

Polyamine Levels in Leaves of Hyacinth Bean (*Lablab purpureus*) and Their Relation to Drought-Tolerance

¹Myrene R. D'souza, ²S. Kokila and ²V.R. Devaraj

¹Department of Chemistry, Mount Carmel College, No. 58, Palace Road, Bangalore, India

²Department of Biochemistry, Central College, Bangalore University, Bangalore, India

Abstract: The levels of diaminoputrescine (DAP), putrescine (Put), spermidine (Spd) and spermine (Spm) were evaluated as possible biomarkers for drought stress. *Lablab purpureus* cv. HA-4 was subjected to drought stress for a period of eight days during the initial growth phase under strictly controlled growth conditions. Drought stress resulted in significant reduction in dry and fresh weight, leaf surface area, root and shoot length and relative water content (RWC). The plant showed ability to rehydrate and grow upon re-watering and the polyamines levels and RWC correlated with drought tolerance in the plants.

Key words: Hyacinth bean • Putrescine • Spermidine • Spermine • Relative water content

INTRODUCTION

Many strategies are adapted by plants as a resort to abiotic environmental stresses. These adaptive mechanisms include changes in physiological and biochemical processes. Among them, the accumulation of polyamines has drawn much attention. Polyamines, mainly putrescine (Put, a diamine), spermidine (Spd, a triamine) and spermine (Spm, a tetramine), are polycationic compounds of low molecular weight which accumulate under a variety of abiotic stress conditions [1]. They are regarded as plant growth regulators involved in a broad spectrum of physiological processes, such as embryogenesis, cell division, morphogenesis, vascular differentiation, root initiation, shoot formation, flower initiation and development, fruit ripening, senescence and embryoid formation in tissue cultures [2, 3]. They also play an integral part in plant stress response either in free or conjugated form [4]. Many of these functions are similar to those mediated by known plant hormones such as auxins, cytokinins, gibberellins, abscisic acid and ethylene. However, the physiological role of stress-induced polyamine accumulation remains unknown. Polyamines titres, altered under abiotic stress, are dependent on several factors, such as plant species, tolerance or sensitivity to stress and the duration of stress.

The involvement of polyamines in the response to drought stress was reported in several publications [4-8]. Mannitol-induced osmotic stress increased Put, Spm and Spd contents in wheat after one week of drought [9]. Drought stress induces a greater increase in Put synthesis in drought-tolerant sugarcane varieties than in sensitive ones [10]. Similarly, osmotic stress causes a greater increase in Put and Spd contents in the tolerant species *Lycopersicon pennellii* than in the sensitive *L. esculentum* [11]. An increase in Spd synthase activity and consequently Spd content in leaves of *Arabidopsis* has been found to be associated with tolerance to various other abiotic stress such as chilling, freezing, salinity, hyperosmosis, drought and paraquat toxicity [5]. The transformation of tobacco with S-adenosylmethionine decarboxylase led to increased polyamine biosynthesis and improved drought tolerance [12]. Exogenous Spd and Spm was found to stimulate elongated growth and reduce membrane damage in jack pine seedlings under drought conditions [13].

Under stress, plants show specific reaction responses in terms of polyamine fluctuation. Thus, changes in cellular polyamines under stress provide clues on its possible implication in stress response; however there is no evidence for its role in counteracting stress. Therefore, in order to understand drought-induced changes in the titers of polyamines and to achieve a

possible correlation between the levels of polyamines and the degree of drought tolerance in Hyacinth bean, the present study was undertaken.

MATERIALS AND METHODS

Plant Material and Growth Conditions: The seeds of *Lablab purpureus* cv. HA-4 were procured from National Seed Project, University of Agricultural Science, Bengaluru, India. They were surface sterilized with 0.1% (w/v) mercuric chloride for 30 s, rinsed immediately with large volume of sterile distilled water and imbibed overnight in distilled water. The overnight-soaked seeds were sown in trays containing vermiculite and acid-washed sand (1:1 w/w) and irrigated daily for ten days with distilled water. The germination was carried out under natural greenhouse conditions; day/night temperature and relative humidity were: 30/25°C and 75/70% respectively. The average photoperiod was 12 h light/12 h dark.

Drought stress application and experimental design: Drought stress was induced by withholding water to seedlings, ten days after germination (DAG). Leaf and root samples were collected at 2, 4, 6 and 8 days after stress (DAS) and 5 days after re-watering (DAR) and frozen until further analysis. For determination of RWC and fresh and dry weights, samples were used immediately after collection. The experimental design used was carried out at random factorial scheme, with 5 evaluation points (2, 4, 6, 8 DAS and 5 DAR) and done in triplicate. Seedlings watered twice daily were used as control.

Determination of Relative Water Content (RWC): The relative water content was estimated according to the method of Turner and Kramer [14] using the equation: $RWC = (FW - DW) \times 100 / (TW - DW)$. Leaf discs of 10 mm diameter were weighed to determine the fresh weight (FW), soaked in distilled water at 25°C for 4 h to determine the turgid weight (TW), then oven dried at 80°C for 24 h to determine the dry weight (DW). Similarly, entire shoot and root was taken for analysis and RWC was computed as before.

Polyamine Analysis: Total free polyamine levels were determined in leaves and roots of Hyacinth bean according to the method of Flores and Galston [15] with slight modification. Each 100 mg of tissue was homogenized with 1.0 ml 5% (w/v) cold perchloric acid using a cooled mortar and pestle. The homogenates were

kept in an ice bath for 1 h and then centrifuged at 15,000g for 30 min at 4°C, the supernatant was transferred to new plastic vials and were stored at -70°C for polyamine quantification. Plant extracts were benzoylated. 1.0 ml of 2 M NaOH was mixed with 500 μ l supernatant. After the addition of 10 μ l benzoyl chloride, samples were vortexed for 20 s, incubated for 20 min at 37°C. Following the high temperature incubation, 2 ml of saturated NaCl was added. Benzoyl-polyamines were extracted in 2 ml diethyl ether and vortexed for 10 s. After centrifugation at 12,000g for 5 min at 4°C, 1 ml of the ether phase was collected, evaporated to dryness under a stream of warm air and redissolved in 100 μ l methanol. The benzoylated extracts were filtered through a 0.22 μ m membrane filter and then separated by HPLC (Shimadzu) on a reverse-phase C₁₈ column (Alex-Octadecylsilane), 5 particle diameter, 4.6 \times 250 mm using methanol:water (64:36) at a flow rate of 1ml/min. Eluted peaks were detected by a spectrophotometer (Shimadzu, UV 254 nm) recorded and integrated by an attached computer. Standards (DAP, Putrescine, Spermine and Spermidine) and plant extracts were determined with the same method.

Statistical Analysis: The experiment was performed using a randomized design. All data are expressed as means of triplicate experiments unless mentioned otherwise. Comparisons of means were performed using PrismGraph version 3.02. Data were subjected to a one-way analysis of variance (ANOVA) and the mean differences were compared by lowest standard deviations (LSD) test. Comparisons with $P \leq 0.05$ were considered significantly different. Each data point represent mean of three replicates analyzed twice and each value is therefore mean of six estimations (n=6).

RESULTS AND DISCUSSION

Plant growth and productivity are primarily dependent on the water availability, a decrease of which has negative effects on plant growth, photosynthesis, solute transport and accumulation. The imposition of drought by withholding water for 8 days and subsequent macroscopic observation of Hyacinth bean revealed increased damage in the form of wilted, chlorotic leaves, which appeared to recover after rewatering. The decrease in growth can be attributed to the reduced cell elongation resulting from decreased turgor, cell volume and cell growth [16]. The plant showed reduction in number of leaves and surface area of leaves as well as curling due to loss of turgidity. The stressed plants failed to grow the second pair of leaves even 8 DAS (Fig. 1). Drastic

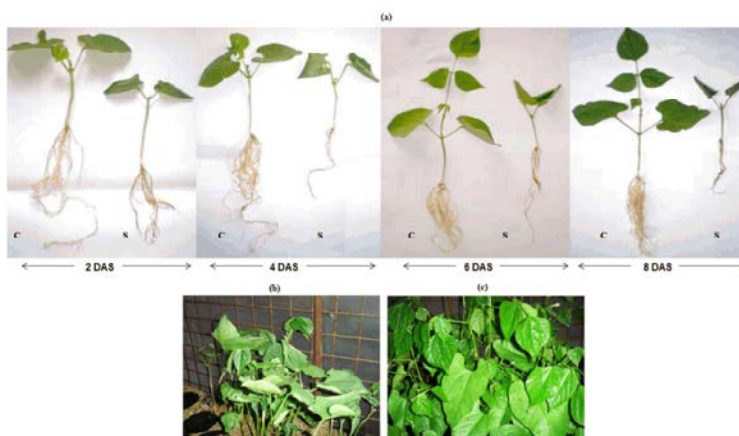


Fig. 1: Effect of drought and rewatering in Hyacinth bean seedlings. (a): Morphological traits of control and stressed seedlings; (b): 6DAS; (c) 5 days after recovery.

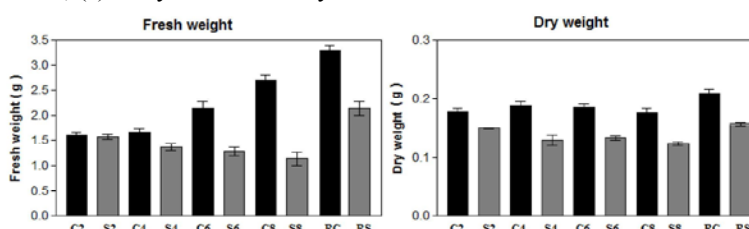


Fig. 2: Fresh weight and dry weight of Hyacinth bean seedlings under 2, 4, 6 and 8 DAS. C2 to C8 controls, S2 to S8 stressed plants, RC (control for RW), RW (rewatered).

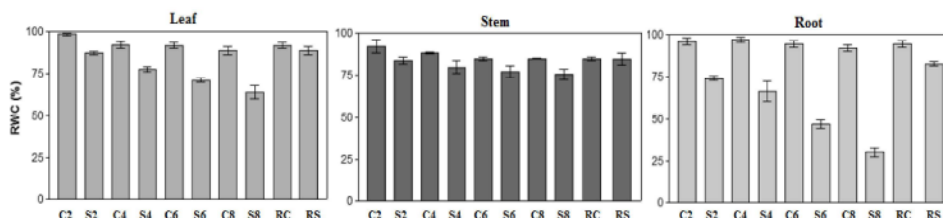


Fig. 3: Relative water content (RWC) in leaf, stem and root tissue of Hyacinth bean seedlings under 2, 4, 6 and 8 DAS. C2 to C8 controls, S2 to S8 stressed plant, RC (control for RW), RW (rewatered).

reduction in fresh and dry weight of the plant was seen 4 DAS (Fig. 2). Other physical features such as length of stem, root and leaves exhibited reduction with increasing time of drought stress. The effect was more pronounced in roots. The growth arrest seen in roots and leaves can be considered as a possible mechanism to preserve carbohydrates for sustained metabolism, prolonged energy supply and for better recovery after stress relief [17].

Metabolic activity and leaf survival is determined by the relative water content (RWC) of the plant [18]. RWC in Hyacinth bean was found to decrease with increase in the time of exposure to drought stress. The RWC of roots showed a steeper decline when compared to RWC of leaves and stem (Fig. 3). It is believed that retardation of root growth and the decrease in its activity [19] combined

with stomatal closure caused by the accumulation of abscisic acid produced in roots and then accumulated in guard cells in response to water deficit are the causes of decrease in RWC [20]. However, on re-watering, the plants recovered fully in terms of RWC (Fig. 3) illustrating the importance of plasticity in the leaf area for maintaining control over water use [21]. Despite the decline in RWC during drought, leaf growth resumed after the relieving of stress, thereby suggesting that the basic components of leaf growth were not completely damaged.

Polyamine metabolism has been reported to play an important role in drought tolerance [6, 8, 22]. Put, Spd and Spm began to raise 2DAS in Hyacinth bean, with Spd showing a more pronounced increase (Fig. 4). Put at an early stress stage provided better adaptation to drought stressed rice cultivars [8]. The enhancement of Spd in

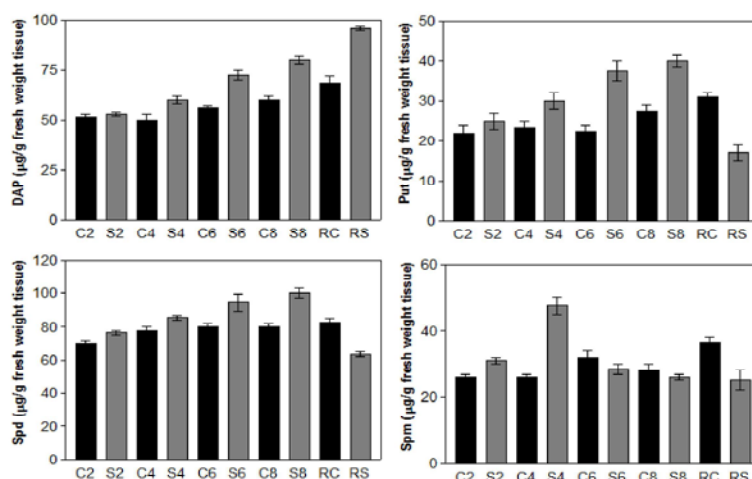


Fig. 4: Levels of polyamines in leaves of Hyacinth bean plants. Data plotted are mean \pm SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$).

Hyacinth bean conforms to results obtained in transgenic *Arabidopsis thaliana* where it was proposed to be a signaling regulator in stress signalling pathways [5]. Spm levels on the other hand began to decline significantly 4DAS, which was accompanied by the accentuated stress injury. Spm is known to contribute to intrinsic gating and rectification of inward rectifier K^+ channels [23]. Under drought, elevated Spm content modulates the activities of certain ion channels and raises cytoplasmic Ca^{2+} concentrations, which inactivates the K^+ inward rectifier at the plasma membrane, thereby stimulating stomatal closure [22], declining transpiration rates and helping to maintain turgor. Other functions of polyamines involve protection of the plasma membrane against stress damage by maintaining membrane integrity [24], preventing activation of superoxide-generating NADPH oxidases [25] and inhibition of protease and RNase activity [26]. Also, the increase in DAP levels upon stress (Fig. 4) could be explained by the fact that Spm is oxidized to signaling molecules, H_2O_2 and DAP [27]. After subsequent re-watering, polyamine levels were found to decrease relative to control, while that of DAP, a product of polyamine metabolism was found to increase.

Plants are known to use more than one mechanism to adapt to unfavourable environmental conditions. For example, under drought stress, Hyacinth bean enhances drought tolerance through osmotic adjustment, restoration of oxidative balance and other metabolic and structural adaptations [28]. Despite the decline in RWC during drought, leaf growth resumed upon re-watering. This implies that the basic components of leaf growth

were not completely damaged under drought stress possibly due to accumulation of compatible solutes and polyamines. The increased sensitivity as noted by the levels of various polyamines was reversed following re-watering indicating a great elasticity in the plant's capacity to cope with stress. Understanding how plants respond to episodic drought and watering pulse and the underlying mechanism could prove to be remarkably helpful towards the implementation of vegetation management practices during changes in climatic conditions common to tropical ecosystems.

REFERENCES

1. Krishnamurthy, R. and K.A. Bhagwat, 1989. Polyamines as modulators of salt tolerance in rice cultivars. *Plant Physiology*, 91: 500-504.
2. Liu, J.H., K. Nada, C. Honda, H. Kitashiba, X.P. Wen, X.M. Pang and T. Moriguchi, 2006. Polyamine biosynthesis of apple callus under salt stress: importance of the arginine decarboxylase pathway in stress response. *J. Exp. Bot.*, 57: 2589-2599.
3. Tonon, G., C. Kevers, O. Faivre-Rampant, M. Graziani and T. Gaspar, 2004. Effect of NaCl and mannitol iso-osmotic stresses on proline and free polyamine levels in embryogenic *Fraxinus angustifolia* callus. *J. Plant Physiol.*, 161: 701-708.
4. Alcázar, R., F. Marco, J.C. Cuevas, M. Patron, A. Ferrando, P. Carrasco, A.F. Tiburcio and T. Altabella, 2006. Involvement of polyamines in plant response to abiotic stress, *Biotechnol. Lett.*, 28: 1867-1876.

5. Kasukabe, Y., L. He, K. Nada, S. Misawa, I. Ihara and S. Tachibana, 2004. Over expression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. *Plant Cell Physiol.*, 45: 712-722.
6. Nayyar, H., S. Kaur, Smita, S. Kumar, K.J. Singh and K.K. Dhir, 2005. Involvement of polyamines in the contrasting sensitivity of chickpea (*Cicer arietinum* L.) and soybean (*Glycine max* (L.) Merrill.) to water deficit stress. *Bot. Bull. Acad. Sin.*, 46: 333-338.
7. Simon-Sarkadi, L., G. Kocsy, A. Varhegyi, G. Galiba and J.A. Ronde, 2006. Effect of Drought Stress at Supraoptimal Temperature on Polyamine Concentrations in Transgenic Soybean with Increased Proline Level. *Verlag der Zeitschrift für Naturforschun*, pp: 833-839.
8. Yang, S.H., L.J. Wang and S.H. Li, 2007. Ultraviolet-B irradiation induced freezing tolerance in relation to antioxidant system in winter wheat (*Triticum aestivum* L.) leaves. *Environ. Exp. Bot.*, 60: 300-330.
9. Galiba, G., G. Kocsy, R. Kaur-Sawhney, J. Sutka and A.W. Galston, 1993. Chromosomal localization of osmotic and salt stress-induced differential alterations in polyamine content in wheat. *Plant Sci.*, 92: 203-211.
10. Zhang, M.Q., R.K. Chen and S.L. Yu, 1996. Changes of polyamine metabolism in drought-stressed sugarcane leaves and their relation to drought resistance. *Acta Phytophysiol. Sin.*, 22: 327-732.
11. Santa-Cruz, A., M.T. Estan, A. Rus, M.C. Bolarin and M. Acosta, 1997. Effects of NaCl and mannitol isoosmotic stresses on the free polyamine levels in leaf discs of tomato species differing in salt tolerance. *Plant Physiol.*, 151: 754-758.
12. Waie, B. and M.V. Rajam, 2003. Effect of increased polyamine biosynthesis on stress responses in transgenic tobacco by introduction of human S-adenosylmethionine gene. *Plant Sci.*, 164: 727-734.
13. Rajasekaran, L.R. and T.J. Blake, 1999. New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. *Plant Growth Regul.*, 18: 175-181.
14. Turner, N.C. and P.J. Kramer, 1980. *Adaptation of Plant To Water And High Temperature Stress*. Wiley, New York, pp: 207-230.
15. Flores, H.E. and A.W. Galston, 1982. Analysis of polyamines in higher plants by high performance liquid chromatography. *Plant Physiol.*, 69: 701-706.
16. Hussain, M., M.A. Malik, M. Farooq, M.Y. Ashraf and M.A. Cheema, 2008. Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.*, 194: 193-199.
17. Mahajan, S. and N. Tuteja, 2005. Cold, salinity and drought stresses: An overview, *Arch. Biochem. Biophys.*, 444: 139-158.
18. Nayyar, H. and D. Gupta, 2006. Differential sensitivity of C3 and C4 plants to water deficit stress: association with oxidative stress and antioxidants. *Environ. Exp. Bot.*, 58: 106-113.
19. Tarumkeng, R.C. and Z. Coto, 2003. Effects of drought stress on growth and yield of soyabean. *Kisman, Science Philosophy PPs 702*, Term paper, Graduate School, Borgor Agricultural University (Institut Ppertanian Bogor).
20. Khan, N.A., S.S. Samiullah and R. Nazar, 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress, *J. Agro. Crop Sci.*, 193: 435-444.
21. Blum, A., 1996. Crop responses to drought and the interpretation of adaptation. *Plant Growth Regul.*, 20: 57-70.
22. Yamaguchi, K., Y. Takahashi, T. Berberich, A. Imai, T. Takahashi, A. Michael and T. Kusano, 2007. A protective role for the polyamine spermine against drought stress in *Arabidopsis*. *Biochem. Biophys. Res. Comm.*, 352: 486-490.
23. Oliver, D., T. Baukowitz and B. Fakler, 2000. Polyamines as gating molecules of inward-rectifier K⁺ channels. *Eur. J. Biochem.*, 267: 5824-5829.
24. Roy, P., K. Niyogi, D.N. Sengupta and B. Ghosh, 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.
25. Shen, W., K. Nada and S. Tachibana, 2000. Involvement of polyamines in the chilling tolerance of cucumber cultivars. *Plant Physiol.*, 124: 431-439.
26. Bais, H.P. and G.A. Ravishankar, 2002. Role of polyamines in the ontogeny of plants and their biotechnological applications. *Plant Cell Tiss. Organ Cult.*, 69: 1-34.
27. Kusano, T., K. Yamaguchi, T. Berberich and Y. Takahashi, 2007. Advances in polyamine research in 2007, *J. Plant Res.*, 120: 345-350.
28. Myrene, R.D. and V.R. Devaraj, 2011. Specific and non-specific responses of Hyacinth bean (*Dolichos lablab*) to drought stress. *Indian Journal of Biotechnology*, 10: 130-139.