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SEED PRIMING WITH POLYAMINES ENHANCES GERMINATION, GROWTH AND BIOCHEMICAL CONTENTS OF MUNGBEAN (*VIGNA RADIATA* L.)

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ABSTRACT

Background The growth and biological nitrogen fixation ability of leguminous crops is known to be enhanced by the *Rhizobium* inoculation. However, very little is known whether exogenously applied polyamines alone and in mixture with *Rhizobium* inoculation further boost-up germination, growth and nitrogenase activity of legumes.

Methodology The present study was carried out to investigate the effect of polyamines (spermine and putrescine) and abscisic acid (ABA) alone and in combination with *Rhizobium* inoculum on germination and growth rates, and nitrogenase activity of mungbean (cv. NM-92). The ABA, spermine and putrescine were applied as seed soaking treatments where seeds were soaked 6.0 hours prior to sowing for studying their effect on germination rate, germination percentage and fresh weight of seedlings. But in case of drought conditions, spermine, putrescine and ABA were applied as foliar spray each at 10⁻⁵ M.

Results Maximum germination percentage (66.2-100%) and seedling fresh weight (0.78-0.95 g) of mungbean were obtained as a result of seed soaking with spermine + inoculum that were 13-23% and 14-22% higher, respectively compared to control. While, ABA treatment showed reduction in seed germination percentage and fresh weight of seedlings. Compared to all other treatments, effect of spermine alone and in the presence of *Rhizobium* inoculum was more pronounced in reducing proline accumulation and enhancing protein content in mungbean seedlings under normal, drought stressed and re-watered conditions.

Conclusion It can be concluded that application of *Rhizobium* inoculum in combination with polyamines especially spermine significantly increased the germination, seedling growth, nitrogenase activity and protein content in the leaves of mungbean.

INTRODUCTION

Mung bean (*Vigna radiata* L. Wilezek) belongs to family Leguminosae, and is native to India, Burma and other areas of the South Asia (Ahmad et al. 2015). It is an important pulse crop in many Asian countries including Pakistan, where the diet is mostly cereal based. It is mostly grown for its edible seeds, which are cooked, fermented, roasted, sprouted, or milled (Bhatty et al. 2000). It is rich in protein, minerals and vitamins while free from fat. Being short duration crop, usually grown as intercrop or

cover crop (Lambrides and Godwin 2006). The straw and byproducts of mung bean are used as feed for livestock, poultry and fish (Khatik et al. 2007). It is an important green manuring crop and can fix a lot of nitrogen through biological fixation, 30-251 kg ha⁻¹ (Ashour et al. 1991). Mungbean require relatively less water and nutrients and thus can be grown under the conditions of limited water supply and soil fertility (Ranawake et al. 2011). Mungbean capable to tolerate drought stress efficiently because its root system consists of tap roots and deep lateral roots which can extract water under limited water supply

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(Shirvan and Asghairpur 2009).

Agricultural scientists are trying their best to improve productivity of cereal grains which accounts for about 65 percent of the total cropped area. As a result, the productivity of food grains in our country over the last 35 years has a significant increase, achieving a level of 37.2 million tons in 2015-16 (Government of Pakistan 2016) compared to 15.0 million tons in 1980-81 (Hussain et al. 2016). In contrast, pulses are considered less important compared to cereals due to their lower yield and income, and are mostly confined to marginal lands of Pakistan, the production status of pulses is facing static or sometimes negative trend (Rani et al. 2014). This is evident from 15.6, 4.4 and 0.8 percent reduction in production of black gram, lentil and mung bean, respectively (Government of Pakistan 2016). Although, the area of mungbean (146.3×10^3 ha) was 14% higher compared to that in the previous year (127.4×10^3 ha), yet its production (98×10^3 tons) decreased by 0.8 percent. Despite the adoption of new high yielding varieties of mungbean, there is little improvement in its yield. Besides many agromanagement constraints like inappropriate sowing time (Rehman et al. 2009), sowing method (Rasul et al. 2012) and fertilization (Hussain et al. 2011a); stagnant mungbean yield is largely attributed to its susceptibility to various abiotic stresses at different growth stages of the crop (Sehrawat et al. 2013). The marginal soils of Pakistan are mainly affected by drought and salinity. Drought resistance is a complicated phenomenon resulting from the interactions of different metabolic processes or management strategies. One of the mechanisms to improve plant tolerance to drought stress is the exogenous application of some of the naturally occurring plant substances like polyamines and abscisic acid (ABA) is considered one of the strategy to induce stress tolerance in plants (Jakab et al. 2005; Duan et al. 2008).

In addition to well recognized hormones viz. auxin, gibberellins, cytokinins, ethylene and ABA, there are polyamines which seem to have similar effects on plants. They arise from three amino acids, viz., arginine, lysine and methionine. The polybasic acids arginine and lysine furnish the main part of the carbon skeleton of the polyamines, while methionine contributes polyamino groups to the simple diamine putrescines (1, 4 diamino butane) to form, successively, the tri-amine, spermidine and the tetra amine spermine (Galston 1983). Polyamines are believed to be indispensable for normal growth and development of plants (Smith 1985; Galston and Kaur-Sawhney 1995). While, the precise physiological function of polyamines remained uncertain, they have been considered either as candidates for

active regulators of plant growth (Galston 1983), secondary messengers that mediate phytohormone effects (Smith 1985) or hormones (Masse et al. 1989). Polyamines have also been shown to be involved in cell division morphogenesis, senescence and plant responses to a variety of stress conditions (Galston and KaurSawhney 1990 and in dormancy breaking of tree seeds (Szcotka and Lewandowska 1989). Different polyamines are related to different processes. Thus, stress of various kinds induces the accumulation of putrescine. Cell growth and elongation are also correlated with high putrescine and cells in division phase are rich in spermidine and spermine (Galston and KaurSawhney 1995).

The ABA is a natural growth inhibitor and well known growth retardant also known as stress hormone. It enables the plants to withstand against environmental stress. It is sesquiterpenoid in nature. The regulatory role of ABA is best documented for responses to stress, especially in water deficiency. Evidences have suggested that ABA induces physiological changes which can enable the plants to tolerate salt and water stress. It has been reported that exogenously supplied ABA improved the adaptation of isolated cells to osmotic stress, whereas increase in endogenous ABA concentration induced onset of growth after exposure of the cells to the salt stress (Galston and KaurSawhney 1995). Application of synthetic ABA tends to minimize the effects of water stress on plant growth and development. Leaf resistance and water potential are increased when ABA is applied exogenously. Zabadal (1974) suggested that there may be a threshold leaf water potential which triggers off ABA biosynthesis. Evidence that favor the involvement of ABA indicated that endogenous ABA appears to increase slowly with initial reductions in the turgor of the leaf. The ABA influences plant physiology, growth and development in a variety of ways. It plays a central role in stress responses and enhances adaptation to various stresses, such as desiccation and salt stress. In some cases, increased endogenous ABA content has been correlated with decreased hypocotyl growth rates. It has been found that ABA may act as *in vivo* effect of some metabolic events that lead to proline accumulation. In non-stressed barley leaves application of ABA in reasonable doses was found to be responsible for proline accumulation. Ober and Sharp (1994) also demonstrated that increased ABA is required for high rates of proline deposition in *Zea mays* L. This concurrent accumulation of proline in response to endogenous ABA and drought stress has demonstrated that ABA may trigger synthesis of proline under stress conditions. The present experiment was carried out to investigate the potential of polyamines and ABA to improve the

physiology and growth of mung bean under drought and re-watered conditions.

MATERIALS AND METHODS

The petri plate and pot bioassay studies were conducted to determine the germination, nitrogenase activities and seedling growth of mungbean in normal and sub-optimal (drought and re-watered) conditions.

Plant material and growth conditions

Seeds of mungbean (*Vigna radiata* L. Wilezek) cv. NM-92 were collected from seed section of Pulses Program, National Agriculture Research Center (NARC), Islamabad, Pakistan. The inoculum (*Rhizobium leguminosarum* TAL420) used in these experiments was obtained from Microbiology Lab, NARC, Islamabad, Pakistan. The drought was introduced by withholding the supply of water, whereas waterlogged environments were maintained in re-watered conditions. The abscisic acid, spermine and putrescine were applied as seed soaking treatments by soaking seed prior to sowing for 6 hours. In case of drought conditions, ABA, spermine and putrescine were applied as foliar spray, each at the rate of 10^{-5} M. The seeds were sown in earthen pots filled with sand and soil in the ratio of 1: 3. The plants were allowed to grow under natural conditions. The seeds were soaked in petri plates for germination. The petri plates/ pots were placed in a controlled environment growth room maintained at 12 hours photoperiod. Temperature varied from 20-25 °C and humidity ranged between 80-85 percent.

Experimentation

Before sowing, in each of the normal, drought and re-watered conditions, twenty seeds were soaked in petri plate containing aqueous solution of putrescine, spermine and ABA in combination with or without *Rhizobium* inoculum. While in case of control, the seeds were soaked in distilled water with and without inoculum. Thus, each experiment comprised of eight treatments and each treatment was replicated four times. Germination was recorded after every eight hours. Germination percentage was calculated by:

$$\text{Germination percentage} = \frac{\text{Germination count}}{\text{Total seeds sown}} \times 100$$

Five seedlings per petri plate were randomly selected and their weight was recorded after 24, 48, 72 and 96 hours of soaking.

Seedling proline estimation

Proline estimation of fresh seedlings was carried out following the method of Bates et al. (1973). The fresh

plant material (100 mg) was homogenized with 5 mL sulphosalicylic acid (3%) in a clean mortar. Later on the homogenized material was centrifuged at 2000 rpm for 5 minutes and the supernatant was adjusted to 5 mL with distilled water. To supernatant, 5 mL glacial acetic acid and 5 mL acidic anhydride (0.1% in acetone) were added and the reaction mixture was shaken and heated in boiling water bath for 30 minutes, cooled and then extracted with 10 mL of toluene in a separating funnel. The absorption of toluene layer was recorded on Shimadzu Spectrophotometer (Model UV-120-01, Japan) at 520 nm. A reference curve was prepared by using standard proline (Sigma Chemicals, UK).

Seedling protein estimation

Protein content of the fresh seedling was determined following the method of Lowry et al. (1951), using BSA (Bovine serum albumin) as standard. For this, the chemicals viz. phosphate buffer (stock solution), monobasic sodium phosphate (27.6 g) dissolved in distilled water (1000 mL), dibasic sodium phosphate (53.6 g) dissolved in distilled water (1000 mL), monobasic sodium phosphate solution (16 mL) and dibasic sodium phosphate solution (84 mL) were mixed together to obtain the desired pH 7.5 of phosphate buffer, Reagent A: (2 g Na_2CO_3 , 0.4 g NaOH and 1 g Na-K tartarate dissolved in 100 mL of distilled water), Reagent B: (0.5 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolved in 100 mL distilled water), Reagent C: (50 mL of solution A and 1 mL of solution B mixed together), and Reagent D: (Foline phenol was diluted with distilled water in the ratio of 1: 1).

Fresh seedling (0.1 g) was ground in 1 mL of the phosphate buffer pH (7.5) with the help of mortar and pestle and was centrifuged at 3000 rpm for 10 minutes. The supernatant (0.1 mL) of the given sample containing unknown amount of protein was poured in the test tube. After this, total volume of 1 mL was made with distilled water and finally to the solution 1 mL of reagent C was added. Later on the test tube was shaken for 10 minutes and 0.1 mL of reagent D was added after shaking. The absorbance of each sample was recorded at 650 nm after 30 minutes' incubation. The concentration of unknown sample was calculated for the protein content with reference to standard curve made by using standard BSA. The BSA of different concentrations viz. 20, 40, 60, 80, 160, 320, and 640 μg were prepared. The reagents were added in the sequence as described above. Finally, the absorbance of BSA was recorded at 650 nm.

Seedling leaf proline and protein estimation

In case of potted plant experiment, proline and protein estimations of mung bean leaves under

drought and re-watered conditions were carried out according to the methods of Bates et al. (1973) and Lowers et al. (1951), respectively.

Nitrogenase activity in mungbean root nodules

Nitrogenase activity was determined following the method of LaRue and Kurz (1973). For this, nodules (5 g) were incubated in 30 mL Mc. Cartinys vials with rubber stopper. After this, 2 mL of air was removed from the vials with syringe and 2 mL of C_2H_2 was injected. Plant nodules were incubated with C_2H_2 for 90 minutes at 22°C. Thereafter, 2 mL of gas phase were removed and injected in Mc. Cartinys vials which contained 1.5 mL of oxidant solution (40 mL of 0.05 M $NaIO_4$, 2.5 mL of 0.005 M $KMNO_4$, adjusted to 7.5 pH with KOH, diluted to 100 mL). The vials were agitated vigorously on a rotary shaker at 3000 rpm for 90 minutes at 22°C. The 4.0 N $NaAsO_2$ (0.25 mL) and 4.0 N H_2SO_4 (0.25 mL) were added. Nash reagent (75 g of ammonium acetate, 1.5 mL of acetic acid and 1 mL of acetyl acetone) diluted to 500 mL was added and the absorbance for C_2H_2 was prepared. Taking all the reagents, incubated and mixed for the same period and absorbance value of the sample subtracted from the blanks.

Statistical analysis

All the data collected were analyzed by using completely randomized design in case of petri-plate experiment and randomized complete block design in case of pot experiment. Whereas, the treatment means were compared by Duncan's New Multiple range Test at 0.05 level of probability (Steel et al. 1997).

RESULTS AND DISCUSSION

Germination percentage

Results regarding germination percentage and germination rate of mung bean as affected by ABA, polyamines and *Rhizobium* inoculation treatments are presented in Table 1 and Figure 1. Results showed that maximum germination percentage (66.25, 85, 97.5 and 100%) of mung bean at 8, 16, 24 and 32 hours of soaking, respectively were observed with seed soaking in spermine + inoculum whereas ABA treated seeds showed the minimum germination percentage values at all the soaking intervals. The comparison of curves indicated germination rate that compared with control, maximum increase in germination rate of mung bean observed by seed soaking in spermine + inoculum which was followed by putrescine + inoculum whereas a little increase was also shown by the seed treatment with inoculum alone (Figure 1). But all the other treatments caused reduction in germination rate of mung bean.

Rhizobium inoculum showed late stimulatory effect on germination but in combination with all other treatments, inoculation consistently increased the rate of germination. This may be due to the production of growth promoting substances which in turn induced physiological and biochemical changes in seeds suitable for germination. The results are in confirmation with the work done by Galston (1983) who correlated elongation growth with putrescine and cell division with spermine, spermidine etc. The observed maximum increase in the rate and percentage of germination due to the spermine + inoculum could be due to the induction of enzymes responsible for germination by spermine. The retardation in germination by ABA was probably due to its growth inhibitory effect. The results are in line with the work done by Poemery et al. (1994). Exogenous supplied ABA may block the translation of mRNA in seeds, which then inhibit the synthesis of enzymes that are essential for germination. Many scientists have speculated that ABA normally induces dormancy (Taylorson and Hendricks 1977). Gill and Tuteja (2010) and Hussain et al. (2011b) mentioned the improvement in germination of different crops by application of spermine and putrescine. An enhancement in germination percentage and germination rate of maize in response to seed soaking in spermine has also been shown by Huang et al. (2017).

Seedling fresh weight

The Table 1 and Figure 2 showed significant ($p \leq 0.05$) differences among the treatments regarding seedling fresh weight of mung bean at different periods after soaking. Significantly, the highest fresh weight ($0.95 \text{ g seedling}^{-1}$) of mung bean seedlings at 96 hours after seed soaking was recorded with seed soaking in spermine + inoculum whereas minimum seedling fresh weight ($0.61 \text{ g seedling}^{-1}$) with seed soaking in ABA. The comparison of mung bean seedling growth rate among different seed soaking treatments has been shown in Figure 2. Results indicated that compared with control, both the polyamines (spermine and putrescine) boosted up the seedling growth rate. The highest seedling growth rate was noted with spermine + inoculum that was followed by putrescine + inoculum. However, putrescine and spermine remained at 3rd and 4th position, respectively. Contrastingly, ABA both alone and in combination with inoculum resulted in decline in seedling growth rate of mung bean.

Rhizobium inoculum has no significant effect on fresh weight of seedlings for measurement taken 24 hours after soaking but at later stages inoculum showed significant stimulation when used in combination with various treatments and the

combination of spermine + inoculum was found the most effective. The enhancing effect of inoculum exhibited only in combination with the polyamines and ABA could be attributed to the interactive effect of the inoculum and the growth regulator/ polyamines on the process of cell division, cell elongation as well as root growth. The results are in confirmation with earlier findings (Galston 1983) correlated elongation growth with putrescine and cell division with spermine, spermidine etc. Huang et al. (2017) also demonstrated that exogenously applied spermine

through seed priming exhibited significant increased seedling biomass of maize. This view is further supported by the present observation that ABA inhibits cell division (Ho 1983; Watts et al. 1999). Application of ABA might be resulted in the stomatal closure, enhanced water fluxed through roots, reduced leaf growth and hypocotyl growth. These factors ultimately resulted in the reduction of fresh weight but combination of spermine with ABA showed stimulatory effect.

Table 1 Germination percentage and seedling fresh weight of mung bean as influenced by abscisic acid, polyamines and *Rhizobium* inoculation treatments

Treatments	Period after soaking (hours)							
	Germination percentage				Seedling fresh weight (g)			
	8 h	16 h	24 h	32 h	24h	48h	72h	96h
Control (untreated)	53.75e	72.25d	86.25b	97.50a	0.68b	0.75c	0.77e	0.78c
Inoculum	50.00cd	75.00c	86.25b	97.50a	0.69b	0.75c	0.77e	0.79c
Putrescine	48.75d	60.00e	81.25d	96.25a	0.75a	0.80b	0.83b	0.85b
Putrescine + Inoculum	48.75d	70.00d	85.00c	96.25a	0.75a	0.91a	0.87b	0.88b
Spermine	57.50b	80.00b	96.25a	100.01a	0.76a	0.81b	0.83b	0.83b
Spermine + Inoculum	66.25a	85.00a	97.50a	100.03a	0.78a	0.85b	0.94a	0.95a
Abscisic acid (ABA)	41.25f	57.50e	63.75f	95.00b	0.52d	0.58e	0.60e	0.61e
ABA + Inoculum	45.00de	60.00e	78.75e	96.25a	0.60c	0.65d	0.68d	0.70d

Means in a column sharing same letter do not differ significantly at 5% probability

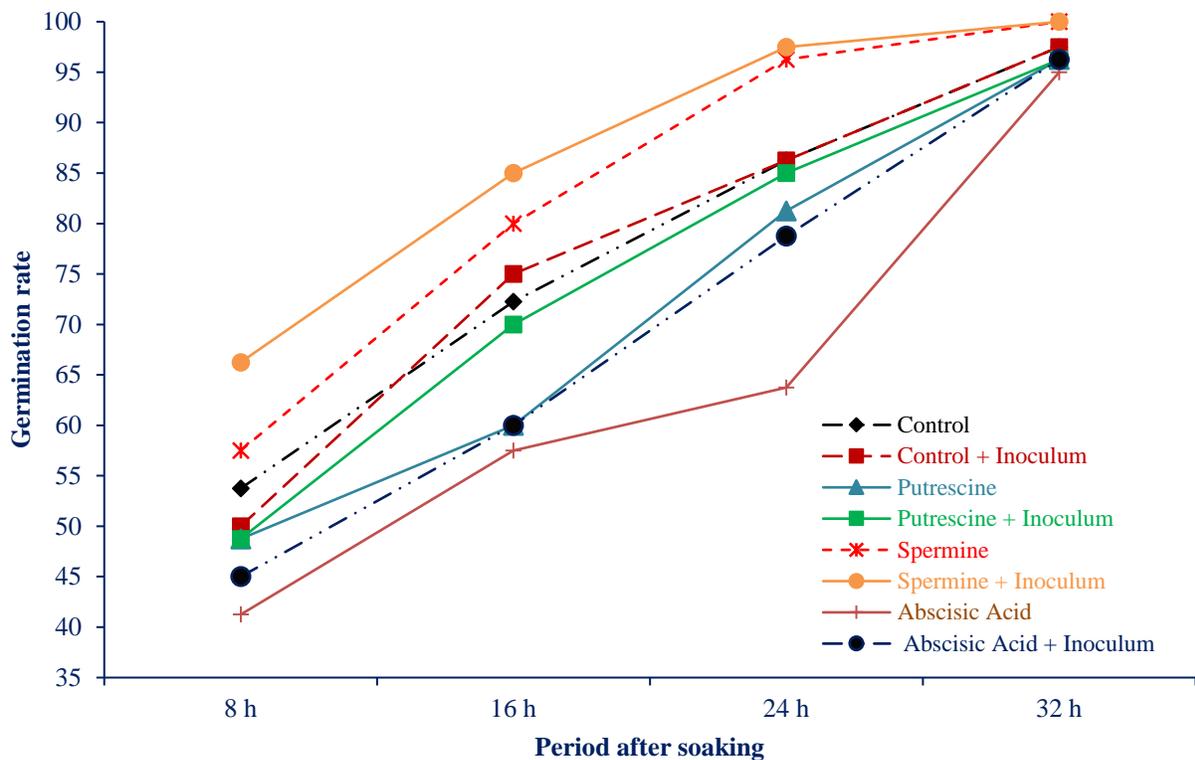


Figure 1 Germination rate of mung bean as affected by abscisic acid, polyamines and *Rhizobium* inoculation treatments

Seedling proline content

It is obvious from the data given in Table 2 that significantly the highest proline content (18.33 $\mu\text{g g}^{-1}$ FW) was observed in ABA treatment whereas, minimum proline contents were found in spermine alone and in combination with inoculum. However, under drought and re-watered conditions, ABA and ABA + inoculum gave significantly the highest leaf proline contents with their respective values of 88.16, 88.50, 27.16 and 25.00 $\mu\text{g g}^{-1}$ FW. The minimum leaf proline contents in drought and re-watered conditions were found in seed treatments with spermine and

spermine + inoculum. The ABA treatment resulted in maximum accumulation of proline. Aspinall and Paleg (1981) reported the accumulation of proline in response to high ABA content, drought stress and salt accumulation. Stewart (1980) also investigated that ABA may act as *in vivo* effect of some metabolic events that lead to proline accumulation in barley. They also revealed that application of ABA can stimulate proline accumulation in non-stressed barley leaves and enhanced glutamate conversion to proline, showed least accumulation of proline perhaps serving itself as osmoregulant or interfered in the biosynthesis

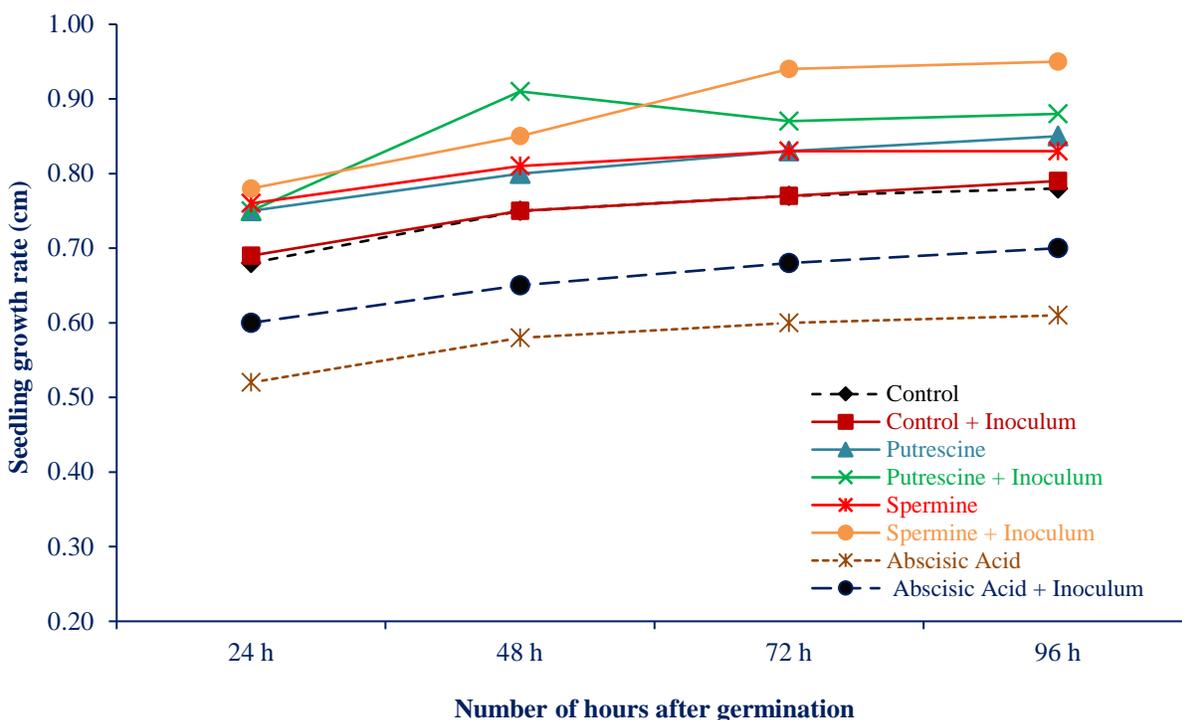


Figure 2 Seedling growth rate of mung bean as affected by absciscic acid, polyamines and *Rhizobium* inoculation treatments

Table 2 Seedling proline and protein contents of mung bean as influenced by absciscic acid (ABA), polyamines and *Rhizobium* inoculation treatments under different growing conditions

Treatments	Growing conditions					
	Proline content ($\mu\text{g g}^{-1}$ FW)			Protein content ($\mu\text{g g}^{-1}$ FW)		
	Normal	Drought	Re-watered	Normal	Drought	Re-watered
Control (untreated)	14.83b	66.16b	17.16b	42.90c	2.88b	11.33d
Control + Inoculum	13.66b	65.33b	13.16c	44.00c	3.00b	16.66c
Putrescine	12.70b	53.76c	18.16b	43.00c	3.70b	12.66d
Putrescine + Inoculum	12.16b	54.66c	13.66c	45.06c	4.00b	17.33c
Spermine	7.46c	34.66d	6.00d	61.76a	13.33a	22.00b
Spermine + Inoculum	7.16c	31.33d	4.160d	63.66a	14.66a	34.66a
Absciscic acid (ABA)	18.33a	88.16a	27.16a	25.53d	1.66c	3.16e
ABA + Inoculum	14.83b	85.50a	25.00a	26.50d	2.16c	5.00e

Means in a column sharing same letter do not differ significantly at 5% probability

Table 3 Nodule nitrogenase activity of mung bean as influenced by abscisic acid (ABA), polyamines and *Rhizobium* inoculation treatments under drought stressed and re-watered conditions

Treatments	Growth conditions	
	Drought	Re-watered
Control (untreated)	6.66d	17.00d
Control + Inoculum	12.83c	23.66c
Putrescine	14.00bc	23.66c
Putrescine + Inoculum	14.06bc	28.00b
Spermine	16.00b	31.00b
Spermine + Inoculum	20.33a	37.33a
Abscisic acid (ABA)	1.33e	14.66d
ABA + Inoculum	4.00d	15.00d

Means in a column sharing same letter do not differ significantly at 5% probability

proline, showed least accumulation of proline perhaps serving itself as osmoregulant or interfered in the biosynthesis of proline. Proline being an osmoregulant and protectant biochemical in plant has been found in an increased level under various stress conditions as compared to normal conditions. There was the marked accumulation of proline under stress. The present work outcomes are in line with the findings of Yamada et al. (2005) who observed marked accumulation of free proline in the drought stress conditions as compared to the optimum growth conditions.

No marked effect of inoculum was observed on proline accumulation. The marked reduction of proline content following spermine as well as putrescine application could be attributed to the fact that being nitrogenous compounds they are involved in osmoregulation or they interfered with the metabolism of proline at certain step of proline biosynthetic pathway. Induction of proline accumulation following ABA and drought as found during present investigation has also been reported earlier by Voetberg and Sharp (1991), Ober and Sharp (2003) and Yamada et al. (2005). But application of ABA under controlled conditions even after re-watering increased proline content in leaves perhaps indicating that plants did not recover after the re-watering as quickly as in control and other treatments. Whereas, in putrescine and spermine treatments the drought induced proline content increase in leaves on re-watering was not observed perhaps due to compartmental action or degradation of proline. Since the putrescine level in shoots of drought stressed *vigna radiata* increased considerably and this increased putrescine content may be responsible for the plant adaptation to drought stress (Friedman et al. 1989).

Seedling protein content

Results revealed significant differences among various treatments with respect to protein contents in

mung bean seedlings (Table 2). The maximum accumulation of protein in seedlings grown in all the normal ($63.66 \mu\text{g g}^{-1}$ FW), drought ($14.66 \mu\text{g g}^{-1}$ FW) and re-watered ($34.66 \mu\text{g g}^{-1}$ FW) conditions was found with seed soaking in spermine + inoculum. However, minimum seedling protein contents were obtained in ABA and ABA + inoculum treatments. Polyamines and ABA markedly affected the protein content of the seedlings. The maximum reduction in protein content due to ABA may be attributed to blockage of transcription and translation for proteins. The results are in line with the work of Slee et al. (1990) who reported that water stress and ABA application induced the incorporation into a 14.4 K_b protein whereas spermine resulted in maximum accumulation of protein in seedlings. Significant reduction in protein content of leaves under drought and ABA alone or combination with inoculum has been recorded that is supported by the investigations of Walton (1980) and Ünyayar et al. (2004). Frota and Tucker (1978) investigated that protein synthesis is significantly reduced in bean shoots when subjected to water stress. But spermine showed maximum protein content perhaps by adding the amino acid pool for protein synthesis or delaying the process of senescence in leaves. Similar was the case in the *Rhizobium* inoculated plants showing marked effect on protein contents of leaves. The *Rhizobium* increased the transport of nitrogen compounds once they are fixed in the module by their *Rhizobium* bacteria following the process of N_2 fixation.

Nitrogenase activity in nodules of mungbean under drought and re-watered condition

The results presented in Table 3 indicated significant differences among different treatments regarding nitrogenase activity in mung bean root nodules. Significantly, the highest nitrogenase activity (20.33 and 37.33) in root nodules of drought stressed and re-watered mungbean seedlings, respectively was observed with spermine + inoculum. However, ABA

and ABA + inoculum treatments gave the minimum nitrogenase activity in mungbean root nodules. Nitrogenase activity was more under re-watered conditions as compared to the drought stress. These results are confirmed by the work done by Kohl et al. (1991) who found irreversible loss of nitrogenase activity when water content of the nodules fell below 80%, perhaps water stress induced the structural and physiological alterations in the root nodule and adversely affected their nitrogen fixing ability. A positive correlation among nitrogenase activity and application of the polyamines was observed. In case of ABA, nitrogenase activity was reduced both under drought and re-watered conditions, may be due to ABA reducing the number of nodules per plant which are the sites of N₂ fixation. Nitrogenase activity was markedly higher in the treatments where inoculum was applied alone and in combination with polyamines. The results are in line with the work of Bandyopadhyay (1992) and Dubey and Tomar (2000) who also observed increased nitrogenase activity when *Rhizobium* inoculum was applied to different legume crops.

CONCLUSION

It is concluded that exogenously applied spermine with *Rhizobium* inoculum may be used under drought stress for osmoregulation as it helped in the accumulation of proline and protein contents. Spermine seed soaking also increased nitrogen fixation in legumes.

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