African Journal of Biotechnology Vol. 10(47), pp. 9673-9679, 24 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2446
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effect of polyamines on thermotolerance and membrane stability of soybean seedling

R. Amooaghaie and S. Moghym

Biology Department, Shahrekord University, POBox: 115, Shahrekord, Iran.

Accepted 24 June, 2011

The role of polyamines in the heat-shock protection of soybean seedling by application of exogenous polyamines (putrescine, spermidine and spermine) and their biosynthetic inhibitors involving CHA (cyclohexylamine) and DFMO (D, L α-diflouromethylornithine) was investigated. Application of polyamines, as a pre-treatment at 28°C for 2 h prior to heat-shock (45°C for 2 h), enhanced the recovery growth of both roots and hypocotyls. Treatment with polyamine biosynthetic inhibitors, that is, CHA and DFMO resulted in thermo sensitization, making seedlings vulnerable to heat-shock. Treatment of polyamines plus inhibitors reduced inhibitory effects and enhanced the growth recovery of seedling. Application of polyamines decreased electrolyte leakage and malondialdehyde from different tissue sections, suggesting protection of membrane integrity. Our results show that in comparisons with the heat-shock control, $CaCl_2$ application in similar to PAs decreased electrolyte leakage and lipid peroxidation from root and hypocotyl tissue sections, and in contrast, in the presence of EGTA (a chelator of calcium), the stress injury intensified and growth were severely inhibited, and electrolyte leakage and MDA content of roots were significantly increased. We suggest that under stress conditions, polyamines may replace calcium in maintaining membrane integrity by binding to phospholipids of the membrane.

Key words: Polyamines, *Glycine max*, electrolyte leakage, heat-shock, lipid peroxidation, seedling growth.

INTRODUCTION

Heat stress due to increased temperature is an agricultural problem in many areas in the world. Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development and may lead to a drastic reduction in economic yield (Porter, 2005; Wahid et al., 2007). The adverse effects of heat stress can be mitigated by developing crop plants with improved thermo tolerance using various genetic approaches. For this purpose, however, a thorough understanding of physiological responses of plants to high temperature, mechanisms of heat tolerance and possible strategies for improving crop thermo tolerance is imperative. Heat stress affects plant growth throughout its ontogeny, though heat-threshold level varies considerably at different developmental stages. For instance, during seed germination, high temperature may slow down or totally inhibit germination, depending on plant species and the intensity of the stress. At later stages, high temperature may adversely affect photosynthesis, respiration, water relations and membrane stability, and also modulate levels of hormones and primary and secondary metabolites. Furthermore, throughout plant ontogeny, enhanced expression of a variety of heat shock proteins, other stress-related proteins and production of reactive oxygen species (ROS) constitute major plant responses to heat stress. In order to cope with heat stress, plants implement various mechanisms, including maintenance of membrane stability, scavenging of ROS, production of antioxidants, accumulation and adjustment of compatible solutes, induction of nitrogen-activated protein kinase and calcium-dependent protein

---

*Corresponding author. E-mail: rayhanehamooaghaie@yahoo.com.

Abbreviations: CHA, Cyclohexylamine; **DFMO,** D,L-α-diflouromethylornithine; **EGTA,** ethylene glycol tetra acetic acid; **EL,** electrolye leakage; **MAD,** malondialdehyde; **ODC,** ornithine decarboxylase; **PAs,** polyamines; **Put,** putrescine; **Spd,** Spermidine; **Spm,** Spermine.

kinase cascades, and most importantly, chaperone signaling and transcriptional activation. All these mechanisms, which are regulated at the molecular level, enable plants to thrive under heat stress. Based on a complete understanding of such mechanisms, potential genetic strategies to improve plant heat-stress tolerance include traditional and contemporary molecular breeding protocols and transgenic approaches are designed (Wahid et al., 2007).

Polyamines (PAs) are low molecular weight polycations found in all living organisms. They are known to be essential for growth and development in prokaryotes and eukaryotes. In plant cells, the diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm) constitute the major PAs. They occur in the free form or as conjugates bound to phenolic acids and other low molecular weight compounds or to macromolecules such as proteins and nucleic acids. As such, they stimulate DNA replication, transcription and translation. They have been implicated in a wide range of biological processes in plant growth and development, including senescence, environmental stress and infection by fungi and viruses. Their biological activity is attributed to their cationic nature (Tiburcio et al., 1993; Galston et al., 1997; Bais and Ravishankar, 2002). The use of PAs biosynthesis inhibitors has shown a causal relationship between changes in endogenous PAs levels and growth responses in plants. These observations led to further studies into understanding the mode of PAs action. Some of the important observations suggest that PAs can act by stabilizing membranes, scavenging free radicals, affecting nucleic acids and protein synthesis, RNase, protease and other enzyme activities, and interacting with hormones, phytochrome, and ethylene biosynthesis (Slocum et al., 1984). Because of these numerous biological interactions of PAs in plant systems, it has been difficult to determine their precise role in plant growth and development. There is a growing appreciation of the role of polyamines in plant stress responses (Bouchereau et al., 1999; Kakkar and Sawhney, 2003) but their role in heat-shock protection of higher plants is less understood.

In this study, the polyamine mediated regulation of the heat-shock response of etiolated soybean seedling was investigated using exogenous applications of polyamines and their biosynthetic inhibitors. Changes in electrolyte leakage and lipid peroxidation of seedling tissues were monitored as measures of cellular membrane integrity.

MATERIALS AND METHODS

Soybean seeds (*Glycine max* (L) Merrill cv. Sahar) were germinated in the dark at 28°C in Petri-dishes on filter paper moistened with 5.0 ml of distilled water. Heat-shock at 45°C for 2 h was applied to germinated seeds with a radicle length of 3 cm in the dark, then seedlings were transferred to 28°C on water for 5 days to observ e recovery growth. For growth evaluations, length of primary roots, hypocotyls and the whole of seedling (root plus hypocotyl) were measured after 5 days recovery period. The tested polyamines (1 mM each) were: putrescine, spermidine and spermine. The biosynthetic inhibitors (4 mM each) were CHA (cyclohexylamine) and DFMO (D,L α-diflouromethylornithine). These were applied as a pre-treatment at the normal temperature of 28°C for 2 h before subjecting the seedlings to heat-shock (45°C for 2 h). Similarly, lysine an amino acid was tested at equivalent concentration to demonstrate that heat-shock protection is really a polyamine related phenomenon and not simply a growth effect from the extra reduced nitrogen and carbon supplied. After each treatment, the seedlings were transferred to 28°C and grown for 5 days. Seedlings kept entirely at 28°C without subjection to heat-shock but pre-treated in the aforementioned manner were also used as a control to observe the effects of polyamines and their biosynthetic inhibitors. Twenty seedlings were used in each experiment and the experiments were repeated on three separate occasions.

Membrane integrity measurements were carried out in relation to heat-shock control, polyamines, calcium and EGTA treatments. Measurements were made either after heat-shock (45°C for 2 h) using excised embryonic axes or after 24 h of recovery growth using sections of primary roots or hypocotyls. Each experiment was repeated thrice. Electrolyte leakage was determined as described by Dionisio-Sese et al. (1999) with some modifications. Embryonic axes (200 mg), roots or hypocotyls (200 mg) were incubated at 28°C for 5 h in test tubes containing 20 ml distilled water and then initial conductivity (E_1) was measured by a conductivity meter. The tubes containing tissues were boiled for 30 min to release all the electrolytes and conductivity (E_2) was measured again. The electrolyte leakage was calculated as follows:

$$EL = (E_1/E_2) \times 100$$

Oxidative damage to lipids was determined as lipid peroxidation in terms of malondialdehyde (Bernheim et al., 1948). Embryonic axes (200 mg), roots or hypocotyls (200 mg) were homogenized in 5.0 ml of TCA (0.1%) and centrifuged at 10,000 g for 10 min. 1 ml of the supernatant was mixed with 4.0 ml of 0.5% TBA in 20% TCA. The mixture was heated at 95°C for 30 min, cooled over ice and centrifuged at 10,000 g for 10 min. The absorbance of the supernatant was recorded at 532 nm and corrected for non-specific absorbance at 600 nm. MDA content was calculated using an extinction coefficient (ε) of 155 $mM^{-1}cm^{-1}$ and expressed as nmol g^{-1} FW.

Statistical analysis

The obtained data were statistically analyzed as a factorial experimental design (SAS, 1996) applying the least significant difference (LSD) at 5% for the comparison among the treatment means. Observations were recorded in 3 replications, and mean values were pooled and standard error (S.E.) was calculated.

RESULTS

The results show that an exposure to 45°C for 2 h caused a 47% decrease in primary root length and a 50% decrease in hypocotyl length (Table 1). The treatment of seedlings with polyamines without a subsequent heat-shock and kept entirely at 28°C revealed a promotory effect up to 13% for hypocotyl length and 18% for root length by application of putrescine, suggesting a role of polyamines in seedling growth. Application of polyamines resulted in enhancement of thermoprotection response. Putrescine was the most effective, followed by

Table 1. Influence of polyamines and lysine on the heat-shock response of soybean seedlings.

Treatment	Root growth (cm)	Hypocotyl growth (cm)	Whole seedling (cm)
28°C	5.52±0.34b	4.94±0.27b	10.46± 0.49b
45°C	2.92±0.34d	2.49±0.27d	5.41± 0.49e
28°C +1 mM Putrescine	6.51±0.12a	5.58±0.24a	12.08± 0.31a
28°C +1 mM Spermidine	5.61±0.23b	5.01±0.15ab	10.62±0.41b
28°C +1 mM Spermine	5.53±0.31b	4.89±0.25b	10.42± 0.33b
28°C +1 mM Lysine	5.49±0.21b	4.97± 0.28b	10.46± 0.45b
28°C +1 mM Putrescine (2h)- 45°C (2 h)	4.91±0.34bc	3.57±0.27c	8.48± 0.49c
28°C +1 mM Spermidine (2h)- 45°C (2 h)	4.15±0.34c	3.25±0.27c	7.36±0.49d
28°C +1 mM Spermine (2h)- 45°C (2 h)	2.99±0.34d	2.52±0.27d	5.51± 0.49e
28°C +1 mM Lysine (2 h)- 45°C (2 h)	2.97±0.38d	2.42±0.32 d	5.39± 0.53e
LSD	0.97	0.65	1.11

Means not sharing the same letters differ significantly at p<0.05.

Table 2. Influence of polyamine biosynthetic inhibitors on the heat-shock response of soybean seedlings and reversal of their effect by polyamines.

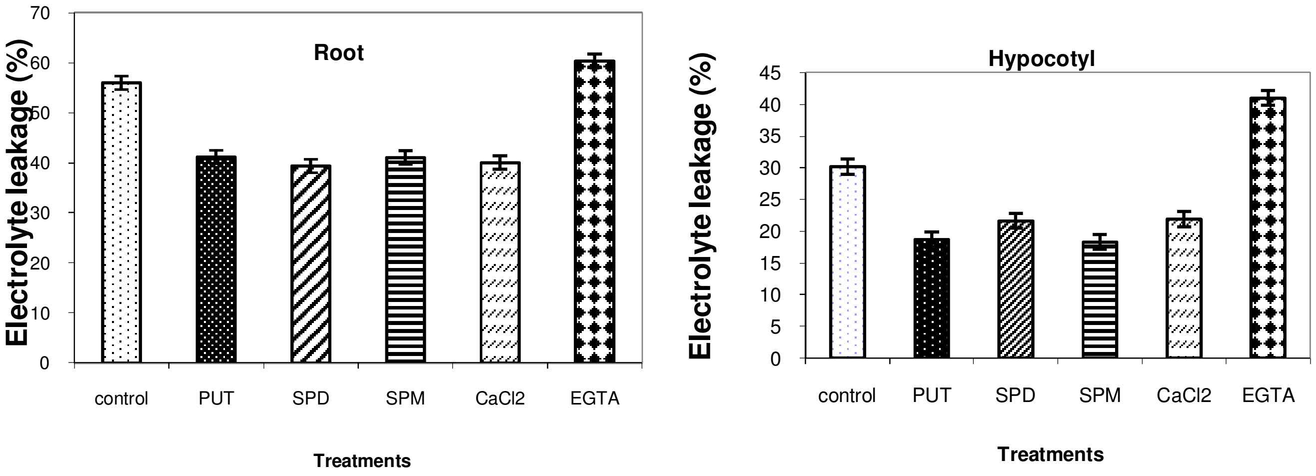
Treatment	Root growth (cm)	Hypocotyl growth (cm)	Whole seedling (cm)
28°C	5.75±0.38a	4.90±0.43a	10.65±0.50a
45°C	2.80±0.38d	2.53±0.43c	5.33 ±0.50d
28°C +4 mM DFMO (2 h)- 45°C (2h)	2.12±0.39e	1.93±0.44de	4.05±0.37e
28°C +4 mM CHA (2 h)- 45°C (2h)	2.06±0.38e	1.65±0.43e	3.71±0.50e
28°C, 4 mM DFMO+1 mM Put (2h)- 45°C (2 h)	4.30±0.39b	3.04±0.44b	7.34±0.37b
28°C, 4 mM CHA+ 1 mM Spd (2h)- 45°C (2 h)	3.45±0.38c	3.08±0.43b	6.53±0.50c
28°C, 4 mM CHA+ 1 mM Spm (2h)- 45°C (2 h)	3.27±0.38cd	2.23±0.43cd	5.50±0.50d
LSD	0.59	055	0.75

Means not sharing the same letters differ significantly at p<0.05.

spermidine. Thermotolerance was induced in both root and hypocotyl tissues, but the roots showed a greater response (Table 1).

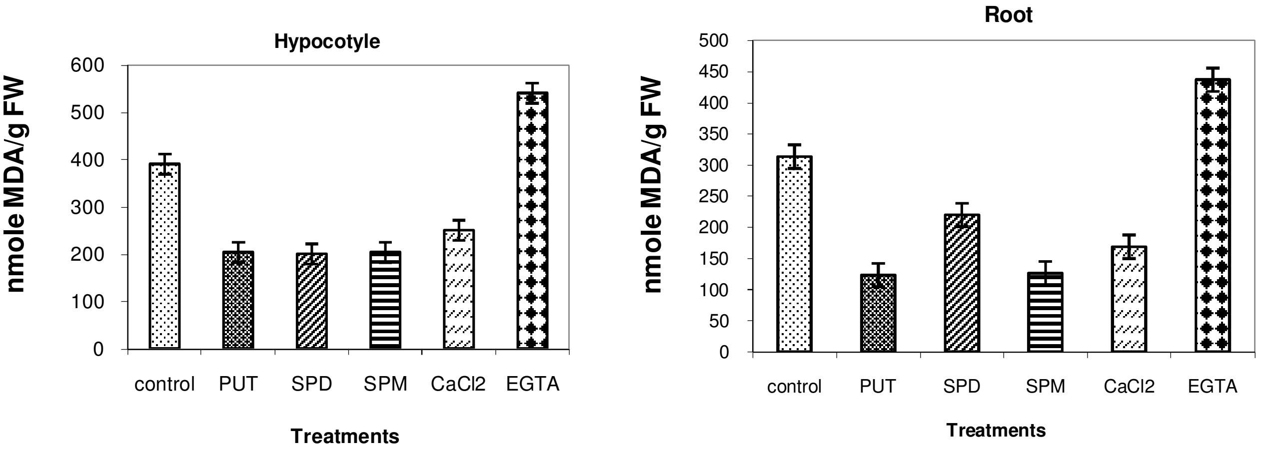
Heat-shock at 45°C for 2 h was applied to germinated seeds with a radicle length of 3 cm in the dark, then seedlings were transferred to 28°C on water for 5 days to observe growth recovery. For growth evaluations, length of primary roots, hypocotyls and the whole of seedling (root plus hypocotyl) were measured after 5 days recovery period. The maximum response of root growth recovery (31%) and hypocotyl growth (22%) was obtained with 1 mM putrescine. Spermidine caused a 20 and 15% recovery of root and hypocotyl growth, respectively. SPM was less effective for seedling growth. The treatment of seedlings with polyamines with a subsequent heat-shock revealed an enhancement of thermoprotection response suggesting a stress adaptive role of polyamines. This point was further proved when lysine, applied at similar conditions as polyamines, failed to elicit a protective response (Table 1).

Our results demonstrate that heat-shock protection is really a polyamine related phenomenon and not simply a growth effect from the extra reduced nitrogen and carbon supplied. Treatment with both the inhibitors rendered the seedlings vulnerable to heat-shock. The inhibitory effects were reversed on adding putrescine and spermidine (Table 2). The tested polyamines (1 mM each) putrescine, spermidine, spermine and biosynthetic inhibitors (4 mM each), CHA and DFMO were applied as a pre-treatment at the normal temperature of 28°C for 2 h before subjecting the seedlings to heat-shock (45°C for 2 h). The effect of polyamines on maintenance of membrane integrity under heat-shock was also investigated. In heat shock stress soybean plants, exogenous Put, Spd and Spm (at 1 mM each) reduced the stress injury (as EL) in roots and shoot than to its control (Figure 1). The



(A) (B)

Figure 1. Changes in electrolyte leakage of root (A) and hypocotyl (B) tissues sections of soybean seedlings following exposure to 45°C for 2h.



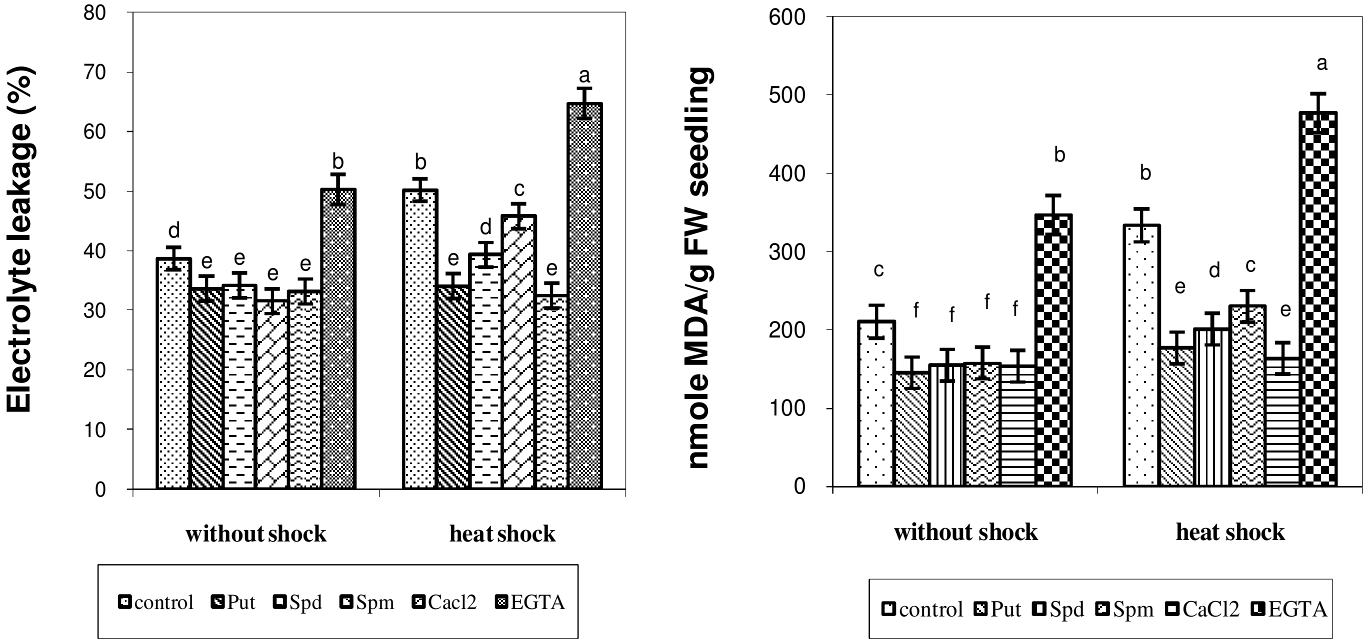
(A) (B)

Figure 2. Changes in lipid peroxidation of root (A) and hypocotyl (B) tissues sections of soybean seedlings following exposure to 45°C for 2 h.

MDA content of shoots and roots soybean showed an appreciable decrease with all the PAs (Figure 2). Our results show that in comparisons with the heat-shock control, $CaCl_2$ application in similar PAs decreased electrolyte leakage (Figure 1) and lipid peroxidation (Figure 2) from root and hypocotyl tissue sections and suggesting protection of membrane integrity. In contrast, in the presence of EGTA (a chelator of calcium), the stress injury intensified, and growth was severely inhibited, and electrolyte leakage and MDA content of roots was significantly increased (Figures 1 and 2). Heat shock increased electrolyte leakage and lipid peroxidation (Figures 3A and B, respectively) from embryonic axes, indicating membrane injury. Our results show that in comparisons with the heat-shock control, putrescine, spermidine and spermine all decreased electrolyte leakage and lipid peroxidation from embryonic axes, suggesting that polyamines protect against damage of membrane integrity.

DISCUSSION

Heat stress is a major growth limiting factor for most crop plants. Prolonged or even a transitory exposure to high temperature leads to altered metabolic functions and



(A) (B)

Figure 3. Comparison of electrolyte leakage and lipid peroxidation of embryonic axes of soybean seedlings with and without heat shock.

growth (Iwaya-Inoue et al., 2004). Our result shows that heat shock stress caused decrease in root and shoot length of soybean seedling (Table 1). Soybean is a thermophilic plant, but episodes of heat stress in temperature of 45°C induced marked changes in seedling growth. Heat shock increased electrolyte leakage and lipid peroxidation (Figure 2) from embryonic axes and suggesting damage of membrane integrity. Many studies indicate that heat induced loss of membrane stability is a major reason for reduced growth of various plant species (Iba, 2002) and has been used as selection criteria for heat tolerance (Marcum, 1998; Rehman et al., 2004). Elevated temperature results in rapid loss of water from the plant surface and causes a state of dehydration. This leads to the disruption of cellular membranes, making them more permeable to ions (Jiang and Huang, 2001) by increased solubilization and peroxidation of membrane lipids under stressful conditions (Iba, 2002). Marcum (1998) noted a positive relationship between cell membrane thermo-stability and shoot dry weight for Kentucky bluegrass cultivars and regarded it as a reliable screening tool for heat stress response. The treatment of seedlings with polyamines without a subsequent heat-shock and kept entirely at 28°C revealed a promotory effect up to 13 and 17%, respectively, for hypocotyle and root length by application of putrescine, suggesting a role of polyamines in growth seedlings. Various studies highlight profound effects of exogenous PAs application as foliar or presowing seed treatment.

The important role of polyamines in root formation and growth has also been demonstrated in other studies (Bais and Ravishankar, 2002; Couee et al., 2004). In this study, the PA absorbed by the seedling stabilized the cellular membrane and produced vigorous seedlings with greater length under heat stress. The pattern of electrolyte leakage indicates the involvement of PAs in the stabilization of cellular membranes. Our results show that in comparisons with the heat-shock control, putrescine, spermidine and spermine all decreased electrolye leakage and lipid peroxidation (Figure 3) from embryonic axes and suggesting protection of membrane integrity.

Our results are in agreement with previous studies (Nayyar et al., 2005), where exogenous polyamines were reported to alleviate stress-induced growth inhibition possibly due to protection of membranes and minimization of oxidative damage. Several lines of evidence have shown that stimulatory effect of exogenous polyamines may be related to their multi-faced nature, which includes working as an antioxidant, a free radical scavenger and a membrane stabilizer (Velikova et al., 2000; Liu et al., 2007). In recent years, the availability of specific inhibitors of PA biosynthesis has allowed us to probe the mechanisms involved and this may help to uncover the physiological significance of PA responses to several processes of growth and development. For example, difluoromethylornithine (DFMO) is a suicide inhibitor of ODC. ADC can be inhibited by the suicide inhibitor, difluoromethylarginine (DFMA). These studies have shown that PAs are involved with a wide range of developmental processes (Tiburcio et al., 1993, Galston et al., 1997, Kaur-Sawhney et al., 2003).

Our experiments show that addition of DFMO (specific inhibitors of ornithine decarboxylase) and CHA (an inhibitor of Spd and Spm synthase), to the seedlings reduced shoot and root growth and that this inhibition was reversed by the addition of exogenous Put, Spd and Spm (Table 2). These results clearly show that PAs are involved in growth and development. The use of PAs biosynthesis inhibitors has shown a causal relationship between changes in endogenous PAs levels and growth responses in plants. These observations led to further studies into understanding the mode of PA action (Kaur-Sawhney et al., 2003). In this study, the participation of polyamines in stress response was corroborated by use of their biosynthetic inhibitors like DFMO and CHA, which increased the stress injury and severely impaired the growth. The loss of thermotolerance by DFMO may be interpreted to mean that ODC pathway of polyamine biosynthesis is operative and is required for thermo-protection and growth recovery. Our results show that pretreatment of soybean seedlings with polyamine inhibitors led to increase the sensitivity of the plant to heat shock. The addition of exogenous PAs to heat shock stressed soybean seedlings retarded growth reduction (Table 2) and inhibited loss of membrane stabilization (Figures 1 and 2). The treatment of seedlings with polyamines with a subsequent heat-shock revealed an enhancement thermo tolerance (Figure 3) and the effect of PAs on seedling growth in heat shock condition was more than normal condition. It is suggesting a stress adaptive role of polyamines. This point was further proved when lysine, applied at similar conditions as polyamines, failed to elicit a protective response (Table 1). Other studies also show that these compounds are involved in plant response to environmental stresses and mineral nutrient deficiencies.

Campos et al. (1991) reported that pretreatment of *Arabidopsis* with DFMA prevented the rise of Spd induced by ultraviolet C irradiation (UV-C), and increased the sensitivity of the plant to UV-C. Application of Spd to the plant partially prevented the damaging effects induced by UV-C (Campos et al., 1991). The addition of exogenous PAs to osmotically stressed oat leaves retarded protein degradation, inhibited loss of chlorophyll and stabilized thylakoid membranes (Besford et al., 1993). Interest has been growing in the possible involvement of polyamines in the defense reaction of plants to various environmental stresses (Bouchereau et al., 1999; Kakkar and Sawhney, 2003). Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and plasma membrane-bound H^+-ATPase in salt-tolerant and salt-sensitive rice cultivars. Velikova et al. (2000) reported that exogenous polyamines have protective role on oxidative stress in acid rain treated bean plants. Nayyar et al. (2005) reported polyamines involve in the contrasting sensitivity of chickpea (*Cicer arietinum)* and soybean (*Glycine max*) to water deficit stress. It has been found that stress-tolerant plants increase their endogenous PAs levels to a much greater extent than sensitive ones. Furthermore, transgenic plants overproducing PAs possess greater stress tolerance (Galston et al*.,* 1997) and exogenous PAs confer protection from a variety of abiotic stresses (Nayyar et al., 2005).

Phenotypic analyses of mutants and transgenic plants with altered PAs levels gives further support to the previous physiological studies with regard to the involvement of these compounds in plant responses to abiotic stresses (Soyka and Heyer, 1999; Roy et al., 2005). The integrity and functions of biological membranes are sensitive to high temperatures. High temperatures modify membrane composition and structure and can cause leakage of ions (Wahid et al., 2007). In this study, we tested the hypothesis that heat shock stress sensitivity in soybean plants is related to changes in the levels and functioning of PAs. Our result confirms that PAs caused greater tolerance to heat shock stress. Our results are in agreement with previous studies, where exogenous PAs were reported to alleviate stress-induced growth inhibition possibly due to protection of membranes and minimization of oxidative damage (Basra et al., 1992; Nayyar et al., 2005). Polyamines being cationic in nature can associate with anionic components of membrane such as phospholipids thereby stabilizing the bilayer surface and retarding membrane deterioration. Polyamines also have radical scavenging properties. Protection of membranes from peroxidation by polyamines could involve both their ability to interact with phospholipids and their antioxidant activity (Roy et al., 2005). Our results show that under heat shock stress conditions, polyamines may mimick role of calcium (Figure 1 to 3) and application of EGTA by chelation of extracellular calcium caused extensive leakage and production of malondialdehyde (Figures 1 and 2). It has also been suggested that under stress conditions, polyamines may replace calcium in maintaining membrane integrity by binding to phospholipids of the membrane (Naik and Srivastva, 1978).

Manipulation of endogenous PAs levels through genetic or exogenous means might enhance the capability to improve stress tolerance in soybean. It is suggesting determining the role of PAs in a specific developmental process; the levels of PAs biosynthetic enzymes are modulated in a specific tissue type by expressing antisense and sense sequences under the control of tissue-specific promoters. Genetic analysis of mutants with either altered concentrations of PAs or altered sensitivity to PAs will play a crucial role in determining the mechanism of PA action. With a better understanding of the role of PAs in the regulation of plant growth and development, it is possible that biotechnological applications, such as control of dormancy, ripening, senescence and stress tolerance, may be developed.

REFERENCES

Basra AS, Cheema DS, Dhillon-Grewal R, Basra RK, Singh S (1992). Proline and polyamines in relation to heat tolerance of tomato. In: Adaptation of vegetable and other food crops to temperature and water stress. AVRDC, Taiwan, pp.493-495.

Bais HP, Ravishankar GA (2002). Role of polyamine in the ontogeny of plants and their biotechnological applications. Plant Cell, Tissue Organ Culture, 69: 1-34.

Bernhiem F, Bernhiem MLC, Wibur K M. (1948). The reaction between thiobarbituric acid and the oxidation products of certain lipids. J. Biol. Chem. 174: 254-264

Besford RT, Richardson CM, Campos JL, Tiburcio AF (1993). Effect of polyamines on stabilization of molecular complexes of thylakoid membranes of osmotically stressed oat leaves. Planta, 189: 201-206.

Bouchereau A, Aziz A, Larher F, Martin- Tanguy J (1999). Polyamines and environmental challenges: recent development. Plant Sci. 140: 103-125.

Campos JL, Figueras X, Boronat A, Pinol MT, Tiburcio AF (1991). Changes in polyamine content of *Arabidopsis thaliana* after UV-C irradiation in Galston GW, Tiburcio AF (eds), Lecture Course on Polyamines as Regulators of Plant Development. Fundation Juan March, pp. 78-80.

Couee I, Hummel I, Sulmon C, Gouesbet G, EL Amrani A (2004). Involvement of polyamines in root development. Plant Cell, Tissue. Organ Culture, 76:1-10.

Dionisio-Sese ML, Shono M, Tobita S (1999). Effect of proline and betaine on heat inactivation of ribulose-1, 5-bisphosphate carboxylase/oxygenase in crude extracts of rice seedlings. Photosynthetica, 36: 557-563.

Galston, AW, Kaur Sawhney R, Altabella T, Tiburcio AF (1997). Plant polyamines in reproductive activity and response to abiotic stress. Bot. Acta. 110: 197-207.

Iba K (2002). Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Ann. Rev. Plant Biol. 53: 225-245.

Iwaya Inoue M, Matsui R, Fukuyama M (2004). Cold or heat tolerance of leaves and roots in perennial ryegrass determined by 1H-NMR. Plant Prod. Sci. 7: 118-128.

Jiang Y, Huang B (2001). Plants and the environment. Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two coolseason grasses. J. Exp. Bot. 52: 341-349.

Kakkar RK, Sawhney VK (2003). Polyamine research in plants-a changing perspective. Physiol. Plant. 116: 281-292.

Kaur-Sawhney R, Tiburcio AF, Altabella T, Galston AW (2003). Polyamines in plants: An overview. J. Cell Mol. Biol. 2: 1-12

Liu JH, Kitashiba H, Wang, J, Ban Y, Moriguchi T (2007). Polyamines and their ability to provide environmental stress tolerance to plants. Plant Biotechnol. 24: 117-126.

Marcum KB (1998). Cell membrane thermostability and whole plant heat tolerance of Kentucky bluegrass. Crop Sci. 38: 1214-1218.

Naik BI, Srivastva SK (1978). Effect of polyamines on tissue permeability. Biochemistry, 17: 1885-1887.

Nayyar H, Kaur S, Kumar S, Singh KJ, Dhir KK (2005). Involvement of polyamines in the contrasting sensitivity of chickpea (*Cicer arietinum)* and soybean (*Glycin max* (L.)) to water deficit stress. Bot. Bull. Acad. Sinica, 46: 333-338.

Porter J R (2005). Rising temperatures are likely to reduce crop yields. Nature, 43: 166-174.

Rehman H, Malik SA, Saleem M (2004). Heat tolerance of upland cotton during fruiting stage evaluated using cellular membrane thermostability. Field Crop. Res. 85: 149-158.

Roy P, Niyogi K, Sen Gupta DN, Gosh B (2005). Spermidine treatment to rice seedlings recovers salinity stress- induced damage of plasma membrane and PM- bound H^+-ATPase in salt- tolerant and salt-sensitive rice cultivars. Plant Sci. 168:583-591.

Slocum RD, Kaur-Sawhney R, Galston AW (1984). The physiology and biochemistry of polyamines in plants. Arch Biochem Biophys. 325: 283-303.

Soyka S, Heyer AG (1999). Arabidopsis knockout mutation of ADC2 gene reveals inducibility by osmotic stress. FEBS Lett. 458: 219-223

Tiburcio AF, Campos JL, Figueras X, Besford RT (1993). Recent advances in the understanding of polyamine functions during plant development. Plant Growth Regul. 12: 331- 340

Velikova V, Yourdanov I, Edreva A (2000). Oxidative stress and some antioxidant systems in acid rain- treated bean plants protective role of exogenous polyamines. Plant Sci. 151: 59-66.

Wahid A, Gelani S, Ashraf M, Foolad MR (2007). Heat tolerance in plants: An overview. Environ. Exp. Bot. 61: 199-223.