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Elucidating the impact of exogenous polymanines in blackgram during water stress

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Abstract
Polyamines, especially putrescine (Put), spermidine (Spd) and spermine (Spm) have been implicated in a wide range of biological processes, including growth, development and apoptosis. They are also associated with responses of plants to drought stress. A pot culture experiment was conducted to study the effect of exogenous application of polyamines – Spermine, Spermidine and Putrescine during water stress in blackgram KKM1. Water stress was imposed from 25 DAS followed by foliar application of Put (0.2 mM), Spd (0.2 mM), and Spm (0.2 mM) on 30 DAS. On 35 DAS, assessed the leaf samples for biochemical attributes - Proline, Chlorophyll, Soluble sugars, Total free aminoacid, RWC, Catalase and Peroxidase. The effect of exogenous polyamine application on growth attributes and yield attributes in blackgram under water stress were recorded. The results demonstrated that polyamines (Spd, Spm, Put) on exogenous application mitigated water stress in blackgram and treatment with spermidine had improved water stress tolerance and there is feasibility of polyamines serving as metabolic marker of water stress.

Keywords: Water stress, exogenous polyamine, blackgram, biochemical attributes

1. Introduction
Global warming is an alarming problem in agriculture and its effect on yield loss has been estimated to be five per cent for every degree centigrade rise in temperature. Plants exhibit multiple mechanisms like optimizing signaling pathway, involvement of secondary messengers, production of biomolecules specifically in response to stress, modulation of various metabolic networks in accordance with stress in order to overcome abiotic stress factors. Many structural genes and networks of pathway were identified and reported in plant systems for abiotic stress tolerance. One such crucial metabolic pathway that is involved in normal physiological function and also gets modulated during stress to impart tolerance is polyamine metabolic pathway. In plants greater accumulation of PAs (Put, Spm, and Spd) during abiotic stress is well documented and is implicated in increased tolerance to abiotic stress. Because they play important roles in diverse plant growth and developmental processes and in environmental stress responses, they are considered as a new kind of plant biostimulant. The prime objective of the study is to evaluate the effect of exogenous polyamine (Putrescine, Spermidine and Spermine) application on biochemical, growth attributes and yield attributes in blackgram under water stress.

2. Materials and Methods
2.1. Plant Materials, Growth Condition, and Stress Treatments
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T_1: Control (Water unstressed with regular watering for every 2 days interval
T_2: Water stress induced by withholding watering for a period of 2 weeks on 25 DAS
T_3: T2 + Foliar application of Spermidine (0.2mM) on 30 DAS
T_4: T2 + Foliar application of Spermine (0.2mM) on 30 DAS
T_5: T2 + Foliar application of Putrescine (0.2mM) on 30 DAS

Water stress was imposed under 30% field capacity from 25 DAS. On 30 DAS, foliar application of Put (0.2 mM), Spd (0.2 mM), and Spm (0.2 mM) along with Tween -20 was given. On 35 DAS, assessed the leaf samples for various biochemical and growth attributes. The experiments were carried out in completely randomised design with 4 replications.

2.2. Biochemical analysis

2.2.1. Proline

Proline (Pro) was determined according to Bates *et al.* (1973) [2]. Leaves were homogenized in 3% sulphosalicyclic acid and centrifuged at 11,500 × *g*. Supernatant was mixed with acid ninhydrin with glacial acetic acid and phosphoric acid. After incubating the mixture at 100 °C for 1h and cooling, toluene was added; chromophore containing toluene was read spectrophotometric ally at 520 nm.

2.2.2. Chlorophyll content

Leaf sample was extracted with 80% v/v acetone (centrifuging at 5,000× *g*). Absorbance's were taken with a UV-visible spectrophotometer at 663 and 645 nm for chl *a* and chl *b*, respectively, and chl content was calculated according to Arnon (1949) [1].

2.2.3. Total soluble sugars

The sample was homogenized with 80% alcohol, and the homogenate was placed at room temperature for 30 min and centrifuged at 4 °C, and then the supernatant was used for total soluble sugar. 2 mL of the supernatant was mixed with 3 mL of anthrone and the mixtures were placed in a boiling water bath for 10 min. After cooling, the absorbance of the mixtures was measured at 620 nm. (Hedge, J E and Hofreiter, B T (1962) [4].

2.2.4. Total free amino acids

The extraction and estimation of amino acids were determined spectrophotometric ally according to Moore and Stein (1948). 0.5 g of sample was taken and it was macerated using 10 ml of 80% ethanol. 0.5 ml of each treatment plant extract was taken and 4ml of Ninhydrin citrate glycerate reagent was added to all the test tubes and heated in boiling water for 10 minutes. Cooled and the absorbance was measured at 570 nm within 1hr against reagent blank.

2.2.5. Soluble protein

0.2 g of the sample was homogenized in 10 ml of phosphate buffer pH (7.0) and then subjected to centrifugation. 0.1 ml of supernatant was treated with 5 ml of Alkaline copper solution followed by addition of 0.5 ml of folin-ciocalteau reagent. The intensity of blue colour was measured spectrophotometric ally at 660 nm. The blue coloured complex was due to reduction of the phospho molybdic and phospho tungstic components in the folin-ciocalteau reagent (FCR) by the amino acids tyrosine and tryptophan present in the protein (Lowry *et al.*, 1951) [6].

2.2.6. Leaf Relative Water Content

RWC was determined according to Barrs and Weatherley (1962). Fresh weight (FW), turgid weight (TW), and dry weight (DW) of leaves were measured, and RWC was calculated using the following formula: RWC (%) = [(FW–DW)/(TW–DW)] × 100

2.2.7. Catalase activity

Catalase activity was determined by monitoring the enzyme-catalyzed decomposition of hydrogen peroxide (H_2O_2) at 240 nm. 0.1 g sample was homogenized with M/150 phosphate buffer at 4 ^{0}C and centrifuged. 0.01 ml of sample extract was mixed with 3ml of H_2O_2- phosphate buffer. The time difference required for decrease in absorbance from 0.45 – 0.4 was noted. The concentration of H_2O_2 was calculated using the extinction co- efficient 0.036 per µmol per mL (Luck, 1974) [7].

2.2.8. Peroxidase activity

1g of fresh tissue was homogenized in 3 ml of ice cold 0.1M phosphate buffer. The homogenate was centrifuged at 5^0 C for 15 minutes and the supernatant was used as enzyme source and the peroxidase activity was measured by adding 0.1 ml of o-dianisidine and 0.2 ml of 0.2M H_2O_2. Increase in absorbance was measured for 3 minutes and enzyme activity was expressed in terms of increased absorbance per unit time per g tissue fresh weight (Malik and Singh, 1980) [8].

2.3. Determination of Growth Parameters

Plant height and root length were measured from each set of seedlings. Ten randomly selected fresh seedlings from each treatment were dried at 80 °C for 48 h, then weighed and considered as DW. The effect of exogenous polyamine application on growth attributes- Shoot length and root length

2.4. Determination of Yield attributes

The number of pods/ plant, yield/plant, TDMP and 100 seed weight in blackgram under water stress were recorded. (Table 2).

2.5. Statistical analysis

The SPSS statistical software and one-way analysis of variance (ANOVA) was used to confirm the variability of the data and the validity of the results.

3. Results and Discussion

The effect of exogenous polyamine application on biochemical attributes during water stress in blackgram is given in Table 1.

Table 1: The effect of exogenous polyamine application on biochemical attributes during water stress in blackgram

Treatments	Chl a mg/g FW	Chl b mg/g FW	Total chl mg/g FW	RWC (%)	Soluble Protein (%)	Proline mmol/ g FW	Total free amino Acids (mg equi of leucine)	Soluble Sugars (%)	CAT (µmol of H_20_2 /min/mg protein)	PER ΔA/min/g FW
T1-Water unstressed	0.745	1.084	1.828	91.67	8.83	50.50	1.76	2.58	3.136	22.25
T2-Water stress induced	0.488	0.591	1.265	86.96	17.78	65.13	5.66	3.77	1.864	21.75
T3 - Water stressed + spd	0.606	0.783	1.389	93.48	14.28	67.75	4.06	2.42	2.693	35.25
T4 - Water stressed + Spm	0.665	0.861	1.525	93.91	9.48	44.50	1.96	2.10	2.317	24.58
T5 - Water stressed + Put	0.668	0.917	1.584	94.26	12.81	40.75	3.36	2.32	2.698	30.75
S.Ed	0.0437	0.084	0.0701	1.301	0.677	13.26	0.294	0.214	0.461	5.018
CD (0.05)	0.0953	0.184	0.153	2.835	1.476	28.89	0.641	0.466	NS	10.933

From the study, it was observed that the chlorophyll content and relative water content were reduced in the water stressed sample and however exogenous treatment of all polyamines contribute for the enhanced chlorophyll content and RWC.

This might be due to the fact that water deficit significantly affected proteins associated with photosynthesis as reported by Zhao *et al.,* 2004 [20]. The results reinforce the role of polyamines with enhanced capabilities of photosynthetic machinery (Sobieszczuk-Nowicka and Legocka (2014) [16]. Polyamines (Spm, Spd, and Put) can regulate the size of the potassium channel and the size of pores in the plasma membrane of guard cells, thereby strongly regulating pore opening and closing. In this way, PAs control water loss in plants (Liu *et al.*, 2000) [5].

PAs can promote photosynthesis, and increase the antioxidant capacity and osmotic adjustment ability of plants (Tian, 2012) [18]. Exogenous polyamines also enhanced the activity of antioxidant enzymes – catalase and peroxidase in all the treatment and this was supported by Parvin *et al.*, 2014 [12] that exogenous Spd added at the same time as NaCl increased cellular contents of Spd, Spm and Pro in Panax ginseng seedlings by activating antioxidant-based defense system, thereby reducing H_2O_2 and superoxide molecule. Antioxidant enzymes can scavenge ROS to prevent membrane lipid peroxidation and stabilize membrane structure (Ouyang *et al.*, 2017) [11].

Tanou *et al.* (2014) [17] suggested that it was due to reprogramming the oxidative status of cells by increased expression of genes producing antioxidant enzymes. Proteomic studies reveal reduced protein carbonylation and tyrosine nitration, and increased protein S-nitrosylation by polyamines. Shi *et al*. (2013) [15] found that exogenous PAs, while mitigating drought and salt stresses, significantly increased the abundance of antioxidant enzymes and several other stress-related proteins in a detailed study with Bermuda grass (*Cynodon dactylon*). In the present study, spermidine treatment had resulted in enhanced antioxidant enzymes.

Moreover, exogenous applications of polyamines resulted in increased proline and free aminoacid and soluble sugar content on comparison with water stressed sample. And, among the polyamines, spermidine treatment reported high proline (67.75 mmol/g FW), free aminoacid 4.06 mg equi of leucine and soluble sugar content of 2.42%. Similar results have been reported in which foliar application of putrescine at an appropriate level triggered physiological processes and induced the biosynthesis of osmotic adjustment substances, such as free amino acids, soluble sugars, and proline which compensate for the negative impacts of drought stress on plant biomass and increase the quality and quantity of bioactive substances (Sánchezrodríguez *et al.,* 2016; Mohammadi *et al.*, 2018) [14, 9].

The effect of exogenous polyamine application on growth and yield attributes during water stress in blackgram is given in Table 2.

Table 2: The effect of exogenous polyamine application on growth and yield attributes during water stress in blackgram

Treatments	Growth attributes			Yield attributes		
	Shoot length (cm)	Root length (cm)	TDMP (g)	Number of Pods /plant	Yield/ plant (g)	100 seed weight (g)
T1 -Water unstressed	21.98	4.73	1.76	36.25	11.6	4.256
T2 -Water stress induced	14.92	5.08	1.48	21.25	8.50	3.188
T3 - Water stressed + 0.2mM Spermidine	21.75	6.38	2.54	31.75	10.16	3.969
T4 - Water stressed + 0.2mM Spermine	19.63	5.15	1.56	29.25	9.51	3.560
T5 - Water stressed + 0.2mM Putrescine	22.6	5.85	1.84	23.25	9.04	3.488
S.Ed	1.606	0.258	0.140	2.095	0.065	0.302
CD (0.05)	3.499	0.563	0.305	5.187	1.477	0.638

In the present study, exogenous application of spd resulted in increased root length (6.38 cm) and total dry matter production (2.54 g). Saleethong *et al.*, 2013 [13] too reported that Spd (for 7 days) at early booting stage of rice (*Oryza sativa* L. ssp. *indica*) prior to treatment with NaCl significantly increased grain yield, Ca^{++} content in the grains, and a higher K^+/Na^+ ratio as compared to the non-treated control plants

Yield attributes are reduced in water stress induced samples and however exogenous application of polyamines resulted in increased yield parameters. Treatment with spermidine showed increased number of pods/plant (31.75), yield per plant (10.16 g) and 100 seed weight (3.969 g). Zeid and Shedeed, 2006 reported that in alfalfa, a Put treatment was shown to improve seed germination and increase all growth indexes (hypocotyl length, root and shoot fresh and dry mass) under drought stress caused by different concentrations of polyethylene glycol (PEG 4000), both in vitro and in a pot experiment.

4. Conclusion

Polyamines (putrescine, spermine and spermidine) play a crucial role in the regulation of cell growth, differentiation, death and function. The results demonstrated that polyamines (Spd, Spm, Put) on exogenous application mitigated water stress in blackgram and treatment with spermidine had improved water stress tolerance and there is feasibility of polyamines serving as metabolic marker of water stress.

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