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TOLERANCE TO CHILLING STRESS IN GERMINATING MUNGBEAN (*VIGNA RADIATA* L. WILCZEK) IS ASSOCIATED WITH INCREASED PHENOLICS AND PEROXIDASE ACTIVITY

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Summary. Chilling stress during germination is one of the major abiotic stresses for mungbean. During germination starch and protein reserves in mungbean cotyledons are degraded, which is hampered by chilling stress. An experiment was conducted using chilling stress tolerant and susceptible mungbean genotypes to investigate the effect of chilling stress on mobilization of cotyledon reserves, production of phenolics and activation of antioxidant enzymes. Comparative histological and biochemical studies showed that tolerant genotypes were more capable of channeling the cotyledon reserves for seedling growth, resulting in higher seedling vigor. In the tolerant genotype WBM 1222, higher autolysis of protein bodies and depletion of starch reserves supported better germination and seedling growth under chilling stress. Higher accumulation of secondary metabolites like phenolics was also associated with chilling tolerance in mungbean. During the recovery period, the tolerant genotype exhibited higher phenolics production. The significant increase in peroxidase activity in WBM 1222 during stress induction and recovery period suggests that peroxidase activity may be used as a biochemical marker for tolerance to chilling stress during germination of mungbean.

Key words: Mungbean; chilling stress; histology; tolerance; peroxidase.

Abbreviations: BSA – Bovine serum albumin; CRD – Completely randomized design; POX – Peroxidase; PPO – Polyphenol oxidase.

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INTRODUCTION

Mungbean or green gram (*Vigna radiata* L. Wilczek) is a major pulse crop in the world. About 90% of mungbean is cultivated in Asia (Chadha, 2010). It is also grown in parts of Southern Europe and South America, where the temperature is higher during germination and growth. In Asia, the crop is generally grown as a summer or spring crop.

Chilling stress is a major bottleneck to both spring and summer mungbean, particularly during germination and early seedling establishment. Although mungbean is the most widely accepted pulse crop used for consumption as sprouted seeds in Europe and America, its cultivation is limited in these continents due to lower adaptation to chilling temperatures (Vaughan and Geissler, 2009). Development of high yielding, chilling stress tolerant genotypes is the only sustainable approach to increase productivity under such agroclimatic situations. To achieve this goal, better understanding of the mechanisms of stress effects are of utmost importance to develop strategies for mitigation of chilling stress.

The biochemical changes in germinating leguminous seeds have been investigated in details (Harris and Chrispeels, 1975; Toyoyaka et al., 2001; Wang et al., 2007; Verma and Sharma, 2010). During germination, mungbean shows high enzymatic activity to convert the storage proteins and starch granules to readily usable energy that drives germination and early growth (Lin and Yao, 1996). Chilling stress leads to a reduction in starch hydrolysis and protein body degradation (Saleh, 2007).

The activities of anti-oxidant enzymes and phenolics in germinating seedlings increase during cold stress in many crop species (Chen et al., 2005). Under chilling stress, marked changes have beeen observed in mungbean cotyledons resulting in higher oxidative stress and activation of oxidase proteins (Darley et al., 1995; Gonzalez-Meler et al., 1999, Saleh, 2007). However, most of these studies used a single susceptible genotype, thus identifying only the stress responses. A comparative study of the cellular responses of stress tolerant and susceptible genotypes is more effective to identify cellular activities leading to chilling stress tolerance. In the present study, two mungbean genotypes, chilling stress tolerant and susceptible, have been subjected to chilling stress to identify the biochemical activities related to chilling stress tolerance during the stress induction and recovery phases.

MATERIALS AND METHODS

Stress treatment

Two genotypes, WBM 1222, a local landrace and Pusa 95-31, a popular high yielding variety were selected for the present study. The experiment was laid in a completely randomized design (CRD) with three replications. Fresh harvested seeds were surface sterilized with 0.1% mercuric chloride for 1 min, rinsed thoroughly using distilled water and germinated in sterile Petri dishes on a moist tissue paper in a plant growth chamber at 28°C for 24 h. The Petri dishes were sealed with paraplast to minimize evaporation loss. For chilling treatment, temperature was lowered to at 4°C for 24 h, with a 12 h day/light photoperiod (Yu

et al., 2003). The germinating cotyledons were then brought to normal growth conditions at 28°C and monitored for the next 72 h. A control experiment was also conducted without stress treatment. Loss in cotyledon weight, percent germination, shoot length and root length were monitored at different stages of germination, D_1 (24 h after 50% germination), D_2 (24 h after chilling stress), D_3 (48 h after germination in the control experiment) and D_4 (48 h after chilling stress). Cotyledon weight of 100 seeds from each experiment was measured after excision of the embryo. All length measurements were taken using digital calipers (Mitutoyo, Japan).

Determination of starch and protein content

Starch in the dissected cotyledons was extracted with hot 80% ethanol to remove soluble sugars. Starch content was determined as glucose content through hydrolysis and multiplying by a factor of 0.9 following Kaur et al. (2001). Starch content was expressed as mg g^{-1} fresh weight. For determination of protein content, dry and germinating seeds were homogenized with Tris-HCL buffer (0.1 M, pH 7.1). Protein content was determined according to Lowry et al. (1951) using BSA as a standard and expressed as mg g^{-1} fresh weight.

Determination of phenolics

Total phenolics were estimated using the Folin-Ciocalteu assay following Howard et al. (2002) using catechol as standard with some modifications. Ortho-dihydroxy phenol (OD-phenol) was estimated following Mahadevan and Sridhar (1986). Briefly, 2 g fresh sample (germinating seeds with roots and shoots) were extracted in 15 ml acetone:water (80:20, v/v), incubated for 1 h at 4°C and centrifuged at 10,000 x *g* for 20 min. The supernatant was collected in a volumetric flask and the pellet was re-extracted with 10 ml acetone:water (80:20, v/v) and the final volume was brought to 25 ml. 100 µl sample was drawn in a test tube and 200 µl of Folin-Ciocalteu reagent was added to it. After 2 min 1.5 ml of 20% sodium carbonate was added. The reaction mixture was incubated at 50°C for 30 min and the absorbance was measured at 765 nm using a spectrophotometer (Perkin-Elmer Lambda 25). Total phenolics were expressed as mg g^{-1} fresh weight.

Enzyme extraction and assays

Germinating seeds with new roots and shoots were homogenized with phosphate buffer (0.1 M, pH 6.8) and centrifuged at 12,000 x *g* for 30 min at 4°C. The clear supernatant was used as enzyme extract. Peroxidase (EC 1.11.1.7, POX) activity was measured according to Kar and Mishra (1976) using pyrogallol as a substrate. POX activity was determined by calculating the change in absorbance (ΔA) over control and expressed as E.U. mg^{-1} protein min^{-1}. Polyphenol oxidase (EC 1.14.18.1, PPO) activity was determined according to Kar and Mishra (1976) using pyrogallol as a substrate and expressed as E.U. mg^{-1} protein min^{-1}.

Histology of cotyledon

Fresh hand sections of cotyledons (0.1 mm thick) were made with sterile sharp razor blades and immediately stained with 1% safranin solution and

mounted using glycerin. The sections were observed under a compound light microscope (Olympus). Photographs were taken using a digital camera (Cannon A480).

Statistical analysis

Differences between means were tested using Student's *t*-test and analysis of variance. All data were analyzed using MS-Excel and SPSS (ver. 12.0).

RESULTS

Effect of stress on germination and seedling vigor

Loss of cotyledon weight due to mobilization of cotyledon reserves is a characteristic feature of seed germination. Significant differences were observed between dry seed weight of the two genotypes (difference in 100 seeds weight 1.11 g, P> 0.01). The effect of stress was prominent on cotyledon weight loss (Table 1). After water uptake, a mean 100-cotyledon weight increase was observed at stage D_2 (11.08±1.32 g). After chilling stress for 24 h, the mean 100-cotyledon weight loss was 1.82±0.24 g, indicating low metabolic activity. The cotyledon weight was further reduced by 1.39±0.43 g 24 h after stress treatment (D_4). In the absence of stress, the mean 100-cotyledon weight loss ($D_1 – D_4$) was higher (6.80±0.83 g) in comparison to stressed conditions (3.21±0.34 g).

Pusa 95-31, a bold seeded genotype recorded higher seed weight during all monitored stages than WBM 1222, a small seeded genotype (Table 1). However, the differences in cotyledon weight varied considerably at each stage. The loss of cotyledon weight was higher in Pusa 95-31 during the

Table 1. Effect of chilling stress on seed morphology and growth of mungbean at different stages of germination and seedling growth.

Stage	Genotype	100 cotyledon weight† [g]	Germination [%]	Shoot length [cm]	Root length [cm]
D_1	WBM 1222	13.29	80.04	0.68	0.11
	Pusa 95-31	17.08	75.38	0.92	0.10
D_2	WBM 1222	11.26	82.26	2.53	0.43
	Pusa 95-31	13.28	77.03	2.11	0.22
D_3	WBM 1222	7.24	88.74	5.39	2.14
	Pusa 95-31	8.53	92.81	6.35	2.68
D_4	WBM 1222	9.51	81.35	3.16	1.44
	Pusa 95-31	12.46	76.35	2.62	0.86
*SE (range)		0.53 – 2.82	2.32 – 6.25	0.07 – 1.76	0.01 – 0.84

Values are means of three replications. D_1: 24 h after germination, D_2: 24 h after chilling stress, D_3: 48 h after germination under non-stressed conditions (control), D_4: 48 h after chilling stress.

†Weight of cotyledon at different durations excluding embryo and seedling.

*Range of standard error.

first 24 h after germination (D_1). At D_2, the cotyledon weight loss was higher in the tolerant genotype compared to the susceptible one (16 mg). Even 24 h after withdrawal of stress treatment (D_4), mobilization of cotyledon reserves was higher in the tolerant genotype WBM 1222 than in Pusa 95-31 (Table 1). In control conditions, the cotyledon weight loss was higher in Pusa 95-31 compared to WBM 1222 at D_3.

Percent germination was higher in WBM 1222 (80±5.8) compared with Pusa 95-31 (75.4±4.1) at D_1 (Table 1). Under chilling stress WBM 1222 exhibited better germination than Pusa 95-31, although under control conditions the percent germination at stage D_3 was higher in Pusa 95-31. The length of shoot was significantly higher in Pusa 95-31 compared to WBM 1222 at stage D_1 and D_3, but upon chilling stress, better shoot growth was observed in the tolerant genotype WBM 1222 during the stages D_2 and D_4. Conversely, the difference in root growth was non-significant at D_1, but a significant effect of stress on root growth was noticed in D_2 and D_4. The chilling stress tolerant genotype WBM 1222 exhibited better root and shoot growth in response to stress.

Changes in starch and protein content

During germination, starch and protein reserves are depleted from the cotyledon to be used for initial seedling growth. Starch and protein content of bold seeded genotype Pusa 95-31 were higher than in WBM 1222, a local landrace having smaller size of the seeds. At stage D_1, cotyledon mean starch content was 9.09±0.43 mg g^{-1} fresh weight. Chilling stress decreased the rate of depletion of cotyledon starch compared to control (Table 2). Under normal growth conditions, the rate of depletion of starch in Pusa 95-31 (2.94

Table 2. Starch, protein, total phenol and ortho dihydroxy-phenol content [mg g^{-1} FW] of tolerant and susceptible genotypes at different stages of germination and seedling growth.

Stage	Genotype	Starch content	Protein content	Total phenol content	OD-phenol content
D_1	WBM 1222	8.78	27.50	5.87	1.10
	Pusa 95-31	9.39	52.45	5.24	0.66
D_2	WBM 1222	6.52	53.11	4.81	0.95
	Pusa 95-31	8.87	23.99	4.40	0.64
D_3	WBM 1222	5.84	42.40	6.55	1.25
	Pusa 95-31	5.63	17.27	5.87	0.86
D_4	WBM 1222	4.58	51.46	6.84	1.19
	Pusa 95-31	6.44	16.00	6.02	0.96
*SE (range)		0.8 – 2.14	0.71 – 4.16	0.12 – 0.33	0.03 – 0.1

Values are means of three replications. Stages are the same as in Table 1 and text.

*Range of standard error.

mg g^{-1} fresh weight) was higher than in WBM 1222 (mg g^{-1} fresh weight). When chilling stress was applied, the depletion of starch reserves at stages D_2 and D_4 was higher in WBM 1222 (2.26 and 4.2 mg g^{-1} fresh weight, respectively) than in Pusa 95-31 (0.52 and 2.95 mg g^{-1} fresh weight, respectively).

At stage D_1, mean protein content in the cotyledons was 39.98 mg g^{-1} fresh weight. Under chilling stress, mean protein content was reduced to 37.55 mg g^{-1} fresh weight and 33.79 mg g^{-1} fresh weight at stages D_2 and D_4, respectively. WBM 1222 had lower cotyledon protein content than Pusa 95-31 at both stages (Table 2). In the absence of chilling stress, the rate of depletion of protein reserves from the cotyledons was similar in both genotypes. However, under chilling stress, the reduction in cotyledon protein content in Pusa 95-31 at D_2 and D_4 stages was recorded to be 1.34 and 0.99 mg g^{-1} fresh weight, respectively. In WBM 1222, the reduction in protein reserves was found to be 3.51 and 12.10 mg g^{-1} fresh weight at D_2 and D_4 stages, respectively. Thus, under chilling stress cotyledons of the tolerant genotype WBM 1222 exhibited higher proteolytic activity resulting in better seedling growth.

Changes in phenolics, POX and PPO

Total phenol content of the tolerant genotype WBM 1222 was higher than in the susceptible genotype Pusa 95-31 at all stages studied (Table 2). Under cold stress, total phenol content decreased during the first 24 h and increased thereafter in both genotypes. The rate of increase in phenol production during the recovery phase (D_4) was significantly higher in WBM 1222 (2.43 mg g^{-1} fresh weight) than in Pusa 95-31 (1.62 mg g^{-1} fresh weight). The pattern of changes in OD-phenol content was similar to that of phenol content, being higher in the tolerant genotype as compared to the susceptible one (Table 2). In response to chilling stress, the tolerant genotype exhibited higher phenolics production than the susceptible genotype, particularly at the recovery phase.

POX activity was lower in WBM 1222 than in Pusa 95-31 at D_1 and increased significantly in both genotypes during germination under normal environment (Fig. 1). In WBM 1222, low POX activity was observed during the first 24 h, which increased sharply in both stressed and normal environment in the next 24 – 48 h. The increase in POX activity during the first 24 h under chilling stress was maintained during the recovery phase. On the other hand, POX activity increased sharply in Pusa 95-31 during the first 24 h, followed by a smaller increase under control conditions in the next 24 h. On the other hand, under chilling stress, POX activity in Pusa 95-31 decreased sharply in the next 24 h and did not increase significantly during the recovery phase.

There was no significant change in PPO activity during germination under both normal and stressed conditions (Fig. 2). PPO activity increased slightly under chilling stress in both genotypes during the first 24 h followed by a steady expression during the recovery phase (D_4).

Cotyledon histology

Bold seeded genotype Pusa 95-31 exhibited a higher amount of storage

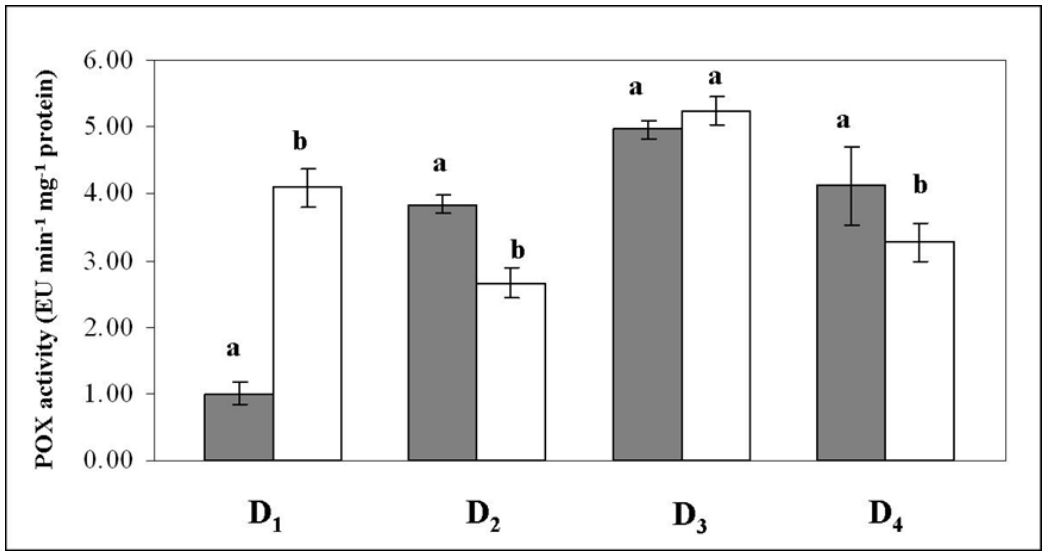

Fig. 1. POX activity in germinating mungbean at different stages ($D_1 – D_4$). Filled bars represent WBM 1222 and open bars represent Pusa-95-31. Bars marked with the same letter at each stage are not significantly different by Fisher's LSD.

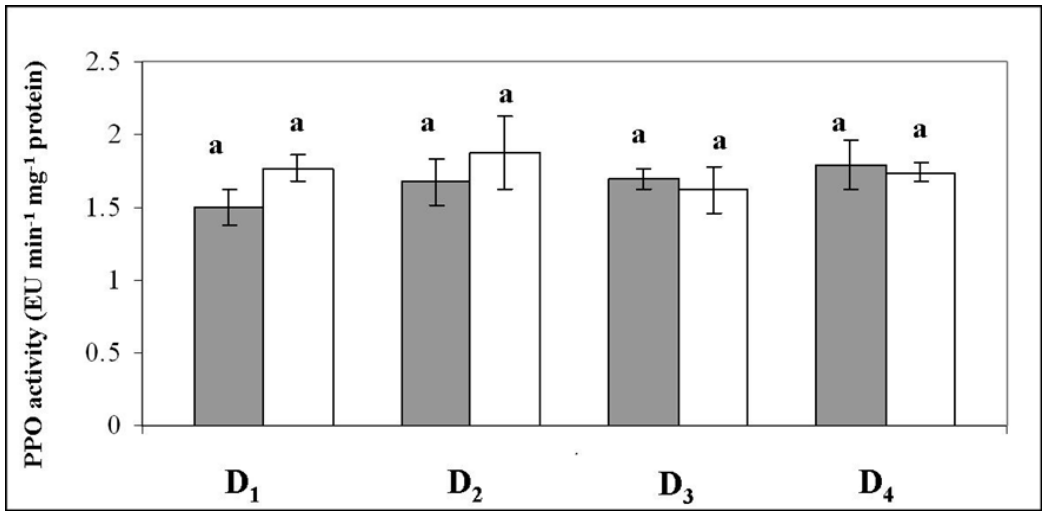

Fig. 2. PPO activity of germinating mungbean at different stages ($D_1 – D_4$). Filled bars represent WBM 1222 and open bars represent Pusa-95-31. Bars marked with the same letter at each stage are not significantly different by Fisher's LSD.

granules (starch granules and protein bodies) at stage D_1 compared to WBM 1222 (Fig. 3). In the tolerant genotype WBM 1222 the degradation of storage bodies continued during stages D_2 and D_4 and formed sectoral regions of storage bodies indicating continued enzymatic activities. On the other hand, Pusa 95-31, which showed lower seedling growth under stress, exhibited less degradation of storage granules. Histological observations at stages D_3 and D_4 in both genotypes showed that degradation of starch and protein bodies

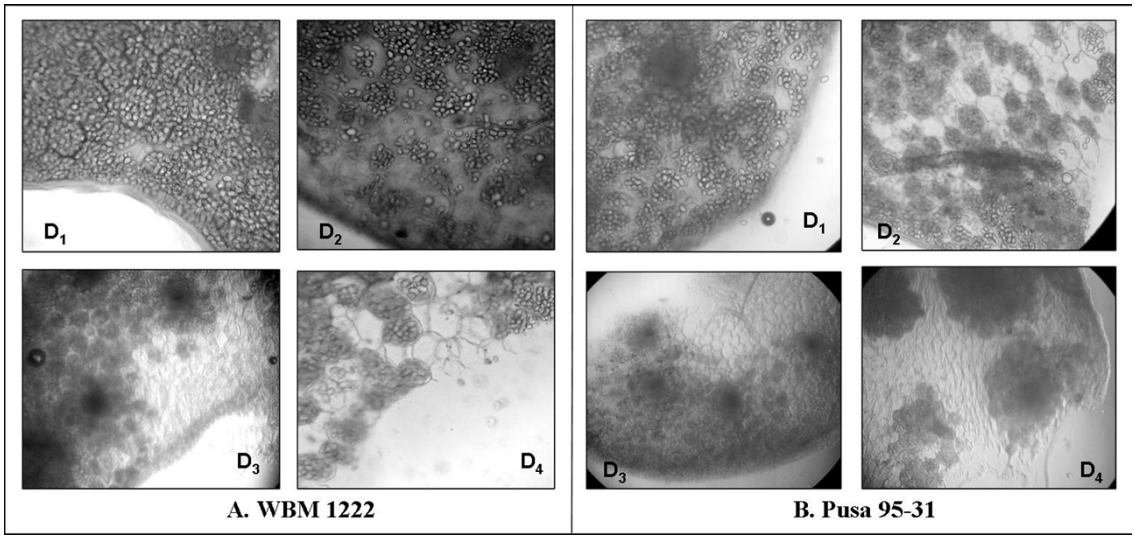

Fig. 3. Degradation of protein bodies and starch granules in germinating mungbean at different stages ($D_1 - D_4$). (A) WBM 1222, (B) Pusa 95-31. Storage bodies and their sectoral degradation are indicated with arrows.

was not synchronous, creating sectoral regions in the cotyledons 24–48 h after germination.

DISCUSSION

Cold stress at the germination stage is a major bottleneck of adaptation of summer mungbean in cooler climates adversely affecting germination and seedling establishment. The better understanding of the cellular metabolic activities during cold stress can help to identify the biochemical pathways involved in stress responses. Few studies have been conducted to examine the cellular metabolic changes in response to chilling stress during germination revealing the effects of stress on germination and growth, translocation of cotyledon reserves and ROS activities in the germinating seedling (Yu et al., 2003; Saleh, 2007). The general conclusions of these studies are that under chilling stress, seedling growth is hampered due to low translocation of protein and starch from the cotyledon. Chilling stress induces also membrane damage, increased ATPase activities and increased activity of active oxygen species (Darley et al., 1995; Saleh, 2007).

The starch reserves in leguminous cotyledons are stored as starch granules, while the proteins are stored as protein bodies. The protein bodies undergo degradation through proteolytic activities keeping the membranes intact through the process of autolysis (Harris and Chrispeels, 1975). The rapid hydrolysis is achieved by already activated hydrolytic and proteolytic enzymes during the early phase of germination through formation of proteolytic vacuoles and lytic vacuoles (Lin and Yao, 1996; Wang et al., 2007). Histological and biochemical evidences from the present study showed that in the tolerant genotypes these activities were higher, resulting in better germination and seedling growth.

In the present study, the tolerant

genotype exhibited better germination and seedling growth under chilling stress than the susceptible genotype with higher protein and starch degradation in the cotyledons. Histological studies showed that in the tolerant genotype, protein bodies and starch granules underwent autolysis more rapidly, exhibiting sectorial degradation pattern. Such patterns were observed earlier by Harris and Chrispeels (1975). Saleh (2007) also reported a significant decline in carbohydrate content in mungbean during germination. This suggests that chilling tolerance is associated with higher proteolytic and starch hydrolytic activities in the germinating cotyledon.

Higher expression of cellular activity under stress is often associated with the expression of ROS degrading enzymes. Peroxidase is a major indicator of mungbean germination as seedlings exhibit very high peroxidase activity during the germination process (Dendsay and Sachar, 1982). It has also been observed that application of hydrogen peroxide induces chilling tolerance in mungbean (Yu et al., 2002; Saleh, 2007). Our observations clearly indicated that tolerance to chilling stress was mediated by higher peroxidase activity, which helped to mitigate the stress-induced damages caused by the active oxygen species. The activity of peroxidase was maintained higher in the stress tolerant genotype during both the stress induction and recovery stages, indicating stress-induced peroxidase activity. Thus, peroxidase activity can be used as a reliable biochemical marker while screening for tolerance to chilling stress in mungbean.

Stress responses are also associated with the production of several secondary metabolites including phenolics. Higher total phenol and OD-phenol activities in the tolerant genotype suggested that the phenolics were involved in the cellular defense pathways against chilling stress in germinating mungbean. Besides, the phenolic compounds play a role in root formation as rooting cofactors (Nag et al., 2001). Higher root elongation coupled with higher phenolic production in the tolerant genotype WBM 1222 indicated that phenolics provided defense against chilling stress by promoting root formation in germinating seedlings. However, overproduction of phenolics leads to cell death. PPO is responsible for the degradation of phenolics produced under stress and thus helps to inhibit high phenolics accumulation and the associated cell damage and death. Elimination of PPO activity in mungbean seedlings and hypocotyls, however, had no effect on OD-phenol production. It was suggested that PPO was not involved in the metabolism of phenolic compounds in mungbean hypocotyls and seedlings (Duke and Vaughn, 1982). Our present study also confirms this observation in both tolerant and susceptible genotypes, but suggests also that due to its constitutive expression PPO may help in limiting overproduction of phenolics. Nag et al. (2001) observed inverse relationship of PPO and phenolics in germinating mungbean seedlings. In the present study, more phenolic compounds were synthesized in the tolerant genotype under chilling stress compared to the susceptible genotype. No significant changes in PPO activity under stress conditions were observed, indicating that

PPO activity cannot be used as a reliable biochemical marker for identification of chilling stress tolerant mungbean genotypes. The constitutive expression of PPO under both stress and control conditions most probably helps to limit overproduction of phenolics under stress conditions.

In conclusion, the present study revealed that in chilling stress tolerant mungbean better germination, growth and seedling vigor were mediated by higher cotyledon reserve mobilization together with increased production of phenolic compounds and higher peroxidase activity that prevented stress induced damage during germination and early seedling growth. These parameters can be used as reliable biochemical markers in screening for chilling stress tolerant genotype identification. Furthermore, isolation of genes associated with higher production of phenolics and increased peroxidase activity will help in further studies of stress response pathways and development of stress tolerant genotypes.

REFERENCES

Chadha M L, 2010. Short duration mungbean: a new success in South Asia. AVRDC – The World Vegetable Center, Regional Center for South Asia (RCSA), ICRISAT Campus, Hyderabad, India. 1–45.

Chen X W, L Yang, Y Jhang, J F Gong, 2005. Effects of low temperature at 10 degrees C on some antioxidant enzyme activities and ultrastructures of hypocotylar cells in mung bean and garden pea. Journal of Plant Physiology and Molecular Biology, 5: 539-544. (*In Chinese*).

Darley C P, J M Davies, D Sanders, 1995. Chill-induced changes in the activity and abundance of the vacuolar proton pumping pyrophosphatase from mung bean hypocotyls. Plant Physiol, 109: 659–665.

Dendsay J P S, R C Sachar, 1982. Hormonal control of peroxidase activity and its relationship with growth in mung bean seedlings. Plant Science Letters, 26: 251–256.

Duke S O & K C Vaughn, 1982. Lack of involvement of polyphenol oxidase in ortho-hydroxylation of phenolic compounds in mung bean seedlings. Physiologia Plantarum, 4: 381–385.

Gonza`lez-Meler M A, M Ribas-Carbo, L Giles, J N Siedow, 1999. The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. Plant Physiol, 120: 765–772.

Harris N, M J Chrispeels, 1975. Histochemical and biochemical observations on storage protein metabolism and protein body autolysis in cotyledons of germinating mung beans. Plant Physiol, 56: 292–299.

Howard L R, N Pandjaitan, T Morelock, M I Gil, 2002. Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. J Agr Food Chem, 50: 5891–5896.

Kar M, D Mishra, 1976. Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plant Physiol, 57: 315–319.

Kaur N, P Kaur, A K Gupta, 2001. Inter organ comparison of amylases and starch content in mungbean seedlings. Acta Physiol Plantarum,

23: 429–435.

Lin Y H, W H Yao, 1996. Mungbean (*Vigna radiata* L. Wilczek) contains some high proteolytic activities already before germination. Bot Bull Acad Sin, 37: 1–7.

Lowry O H, N J Rosebrough, A L Farr, R J Randall, 1951. Protein measurements with the Folin phenol reagent. J Biol Chem, 193: 265–275.

Mahadevan A, Sridhar R, 1986. Methods in Physiological Plant Pathology. 3rd Edn. Sivakami Publication, Madras. India. p. 316.

Nag S, K Saha, M A Choudhury, 2001. Role of auxin and polyamines in adventitious root formation in relation to changes in compounds involved in rooting. J Plant Growth Regul, 20: 182–194.

Saleh A A H, 2007. Amelioration of chilling injuries in mung bean (*Vigna radiata* L.) seedling by paclobutrazol, abscisic acid and hydrogen peroxide. *American Journal of Plant Physiology*, 2: 318–332.

Toyooka K, T Okamoto, T Minamikawa, 2001. Cotyledon cells of *Vigna mungo* seedlings use at least two distinct autophagic machineries for degradation of starch granules and cellular components. J Cell Biol, 154: 973–982.

Vaughan J G, C A Geissler, 2009. The New Oxford Books of Food Plants. Oxford University Press, New York.

Verma G & S Sharma, 2010. Role of H_2O_2 and cell wall monoamine oxidases in germination of *Vigna radiata* seed. Indian J Biochem Biophys, 47: 249–253.

Wang J, Y Li, S W Lo, S Hillmer, S S M Sun, D G Robinson, L Jiang, 2007. Protein mobilization in germinating mung bean seeds involves vacuolar sorting receptors and multivesicular bodies. Plant Physiol, 143: 1628–1639.

Yu C W, T M Murphy, C H Lin, 2003. Hydrogen peroxide-induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. Funct Plant Biol, 30: 955–963.