxidants by quenching potentially harmful triplet chlorophyll and singlet oxygen species that could damage the chloroplast (Passarini *et al.*, 2009; Han *et al.*, 2010). Despite this protective role, some studies have reported a reduction in carotenoid content under low-temperature stress (Fu *et al.*, 2017; Gerganova *et al.*, 2016). The observed decrease in chlorophyll and carotenoid content in bitter gourd seedlings could be attributed to oxidative stress induced by low-temperature exposure. The photosynthetic apparatus is highly sensitive to environmental stresses, with photosystem II (PS II) often being particularly

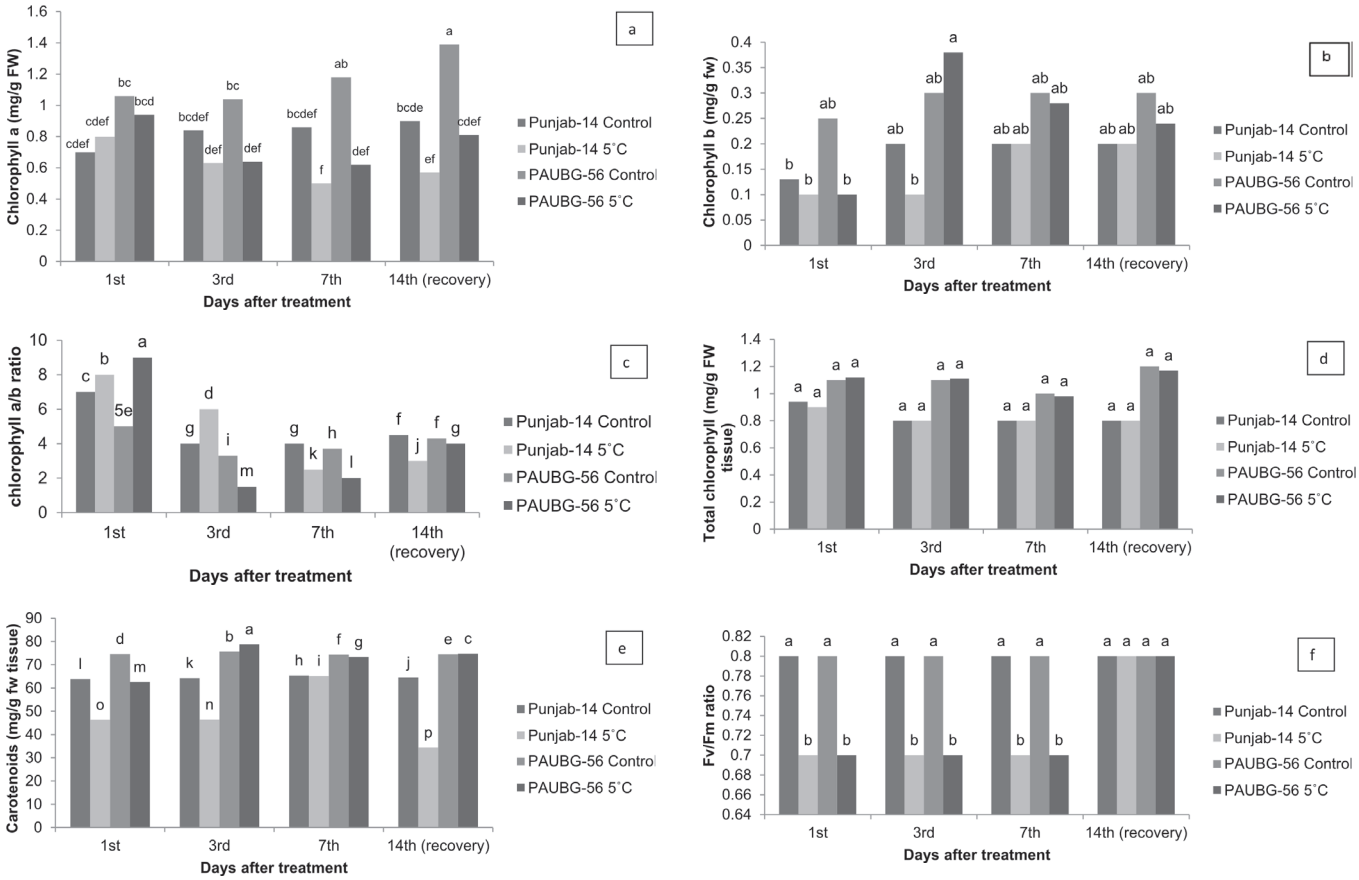

Fig. 3. Effect of low temperature (5°C) on photosynthetic pigments in the leaves of bitter gourd genotype seedlings. Values represent the mean from three seedlings (CD at *p≤0.05*)

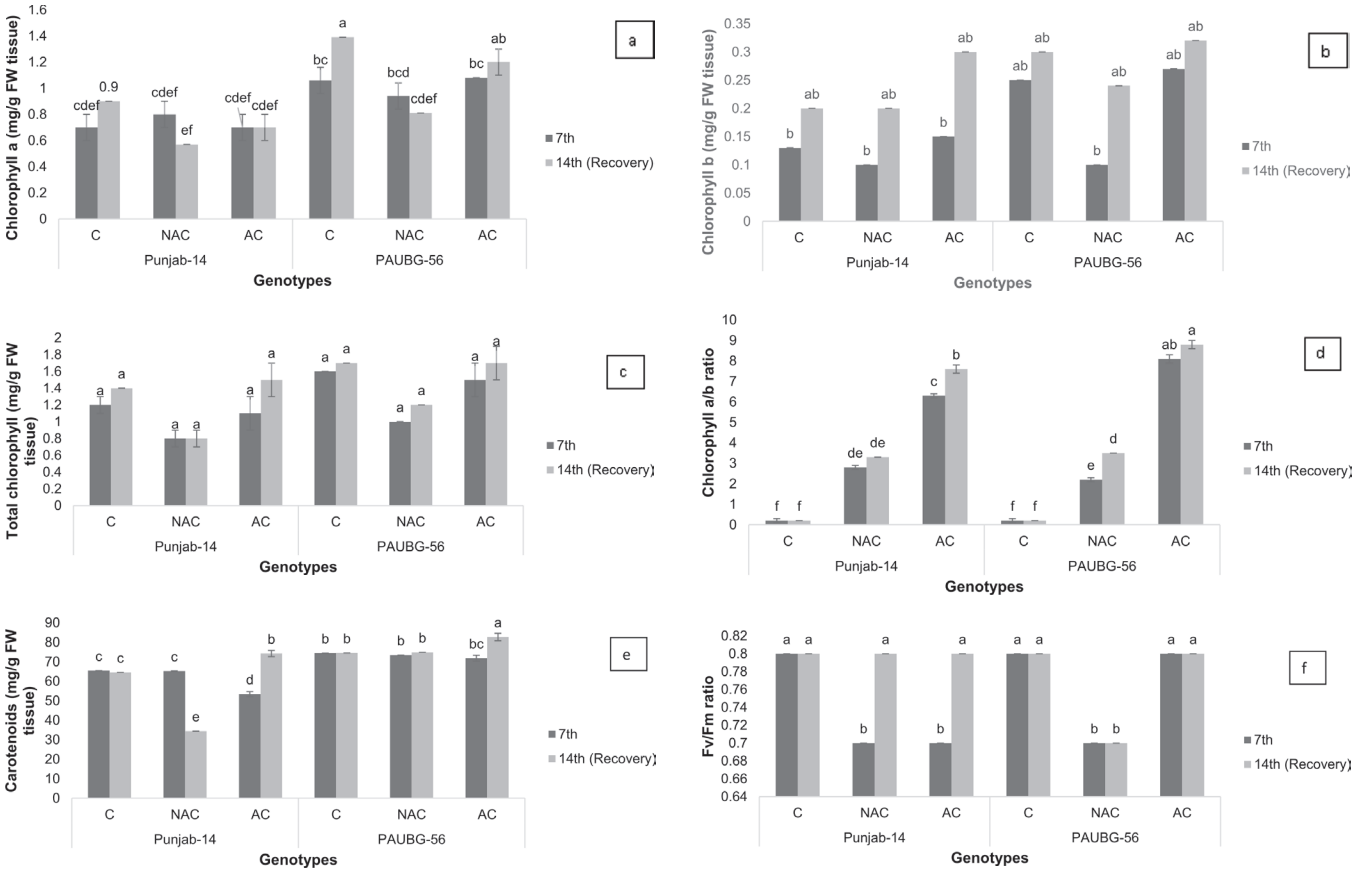

Fig. 4. Effect of cold acclimation (5°C) on photosynthetic pigments in the leaves of bitter gourd genotype seedlings. C: Control, NAC: Non-acclimated at 5°C, AC: Acclimated at 5°C. Values represent the mean from three seedlings (CD at *p≤0.05*).

affected by chilling stress (Zhang *et al.*, 2010). The Fv/Fm ratio serves as an indicator of the susceptibility of PS II to damage, reflecting the efficiency of this photosynthetic system. In the present study, although the Fv/Fm ratio was not drastically altered by cold stress, it still indicated a reduction in the efficiency of PS II. Cold acclimation is known to help maintain the Fv/Fm ratio, thereby preserving PS II efficiency under stress (Hajiboland and Habibi, 2011). This was also observed in the bitter gourd seedlings, where acclimation contributed to better maintenance of the Fv/Fm ratio, highlighting its role in enhancing resilience to low-temperature stress.

Electrolyte leakage increased significantly with exposure to low temperatures, showing a gradual rise throughout the stress period (Fig. 5). Specifically, electrolyte leakage in Punjab-14 increased by 68.5%, while in PAUBG-56 it rose by 40.5% at 5°C. Notably, electrolyte leakage was significantly lower in acclimated seedlings compared to those directly exposed to the cold, when compared to control seedlings (Fig. 6). For instance, electrolyte leakage in acclimated Punjab-14 and PAUBG-56 seedlings increased by 17.7% and 4%, respectively, compared to controls. Moreover, electrolyte leakage was higher in acclimated Punjab-14 seedlings than in the PAUBG-56 genotype. Following a seven-day recovery period, electrolyte leakage in acclimated seedlings decreased below the levels observed in control seedlings, indicating improved membrane stability and resilience in both genotypes after acclimation.

Low-temperature (LT) stress induces the accumulation of reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), superoxide radical ($O_2\bullet^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•), through various chemical reactions (Liu *et al.*, 2013). The accumulation of these ROS under LT stress can lead to membrane lipid peroxidation, which in turn increases malondialdehyde (MDA) content, electrolyte leakage, and ultimately results in membrane instability (Yang *et al.*, 2009; Khaledian *et al.*, 2015; Gulen *et al.*, 2008; Fahimirad *et al.*, 2013). The observed higher electrolyte leakage in the Punjab-14 genotype suggests its susceptibility to cold stress, as susceptible plants

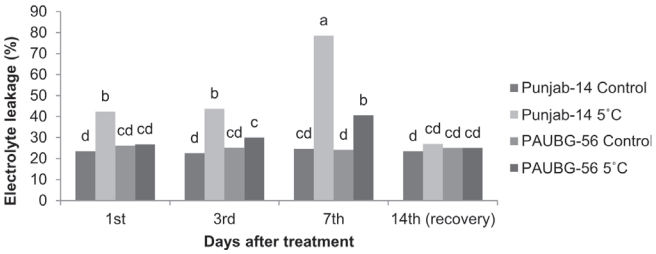

Fig. 5. Effect of low temperature (5°C) on electrolyte leakage in the leaves of bitter gourd genotype seedlings. Values represent the mean from three seedlings (CD at *p≤0.05*).

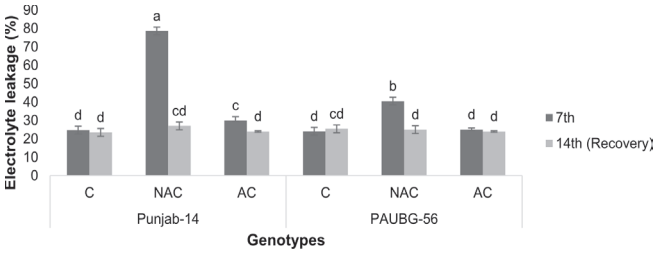

Fig. 6. Effect of cold acclimation (5°C) on electrolyte leakage in the leaves of bitter gourd genotype seedlings. C: Control, NAC: Non-acclimated at 5°C, AC: Acclimated at 5°C. Values represent the mean from three seedlings (CD at *p≤0.05*).

Table 1. Correlation coefficients among different morpho-physiological traits in bitter gourd seedlings under low-temperature stress

	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid	Chl a/b ratio	Electrolyte leakage	fv/fm ratio
Chlorophyll a	1						
Chlorophyll b	0.191	1					
Total chlorophyll	0.887*	0.484*	1				
Carotenoid	0.454*	0.417*	0.614*	1			
Chlorophyll a/b ratio	-0.059	-0.524*	-0.345	-0.378	1		
Electrolyte leakage	-0.501*	-0.141	-0.498*	-0.073	0.192	1	
fv/fm ratio	0.41	0.399	0.491*	0.246	-0.572*	-0.408*	1

* Significant at 5%

typically exhibit greater electrolyte leakage compared to more tolerant varieties (Jiang *et al.,* 2010; Kamran *et al.*, 2015). Elevated electrolyte leakage reflects membrane dehydration and ion leakage induced by LT stress (Gerganova *et al.*, 2016). On the other hand, acclimation has been shown to mitigate electrolyte leakage, thereby enhancing the survival and growth of plants under adverse conditions (Gong *et al.*, 2011; Yadegari *et al.*, 2007; Hajiboland and Habibi, 2011; Aaron and Ryan, 2009). Acclimated plants often exhibit improved membrane stability and reduced oxidative damage, contributing to better resilience against cold stress.

Correlation analysis

Table 1 presents the correlation analysis among various traits under low-temperature stress. The analysis reveals significant correlations between total chlorophyll and individual chlorophyll components: chlorophyll a (r = 0.887) and chlorophyll b (r = 0.484). This indicates that reductions in total chlorophyll content are closely associated with decreases in both chlorophyll a and b levels in seedlings. Furthermore, the Fv/Fm ratio exhibits a strong positive correlation with total chlorophyll content (r = 0.491), suggesting that a decrease in total chlorophyll negatively impacts the Fv/Fm ratio. Electrolyte leakage shows significant negative correlations with chlorophyll a (r = -0.501), total chlorophyll (r = -0.498), and the Fv/Fm ratio (r = -0.572). These negative correlations imply that increased electrolyte leakage is associated with reductions in total chlorophyll, chlorophyll a, and the Fv/Fm ratio. Thus, as the levels of chlorophyll and Fv/Fm ratio decrease, electrolyte leakage tends to increase, highlighting a detrimental impact of low-temperature stress on membrane stability and photosynthetic efficiency.

CONCLUSION

Bitter gourd is a valuable vegetable crop in India, primarily grown during the summer but in demand year-round due to its medicinal properties. Direct exposure of bitter gourd seedlings to low temperatures significantly reduced the survival percentage, particularly in the Punjab-14 genotype. This genotype exhibited increased electrolyte leakage and a gradual decline in chlorophyll a, chlorophyll b, and carotenoid content under cold stress. In contrast, the PAUBG-56 genotype maintained higher levels of these components compared to Punjab-14. Acclimation treatment, however, enhanced the survival percentage of both genotypes to 100%. This improvement is likely attributed to increased levels of chlorophyll a, chlorophyll b, total chlorophyll,

and a higher chlorophyll a/b ratio, along with improved membrane stability. Acclimated seedlings of both genotypes demonstrated better growth, as evidenced by increased root and shoot lengths compared to their non-acclimated counterparts. Thus, acclimating seedlings before exposure to low temperatures is an effective strategy to enhance the cold tolerance of bitter gourd. This approach not only improves survival rates but also promotes better growth during later stages of development under cold stress conditions.

Authors' contribution: Conceptualization of research work (SMKDV), Designing of the experiments (SMKDV), Contribution of experimental materials (PM); Execution of field/lab experiments and data collection (DV, KP); Analysis of data and interpretation (DV, Kumar P); Preparation of the manuscript (Sangha MK, Devi V).

Conflict of interest: Authors declare that they have no conflict of interests.

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