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Biochemical changes in bitter gourd in response to low temperature stress

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ABSTRACT

The present study investigated the effect of low-temperature stress on biochemical parameters in fifteen days old seedlings of bitter gourd genotypes. The seedlings were exposed to 10°C and 5°C for seven days and assessed for recovery at 25°C. Then, the seedlings were exposed to temperatures from 20°C to 5°C for acclimation, decreasing at 2°C/h before LT exposure. Leaf samples were collected on different days post-exposure, post recovery and analysed for biochemical parameters. The survival percentage of seedlings of PAUBG-56 was 10.7% higher than that of Punjab-14 at 5°C. Phenylalanine ammonia-lyase activity, total phenols, flavonols, and total proteins were significantly (CD at 5%) higher in PAUBG-56 than in Punjab-14 seedlings. New protein bands in the 12-62 kDa range were observed in SDS-PAGE in both genotypes, which might be involved in defence against stress. At the seedling stage, both genotypes showed resilience to cold stress, though it was observed to be higher in PAUBG-56. Cold stress caused an increase in phenolics and related enzymes in the seedlings. Acclimation improved cold tolerance in fifteen day old seedlings of both genotypes.

Keywords: *Momordica charantia*, Biochemical parameters, Bitter gourd seedlings, Acclimation, Antioxidants.

INTRODUCTION

Bitter gourd belongs to genus *Momordica* in the family Cucubitaceae. It is a flowering vine also known as karela, bitter melon, balsam pear. It thrives in the tropical, subtropical and temperate regions, but now cultivated all over the world because of the medicinal and dietary value. In addition to being good source of folate and vitamin C, bitter gourd is nutritionally comparable to other cucurbits. The vine tips of bitter gourd are rich in vitamin A and phenolics especially gallic acid. A number of other bioactive compounds, *viz*. saponins, peptides and alkaloids are also present in higher amounts in bitter gourds (Anilakumar *et al*., 2). Each part of bitter gourd including roots, vines, stems, leaves, seeds, unripe and ripe fruit have potential for management of diabetes. In addition to this in many countries, bitter gourd is also used to cure malaria. Generally bitter gourd is an annual crop but can also grow as perennial crop in areas having mild, frost-free winters. Under temperate field conditions, summer is the best season for the germination and production of bitter gourd. Minimum temperature required by the plants for its normal growth is 18°C during early periods, but optimal temperatures ranged from 24–27°C. Though low temperature retards the growth of bitter gourd yet it is more tolerant to cool temperatures compared to other gourds. A well drained sandy loam soil with a good irrigation system and appropriate amount of fertilizers are required for optimum growth of bitter gourd (Anonymous, 3). In Punjab it is mainly grown in summer season. The crop is available in market in winters at high price. The crop has round the year demand among people because of its medicinal value (Thakur and Sharma, 17). Despite this, due attention has not been paid towards a need based crop improvement programme. There is a prime need for its improvement and to develop varieties of bitter gourd that are suitable for cultivation at low temperature. Low temperatures (LT) stress is one of the abiotic stresses in plants that affect the cell survival, cell division, photosynthesis and water transport with negative effect on plant growth eventually constraining crop productivity. LT stress is categorised as, chilling stress where low temperature (0-15°C) cause injury without ice crystal formation in plant tissues; and freezing stress (<0°C) where in ice formation occurs within plant tissues. Both stresses together are termed as low temperature or cold stress (Singh *et al*., 15). In general, plants originating from tropical and subtropical region are sensitive to LT, whereas temperate plants showed chilling tolerance to variable degrees. To overcome stress generated by low temperature exposure, plants trigger cascade of events that enhance their tolerance through inducing biochemical and physiological modifications (Megha *et al*., 12). Various studies indicated the deleterious effect of low temperature on the plants. Acclimation helps to enhance the tolerance in plants. The investigation was undertaken to study biochemical changes and

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tolerance of bitter gourd genotypes to low temperature and under acclimation conditions.

MATERIALS AND METHODS

The present experiment was carried out in Punjab Agricultural University, Ludhiana during 2016-17. The plant material consisted of two bitter gourd genotypes; Punjab-14 and PAUBG-56. They were grown in plastic trays, containing mixture of coco peat, vermiculite and perlite in green house. Each tray contained 50 seedlings. Seedlings were watered every alternate day. After 15 days of germination (DAG) the seedlings were exposed to low temperatures (5°C and 10°C). Leaf samples were collected on 1st, 3rd and 7th day after stress and seven days after recovery, and stored at -20°C till further analysis. During acclimation, the seedlings were exposed to temperatures from 20°C to 5°C decreasing at the rate of 2°C/hr before LT exposure. Survival percentage after the low temperature stress was recorded.

Phenylalanine ammonialyase (PAL) (EC 4.3.1.24) assay was done by the method of Zucker *et al*. (20). Total phenol content was estimated with the method of Swain and Hillis (16). Flavonols were estimated using method of Balabaa *et al*. (4). Total protein was measured by the method of Lowry *et al*. (11). Proteins were separated by SDS-PAGE (Sodium dodecyl sulfate-Polyacrylamide Gel Electrophoresis) by the method of Walker (18). Electrophoresis was carried out in discontinuous system using 10% separating gel and 4% stacking gel. The molecular weight of protein subunits was measured by comparing sample bands to the standard protein molecular weight marker bands. Data analysis was done using CPCS1 statistical software.

RESULTS AND DISCUSSION

Effect of low temperature was reflected in decreased survival percentage of the seedlings at low temperature (Fig. 1). PAUBG-56 exhibited slightly higher survival percentage than Punjab-14 indicating the former to have better adaptability to low temperature stress.

PAL activity increased significantly in response to LT stress in leaves of the seedlings. The increase was more in PAUBG-56 at 5°C whereas at 10°C it was comparable (Fig. 2a & b). After recovery period the activity remained higher (17 and 24%) in acclimated seedlings (Fig. 2g). Phenylalanine ammonia-lyase (PAL) is the key enzyme of the phenylpropanoid pathway, converting L-phenyalanine (substrate) into *trans*-cinnamic acid, a precursor of phenolics. Phenolics protect plants against ROS by acting as antioxidants. Dong *et al*. (6) reported that chilling stimulated the expression of genes for phenylalanine ammonia-lyase (PAL) and lead to concomitant increase in the enzymatic activity in cucumber seedlings. Increase in PAL will lead to increase in total phenols. Total phenols registered increase under LT stress, but flavonols decreased significantly (CD at 5%) in Punjab-14 seedlings. Greater increase in phenol content was exhibited in PAUBG-56 seedlings and the content remained higher after recovery too (Fig. 2c & d). Acclimated seedlings had greater PAL activity as well as total phenols in Punjab-14 seedlings (Fig. 2h), which was higher even after recovery. Flavonols depicted increase at both the temperatures in PAUBG-56 (Fig. 2e & f). The increase was higher in acclimated seedlings too. The increase was higher in Punjab-14 (56%) samples compared to PAUBG-56 (38.5%) (Fig. 2i). After recovery also, the content was higher in samples of Punjab-14 seedlings. Esra *et al*. (5) reported that phenolics accumulated in seedling leaves under LT stress in pepper (*Capsicum annum* L.). In acclimated plants increase in PAL activity and accumulation of different phenolics are thought to play an important role in creating cold tolerance. Hajiboland and Habibi

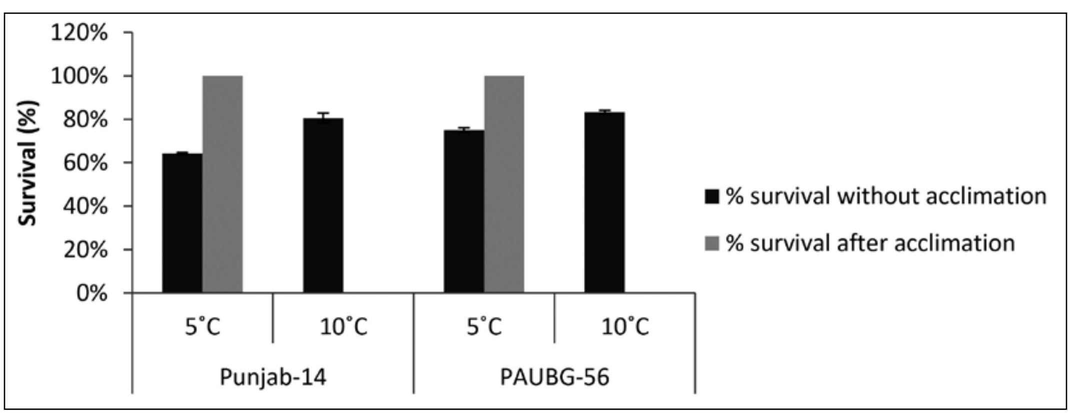

Fig. 1. Effect of low temperature and cold acclimation on survival percentage of bitter gourd seedlings.

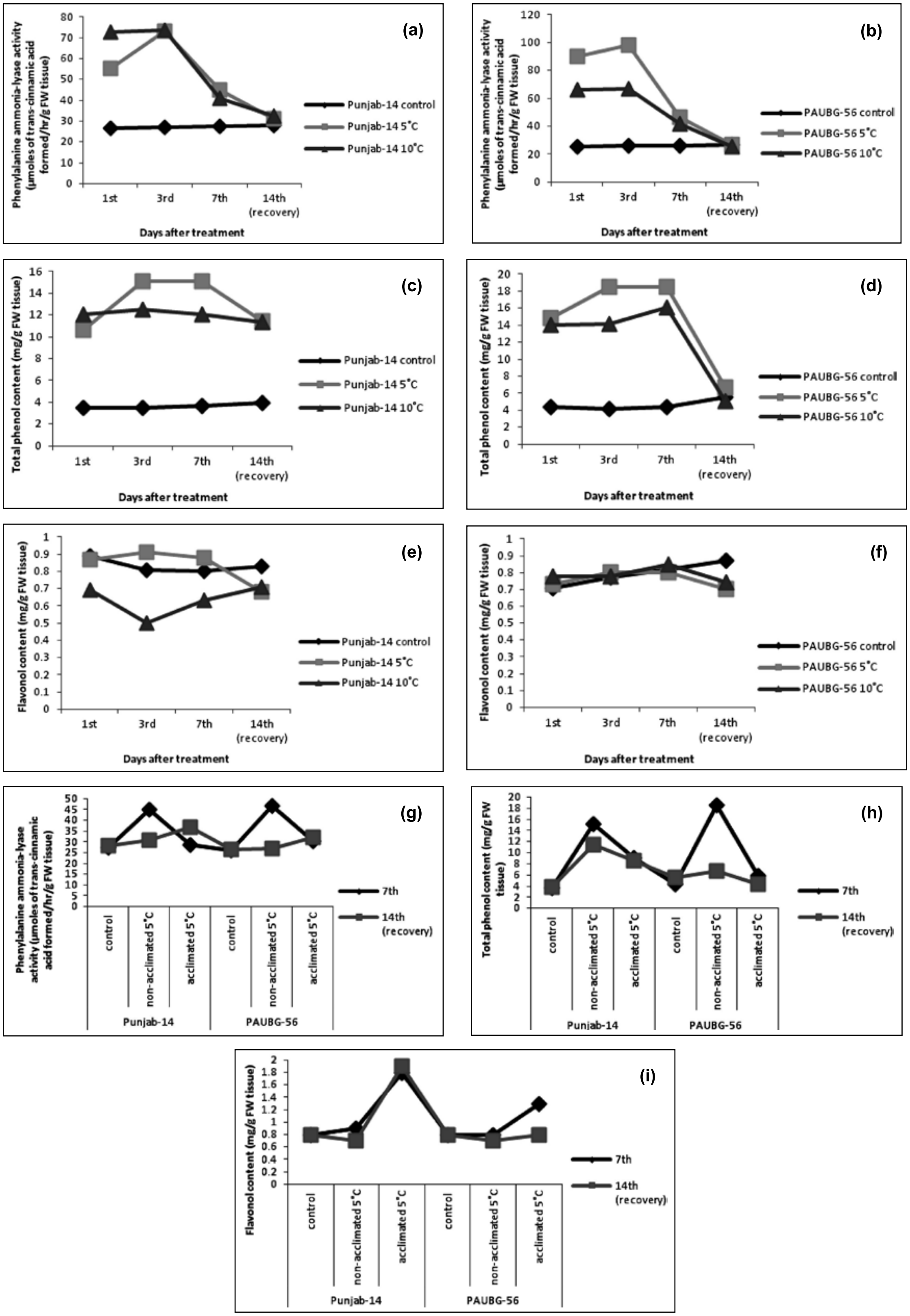

Fig. 2. Effect of low temperature stress and acclimation on phenylalanine ammonia-lyase activity and phenolics content in seedling leaves of bitter gourd genotypes.

(8) reported that PAL activity was increased in winter wheat cultivar under acclimation and more phenolic content accumulated in seedling leaves. Schulz *et al*. (14) showed that flavonoids accumulation increased in *Arabidopsis thaliana* after cold acclimation and all acclimated plants performed better under cold stress. Thus it could be inferred that increase in PAL activity, total phenols and flavonoids in response to cold stress in bitter gourd genotypes PAUGB-56 and Punjab-14 might help in their better stress tolerance.

Low temperature stress results in the synthesis of new proteins in plants. LT stress caused accumulation of total soluble proteins in both the genotype seedlings. The content increased initially in leaves of Punjab-14 but later registered decline. In Punjab-14seedlings, the peak was attained on the 1st day of stress; however it increased significantly upto 7th day of stress in PAUBG-56 genotype leaves (Fig. 3a & b). Total protein content in acclimated seedlings of Punjab-14 and PAUBG-56 increased by 31% and 33% respectively post LT exposure. After recovery, the higher level was still observed in acclimated seedlings as compared to their controls (Fig. 3c).

Low temperature stress caused loss of some proteins (lane 1 and 2) and appearance of new protein bands (lane 4, 5 and 6) in protein separation on SDS-PAGE. Numbers of new protein bands were observed in both the genotypes which were found to lie in the molecular weight range of 12-62 KD (Fig.4). In acclimated samples (Lane 5 and 6) too similar types of bands were observed.

Aghaee *et al*. (1) reported increase in total soluble proteins in response to low temperature stress in temperature sensitive rice genotypes. Also the soluble protein content remained unchanged in cold tolerant varieties. Our results are also confirmed by the study of Yadegari *et al*. (19), who reported that total protein content increased in both acclimated and non-acclimated seedlings of soyabean in response to low temperature exposure. During cold stress and cold acclimation various heat-shock proteins (HSPs) are induced in leaves (Janska *et al*., 10). This protein class is involved in stabilizing protein conformation and thus is essential to sustain cellular homeostasis under chilling stress (Janmohammadi *et al*., 9). Janska *et al*. (10) results revealed that during the cold acclimation, the production of ice nucleation substances in leaf and crown was suppressed, correlating with the rapid up-regulation of genes encoding the major antifreeze (chitinases, glucanases and thaumatin-like proteins) and ice recrystallization inhibition proteins. Sarhadi *et al*. (13) results showed induction of cold regulated (Cor)/ Lea (10-30 KDa) and antifreeze proteins (AFPs) during cold acclimation in the freezing tolerant

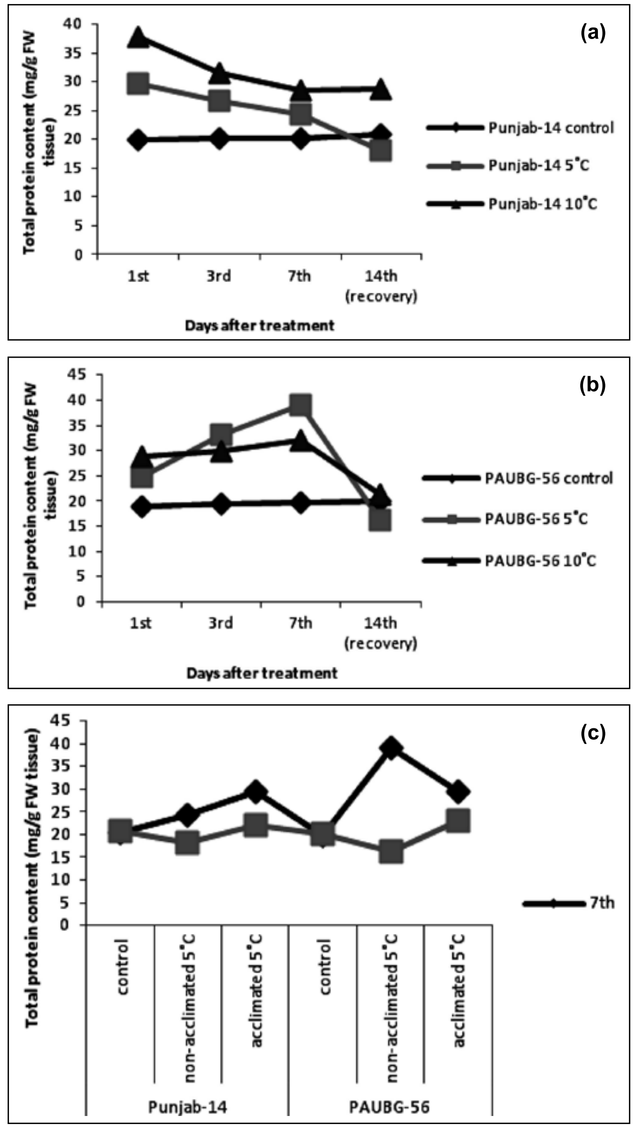

Fig. 3. Effect of low temperature and cold acclimation on total protein content in seedling leaves of bitter gourd genotypes.

wheat genotypes. Comparable results were also observed in diploid wild wheat (*Triticum urartu* L.) where cold acclimation increased the abundances of ROS scavenging proteins; LEA/RAB proteins and dehydrins (22-60 KDa) (Gharechahi *et al*., 7). Different band positions between Punjab-14 and PAUBG-56 indicate their specific response towards the low temperature stress.

Thus increased PAL activity, higher level of phenolics and induction of new proteins as seen in SDS-PAGE might be contributing to increased survival of the two bitter gourd genotypes at low temperature. Further although both the genotypes exhibited tolerance against low temperature yet

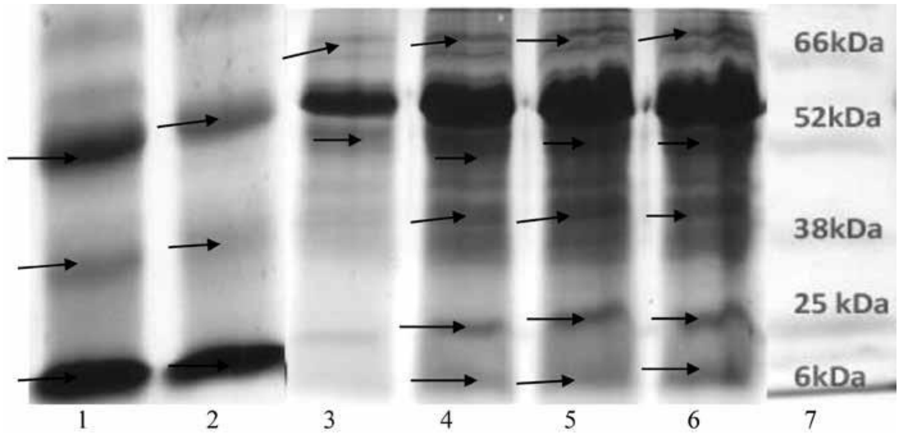

Fig. 4. SDS-PAGE of normal, LT stressed and acclimated seedlings of different bitter gourd genotypes.

1- Control punjab-14 3-Punjab-14 at 5˚C after 7 days stress 5-Acclimated Punjab-14 7-Protein marker ladder
2-Control PAUBG-56 4-PAUBG-56 at 5˚C after 7 days stress. 6-Acclimated PAUBG-56

PAUBG-56 registered better performance. Acclimation further improved the performance of the seedlings at low temperature. Further investigations should be carried out under natural conditions in field to validate the lab studies.

AUTHORS' CONTRIBUTION

Conceptualization of research (Sangha M.K.); Designing of the experiments (Sangha M.K. and Devi V.); Contribution of experimental materials (Pathak M.); Execution of field/lab experiments and data collection (Devi V. and Kumar P.); Analysis of data and interpretation (Devi V. and Kumar P.); Preparation of the manuscript (Devi V. and Sangha M.K.).

DECLARATION

The authors declare no conflict of interest.

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