



POLYAMINES INDUCED CHANGES IN WATER STRESSED SOLANUM SURATTENS, BURM.F

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ABSTRACT

Seedlings of *Solanum surattense* Burm.F, a commonly used medicinal plant, were grown under water stressed ($\psi=3.2\text{Mp}_d$) condition for 7 days. After 7 days under water stress plants were sprayed with spermine ($100\mu\text{M}$), a polyamine. Water stress has caused considerable reduction in leaf area, leaf fresh and dry weight, stem fresh and dry weight, root fresh and dry weight. It is paralleled with the reduction in chlorophyll, carotenoid, protein, glucose and starch content in *Solanum surattense*. Similarly, a significant increase in the content of proline and free amino acids. However after the spermine application, all the parameters analysed viz., growth parameters, chlorophyll, carotenoid, protein, glucose, and starch content were found to be increased than the water stressed seedlings and decreased in the content of proline and free amino acids indicating the remedial effect of polyamine on water stressed plants.

KEY WORD: medicinal plant, chlorophyll, carotenoid, protein, glucose.

INTRODUCTION

India has rich medicinal plant diversity. These medicinal plants form the source for even increasing pharmaceutical cosmetics and biotechnology industry. Among the medicinal plants, *Solanum surattense* is one of the most significant medicinal plants in Ayurveda and other traditional systems of medicine. Drought is the environmental constraint that limits the productivity of many crops and it affects both quality and quantity of the yield adversely (Sorte et al., 1993). Drought brings about the reduction in growth rate, stem elongation, leaf expansion and stomatal movements (Hsiao, 1973). The concentrations of various secondary plant products are strongly depending on the growing conditions and it is obvious that especially stress situations have a strong impact on the metabolic pathways responsible for the accumulation of the related natural products. The responses of plants to environmental stresses are complex and involve physiological and biochemical changes. Plants tolerate drought (D) stress by modifying their morphological and anatomical features, through physiological adaptations, or by biochemical means and molecular adjustments at the whole plant level. Biochemical adaptation may involve both primary and secondary metabolism.

However the water stress caused changes in the number of physiological and biochemical processes governing plant growth and productivity (Daie, 1988). Polyamines (PAs) (putrescine, spermidine and spermine) are phytohormones, with aliphatic nitrogen structure present in every living life form including plants. Zhou et al. (2015) demonstrated that polyamines are engaged with numerous physiological procedures, for example, cell development and advancement and react to pressure resilience and to different environmental factors. In many cases the relationship of plant stress tolerance was noted with the production of conjugated and bound polyamines as well as stimulation of polyamine oxidation. Genetic manipulation of crop plants with genes encoding enzymes of polyamine biosynthetic pathway provided better stress tolerance to crop plants.



The exogenous application of PAs is also another option for increasing the stress tolerance potential in plants. Endogenous polyamine (PA) played a critical role in tolerance to water stress in plants acting as a signalling molecule activator. Water stress caused increase in endogenous PA content in leaves, including putrescine (Put), spermidine (Spd), and spermine (Spm). Exogenous application of Spd induced the instantaneous H₂O₂ burst and accumulation of cytosolic free Ca²⁺ and activate NADPH oxidase and CDPK gene expression in cells. To a great extent, PA biosynthetic inhibitor reduced the water stress-induced H₂O₂ accumulation, free cytosolic Ca²⁺ release, antioxidant enzyme activities and genes expression leading to aggravate water stress-induced oxidative damage, while these suppressing effects were alleviated by the addition of exogenous Spd, indicating PA was involved in water stress-induced H₂O₂ and cytosolic free Ca²⁺ production as well as stress tolerance.

Dehydrin genes (Y2SK, Y2K, and SK2) were showed to be highly responsive to exogenous Spd. PA-induced antioxidant defence and dehydrin genes expression could be blocked by the scavenger of H₂O₂ and the inhibitors of H₂O₂ generation or Ca²⁺ channels blockers, a calmodulin antagonist, as well as the inhibitor of CDPK. So work was carried to study the effect of polyamine on the change of growth and biochemical parameters in water stressed Solanum surattense Burm.F.

MATERIALS AND METHODS

Healthy seeds of Solanum surattense purchased from the local market and were sown in plastic container containing black, red and garden soil in the ratio of 1:1:1 and were grown in laboratory condition. (Temperature 28± 2°C, relative humidity 65± 15%, pH=6.8, maximum irradiance (PAR) 900 μmol m⁻² s⁻¹, photoperiod 14). After 30 days the seedlings are exposed to the following treatments.

Control seedlings were watered daily. For water stress withheld water for 7 days (minimum leaf water potential ψ -3.2Mp_a according to Muthuchelian et al. (1997)) daily monitored by a thermocouple psychrometer (wascor PR-SSI USA). For Spermine control seedlings were watered daily and sprayed with spermine (100μM) for a week until the leaves were completely wet. For Spermine and water stress seedlings, spermine (100μM) was applied to water stressed seedlings for a week. The seedlings were harvested after 21 days (3 weeks) of their respective treatment growth of biochemical characteristics were studied.

RESULT AND DISCUSSION

Solanum surattense, Burm.F. seedlings were exposed to water stress (ψ =-3.2Mp_a) for 7 days showed a significant reduction in leaf area, fresh weight and dry weight of leaf (fig 1,2&3). In addition similar reductions in fresh weight and dry weight of stem (fig.4&5) and fresh and dry weight of root (fig 6&7) were also observed. Similar results were reported in the shoot and root length of water stressed paddy plants by sorte et al. (1993). Whilhead (1983) found that frequent defoliation and drought reduced substantially the root and shoot weight of white clover grown in field. Upreti et al. (1998) reported that the plant under water stress condition showed a significant reduction in growth level.

Leaf area, fresh weight and dry weight of leaf were significantly increased in water stressed seedling sprayed with spermine. Polyamine treated seedlings showed enhance in the fresh and dry weight of stem, fresh and dry weight of root.

The leaf area was analysed by tracing the area on a graph sheet. Fresh weight and dry weight were measured using an electronic balance.

Leaf tissues were extracted with 100% acetone for the complete release of chlorophyll, carotenoids. The amount of total chlorophyll and carotenoids was quantified using the formulae of Arnon (1949). The total soluble protein content was analysed following the method of Lowry et al. (1951). Glucose content was analysed following the method of Jayaraman (1981). The free amino acid and starch contents were analysed following the procedure of Jayaraman (1981). The proline content was analysed following the method of Bates et al. (1973).

A conspicuous increase in fresh and dry matter content of leaf, stem and root is attributed due to the application of growth regulators (Ries and Wert, 1982; Muthuchelian et al. (1997). Water stress caused inhibition of chlorophyll pigments (Table-1). The reduction in chlorophyll pigment might be due to preferential photo oxidation of chlorophyll a (Guralnick and Ting, 1987). Chlorophyll gets bleached during water stress, leads to metabolic upset such as protein degradation (Parsons et al., 1989). Seedling exposed to water stress conditions showed significant reduction in protein, starch and glucose content (Table-2). But increase in the free amino acids and proline content was also observed (Table2). The loss of protein during water stress may be due to decline in RNA syntheses as reported by Nir et al. (1990). Protein, Glucose and starch content were increased in seedlings sprayed with spermine. These parameters also increased in water stressed seedlings sprayed spermine (Table-2). Water deficit leads to decreases in crop yields which are associated with changes of various physiological and molecular factors. Recent evidence indicates that polyamine (PA) is closely involved in increase in growth and development (Krasuska et al., 2014; Pottosin et al., 2014), as well as stress tolerance in plants (Yiu et al., 2009; Do et al., 2014). Maintenance of PA levels and metabolism, whether through exogenously applied putrescine (Put), spermidine (Spd) and spermine (Spd), or transgenic approaches with PA biosynthesis genes, has been found to promote plant stress tolerance due to their roles in protecting membranes, maintaining osmotic adjustment, and promoting tolerance-related gene expression and protein levels (Tang and Newton, 2005; Shi et al., 2013; Li et al., 2014a).

The increased accumulation of proline in plants subjected to water stress condition may be used as an index of screening soybean cultivars for drought tolerance (Singh and Gupta, 1983). Accumulation of free amino acid and proline were significantly reduced by polyamine treated seedlings and water stressed seedlings sprayed with spermine (Table 3). Thus, these results indicate that polyamine - spermine may be a protectant against drought stress. Polyamine has alleviating effect against water stress. So it is concluded that polyamine can be used as a reviving agent for stress as well as a growth promoting hormone in medicinal plants.

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Table 1: Ameliorating Effect of Polyamines on the Photosynthetic Content of Water Stressed Solanum Surattense, Burm.F.

Biochemical Parameters	Control(C)	Water stress (W)	Polyamines (P)	Water stress+ Polyamines (W+P)
Chlorophyll a (mg/g LFW)	1.226±0.0023 (100)	0.7894±0.008 (64)	2.08±1.03 (196)	1.26 ± 0.0003 (102)
Chlorophyll b (mg/g LFW)	0.7751± 0.0025 (100)	0.2305 ± 0.005 (29)	0.9395±0.00035 (120)	0.5066± 0.00035 (65)
Total Chlorophyll (mg/g LFW)	2.001 ± 0.0025 (100)	1.099 ± 0.0076 (50)	3.017 ± 0.0015 (150)	1.76 ± 0.00076 (87)
Carotenoids (mg/g LFW)	0.6450±0.00045 (100)	0.3270±0.0004 (50)	0.8604±0.00068 (133)	0.4536 ± 0.0026 (70)

Values are the mean of 3 Samples. Mean ± SD, n=3.

Values in parentheses represent the percentage increase (or) decrease over the control.

Table 2: Ameliorating Effect of Polyamines on the Biochemical Content of Water Stressed Solanum surattense, Burm.F.

Biochemical Parameters	Control (C)	Water stress (W)	Polyamines (P)	Water stress+ Polyamines (W+P)
Starch (mg/g LFW)	2.66± 0.045 (100)	2.5 ± 0.15 (93)	3.0 ± 0.25 (112)	4.2 ± 0.2 (157)
Glucose (mg/g LFW)	60 ± 3.60 (100)	30 ± 3.60 (50)	95 ± 2.0 (158)	100 ± 1.52 (166)
Protein (mg/g LFW)	10.5 ± 0.25 (100)	8 ± 0.36 (76)	11.25 ± 0.02 (107)	12 ± 0.28 (114)
Proline (µg/g LFW)	0.258±0.003 (100)	1.334± 0.002 (517)	0.545± 0.003 (212)	0.524±0.0020 (203)
Freeaminoacids (µmoles gLFW)	21.6 ±0.26 (100)	24 ± 0.23 (112)	11.6 ±0.15 (53)	13.6 ± 0.15 (62)

Values are the mean of 3 Samples. Mean ± SD, n=3. Values in parentheses represent the percentage increase (or) decrease over the control.

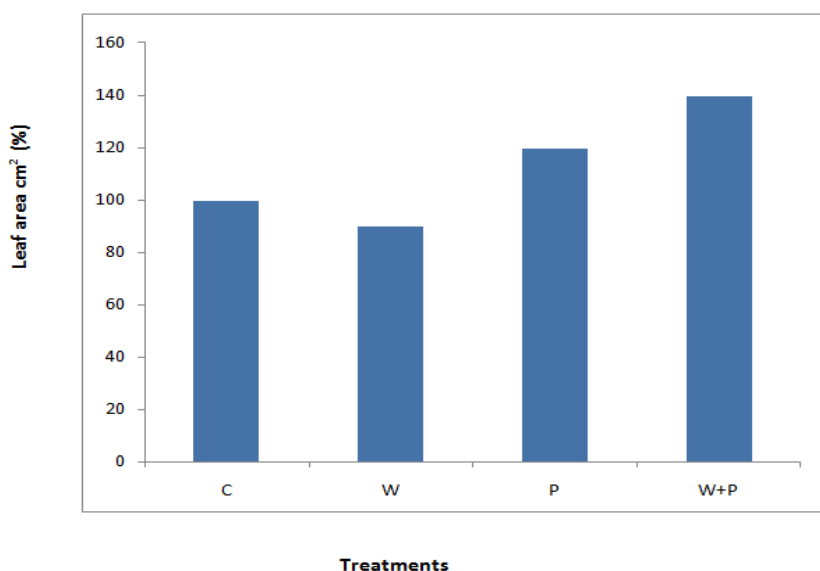


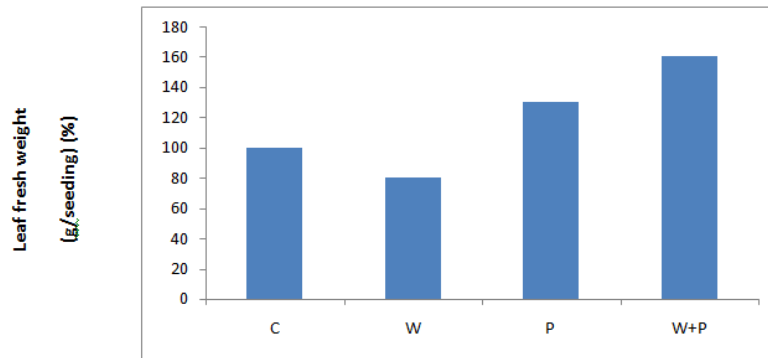
Fig. 1. Effect of polyamine and water stress on the leaf area of S. Surattense. The values are mean of 10 independent measurements. Mean ± SD. n = 10.

C - Control

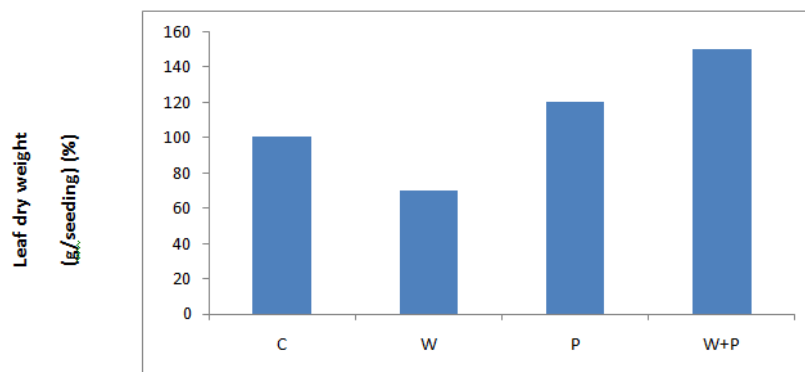
W - Water Stress

P - Polyamine

W+P - Water Stress + Polyamine



Treatments
Fig.2



Treatments
Fig.3

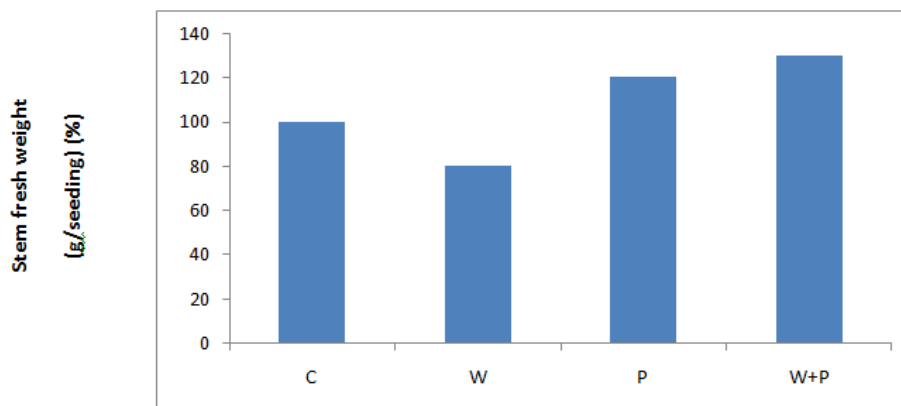
Fig. 1, 2 & 3. Effect of polyamine and water stress on the leaf area (1) and leaf fresh (2) and dry (3) weight of *S. Surattense*. The values are mean of 10 independent measurements. Mean \pm SD. n = 10.

C - Control

W – Water Stress

P – Polyamine

W+P - Water Stress + Polyamine



Treatments
Fig.4

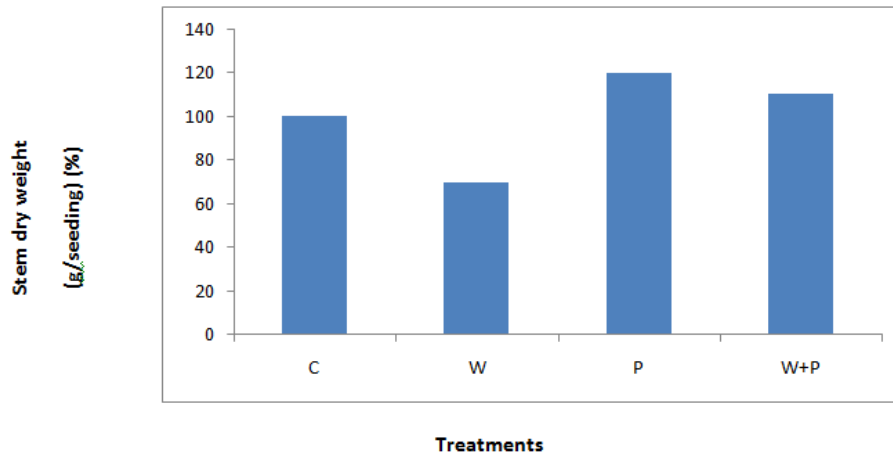


Fig.5

Fig. 4, 5. Effect of polyamine and water stress on the Stem fresh (4) and dry weight (5) of *S. Surattense*. The values are mean of 10 independent measurements. Mean \pm SD. n = 10.

C - Control

W – Water Stress

P – Polyamine

W+P - Water Stress + Polyamine

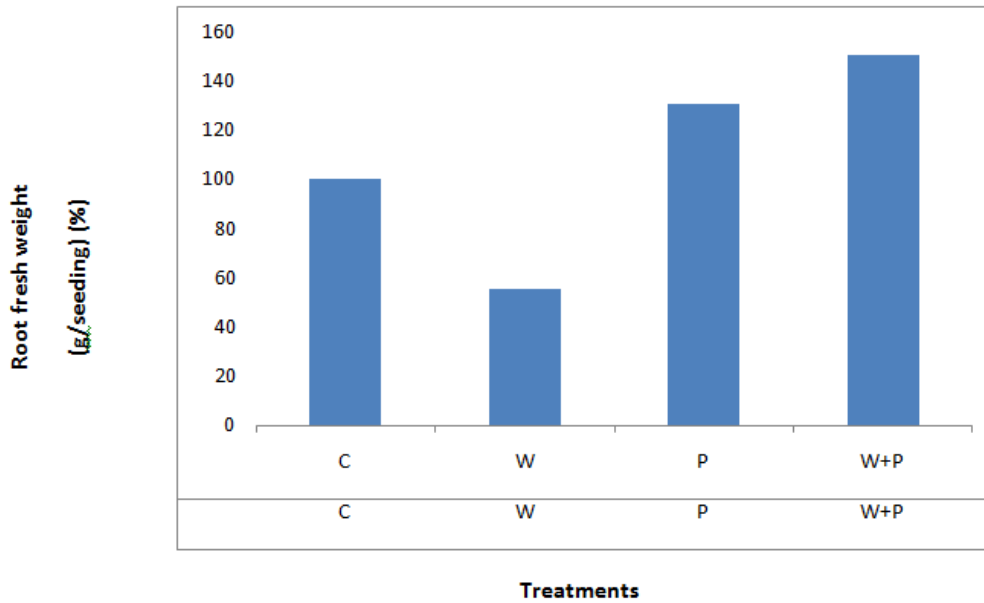


Fig.6

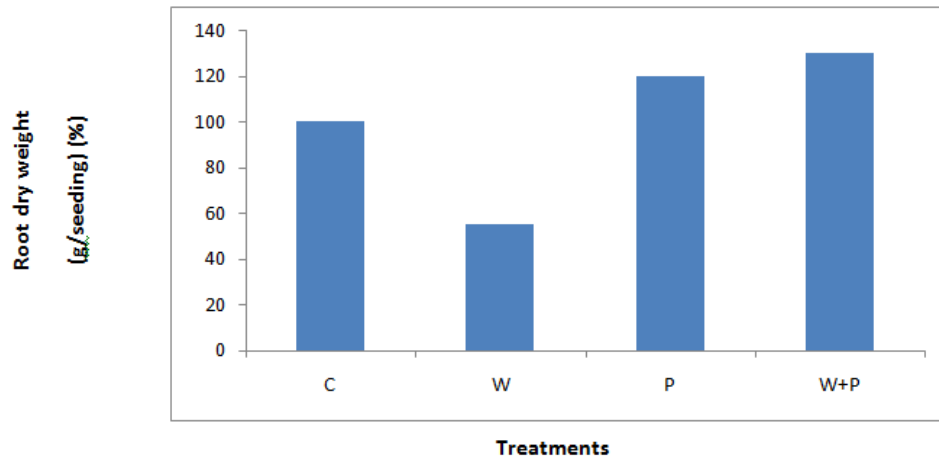
**Fig.7**

Fig. 6,7. Effect of polyamine and water stress on the Root fresh (6) and dry weight (7) of *S. Surattense*. The values are mean of 10 independent measurements. Mean \pm SD. n = 10.

C - Control

W - Water Stress

P - Polyamine

W+P - Water Stress + Polyamine