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Effects of Spermine and Abscisic acid on growth and Biochemical contents of *Vigna mungo* L. under high temperature and salt-stress.

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Abstract

Effects of Abscisic acid (ABA) and Spermine were studied on the two varieties of *Vigna mungo* L. (Cv.M₁ and Cv.M₂) under heat shock and salt-stress. The combined treatments of ABA, salt - stress (NaCl 25 mM) & Heat shock (40°C) for 3 h in Cv. M₂ have shown maximum 1AA-Oxidase activity in roots and the highest increase in number of seeds plant⁻¹. Protein contents of the seeds in both the varieties increased under ABA treatment made alone, while maximum reduction was observed under salt stress in Cv. M₁ and under heat shock in Cv.M₂. Spermine tend to increase the number of seeds plant⁻¹ and 100-seed weight under salt stress in both the cultivars. Salt stress appears to retard the yield parameters in both the varieties but the magnitude of inhibition was more pronounced in cv.M₁.

Introduction

Agricultural crop productivity results from complex interaction of plants with climate and soil. Soil salinity is one of the major problems in arid and semi-arid regions of the world, which hamper the agriculture out put by lowering the yield of various crops. Mash (*Vigna mungo* L.) is an important crop in the agriculture sector of Indo-Pak subcontinent. It contains high protein contents (25-32%) which is a major source of human diet. The yield of mash in the world including Pakistan is very low partly due to the poor agricultural practices, the use of low indigenous genotypes and the use of marginal land. Significant changes in leaf proteins were also observed due to salt stress as well as after heat shock (Kuznetsov *et al.*, 1993).

Use of Polyamines and plant growth regulators have immense importance in crop production under adverse environmental conditions. Kumar *et al.* (1997) reported that polyamines have been implicated in a wide range of biological process, including growth, development and stress responses. Flores and Galston (1982) have observed that Polyamines protect the plants under stress conditions. The effect of salinity on plant leaves can also be markedly decreased by using spermidine (Ivanova *et al.*, 1991). The maize seedlings, pre-incubated in polyethylene glycol solution, increased the thermo-tolerance in plants. (Bonnham-Smith *et al.*, 1987).

Krishna Murthy, (1991) observed that foliar application of Abscisic acid (ABA) play a regulatory role, especially under stress conditions. Prakash and Prathapasenan (1990) and Cramer (1990) pointed out that salinity elevates ABA concentrations in the root, Shoot and callus of *Brassica* Sp. and *Oryza sativa* L. In tobacco cell culture, resistance against salinity increased after initial high temperature treatment (Hamington and Alm 1988) whereas, putrescence prevented the degradation of chlorophyll, protein, RNA and DNA contents. The changes, when the plants are exposed to excess salt and high temperature (Smith, 1979) concentration of polyamine.

The present study was aimed to investigated the effects of polyamines (Spermine) and (ABA) on the two cultivars of *Vigna mungo* (L) under high temperature stress and salt stress, on the leaf and seed protein contents, IAA-oxides activity of roots and the yield parameters.

Materials and Methods

Seeds of *Vigna mungo* L. were cultivated in earthen pots containing sand and soil, (1:3). After six weeks, the plants were treated with ABA (10-6 M) and Spermine (10-6M) as foliar spray and Salt stress NaCl (25mM) was applied as root treatment. High temperature treatment to six weeks old plants was made for 3h at 40°C for 7 days. The following treatments were made:

T1: Control	T7: Spermine Heat Shock
T2: ABA	T8: ABA + Salt + Heat Shock
T3: Spermine	T9: Spermine + Salt + Heat Shock
T4: ABA + Salt	T10: Salt
T5: Spermine + Salt	T11: Heat Shock
T6: ABA + Heat Shock	T12: Salt + Heat Shock

After each treatment the samples were analyzed for leaf protein and fresh seed protein by the method of Lowry *et al.* (1951). IAA-Oxidase activity of the roots were estimated by the method of Malik and Singh, (1980).

Statistical Analysis: Data collected were subjected to analysis of variance (Steel and Torry, 1960) and the significant differences between the means were determined by using Duncan's multiple Range Test (Duncan, 1955).

Results and Discussion

Yield parameters: In both the cultivars of the *Vigna mungo* L. salt stress was inhibitory to seeds plant⁻¹ where as Spermine in combination with salt stress has increased the number of seeds plant⁻¹ In Cv. -M₁. These results coincide with the findings of Sawhney (1990) and Sherman (1987) that polyamines provide salt resistance for normal growth of plants and results in greater number of seeds plant⁻¹. However, the reduction in yield due to salinity stress and heat shock may be due to less vegetative growth, and development of the plant to yield the poor seeds including inhibition in the weight of seeds. These findings confirm the previous results that water and salinity stress effect the seed yield due to plasmolytic effect of water and salinity

Table 1: Duncan Multiple Range Test (DMRT) of twelve treatments mean for 100-seed wt. in *Vigna mungo* L. cv. M₁ and seed plant⁻¹ in *Vigna mungo* L. cv. M₃.

Treatment	100-Seed weight	Seed per plant ⁻¹
Control	0.900c	3.127abc
ABA	2.340ab	1.630d
Spermine	1.207bc	2.510bcd
ABA + Salt	2.483ab	2.883abc
Spermine + Salt	3.213a	3.073abc
ABA + Heat Shock	0.6767c	2.907abc
Spermine + Heat Shock	0.7967c	3.420ab
ABA + Salt + Heat Shock	1.827Bc	3.383abc
Spermine + Salt + Heat Shock	1.780Bc	3.350abc
Salt	1.920abc	2.387cd
Heat Shock	1.8070bc	3.520ab
Salt + Heat Shock	0.9333c	3.570a

All such means that share a common English letter are non-significantly different, otherwise they differ significantly at least at P < 0.05

Table 2: Duncan Multiple Range Test (DMRT) of twelve treatments means for protein content (%) in the seeds of *Vigna mungo* L. cv. M₁ and M₃.

Treatment	Protein content(%) of seed	
	M ₁	M ₃
Control	25.67a	23.87ab
ABA	25.65a	24.78a
Spermine	18.81b	21.70abcde
ABA + Salt	22.24ab	19.23de
Spermine + Salt	22.92ab	20.32bcde
ABA + Heat Shock	21.29ab	18.22e
Spermine + Heat Shock	21.04b	22.22abcde
ABA + Salt + Heat Shock	20.66b	18.16e
Spermine + Salt + Heat Shock	20.12b	18.61de
Salt	22.52ab	23.06abc
Heat Shock	22.67ab	20.08cde
Salt + Heat Shock	20.95b	18.036e

All such means that share a common English letter are non-significantly different, otherwise they differ significantly at least at P < 0.05.

stress in soybean (Salinas *et al.*, 1996) ABA induced reduction in yield under heat shock, might be due to additive effect of temperature shock as reported by Knievel (1973) and inhibitory effects of ABA.

IAA-oxidase activity: Salt stress under heat shock and with the foliar spray of ABA (10-6M) has increased the maximum IAA-oxides activity in Cv. M₃ whereas, this treatment has reduced the activity of the enzyme in Cv.-M₃ whereas, this treatment has reduced the activity of the enzyme in Cv.-M₁. This decrease in Cv.-M₁ may be attributed to more IAA production and increased resistance to temperature shock

Table 3: Treatments mean for IAA(μg g⁻¹ weight of fresh roots in *Vigna mungo* L. cv. M₁ and cv. M₃.

Treatment	IAA(μg g ⁻¹ weight of fresh roots	
	M ₁	M ₃
Control	77.59a	77.59a
ABA	52.05ab	52.05ab
Spermine	38.87ab	39.70ab
ABA + Salt	15.80ab	38.87ab
Spermine + Salt	37.23ab	37.23ab
ABA + Heat Shock	9.20b	31.46ab
Spermine + Heat Shock	39.70ab	28.99ab
ABA + Salt + Heat Shock	31.46ab	4.04ab
Spermine + Salt + Heat Shock	14.33ab	22.40ab
Salt	24.04ab	15.80ab
Heat Shock	22.40ab	14.33ab
Salt + Heat Shock	28.99ab	9.209a

All such means that share a common English letter are non-significantly different, otherwise they differ significantly least at P < 0.05

Table 4: Duncan Multiple Range Test (DMRT) of five treatments means for fresh leaf protein(μg g⁻¹ weight in the leaves of *Vigna mungo* L. cv.

Treatment	Mean
control	17.14ab
Spermine + Salt	19.52ab
Salt	14.87a
Heat Shock	16.70b
Spermine + Heat Shock	21.77ab

All such means that share a common English letter are non-significantly different, otherwise they differ significantly least at P < 0.05

1AA-oxidase activity may be due to the effect of salinity per findings of Mittal and Rama (1991). Also ABA production during salt-stress may increase IAA-oxides activity of roots by over-coming the inhibitory effects of the enzyme. Maximum reduction in IAA-oxides activity was observed in Cv.-M₃ only in ABA treatment.

Protein contents: Heat shock appears to be inhibitory to protein contents in Cv.-M₃ as compared to Cv.-M₁. Maximum increase in the leaf protein contents was recorded under the treatment of spermine and heat shock in Cv.-M₁ as Neuman *et al.* (1989) stated that heat shock activated the synthesis of low molecular weight proteins but the same treatment was inhibitory to seed protein. Maximum reduction in the protein content under heat stress may be due to reduced nitrogen accumulation as observed in Anuradha and Sarma (1995) that stress induced protein reduction in soybean. These findings also corroborate the results the spermidine showed the increasing effect on protein content in association of tobacco cells due to spermine and RNA in medium containing protoplast isolated from leaves (Cheong *et al.*, 1986, and Kaur-Sawhney *et al.*, 1986).

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