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Effect of spermine and putrescine on germination and growth of *Vigna radiate* (L.) R. Wilczek seeds

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ABSTRACT

Polyamines are polycationic hydrocarbons that are found in all plant parts that regulates growth and development. In current study, seeds treated with polyamines, spermine and Putrescine were studied to evaluate the growth and development in terms of various physiological and biochemical parameters. For this study, seeds were soaked in different strengths of Spermine and Putrescine (0.01mM, 0.1mM and 1mM) for 48 hours and distilled water was used as control. After sprouting, seeds were transferred into half strength M.S. medium and evaluated at every 48 hours for 10 days. Highest seed germination (95%) was obtained in control after 24 hours and it was observed that germination was less in higher concentrations of polyamines. Out of all treatments, 0.01mM concentration showed maximum concentration compared to 0.1mM and 1mM polyamine concentration. First leaves were observed in Putrescine and Spermine treated seeds after 24 hours whereas, first leaves in distilled water treated seeds were observed after 30 hours. Statistical analysis using One Way ANOVA and Dunn's Post hoc analysis were performed to check the relationship between protein concentration, shoot length, chlorophyll a and b content. It was observed that there is a significant relationship between total protein content and chlorophyll a and b content and also between shoot length and chlorophyll a and b content. However, no significant relationship was observed between shoot length and protein concentration. An attempt has been made to characterize protein profile using SDS-PAGE, however no distinct band was found in treated seeds compared to control.

Keywords: Polyamines, Spermine, Putrescine, *Vigna radiate,* Growth and Development

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INTRODUCTION

Polyamines are organic compounds that presents in every parts of plants. They are polycations and they are found with different numbers of hydrocarbon and primary amino groups. There are three major forms of polyamines that include diamine putrescine, triamine spermidine to tetraamine spermine.[1] These are widespread in living organisms, found in high concentration in actively proliferating cells. Polyamines are responsible for many fundamental processes which include transcription, RNA modification, and synthesis of protein and the modulation of enzyme activities.[1] They are also involved in various physiological functions and their regulation. They are involved in regulation of ion channels and molecule transportation in the cell membrane.[2] Polyamines have positive charge hence they can bind to macromolecules such as DNA, RNA and proteins, hence stabilize them against free radicals activity.[3] Their role against stresses is well established.[4] Exogenous application of polyamines protects the plants from stress by expressing various stress relevant genes. Polyamines also play direct defensive role during stress by eliciting cell death at the sight of pathogen entry.[3]

The biological functioning of various polyamines is similar. Polyamines exist in various forms i.e. free living, conjugated and titers.[5] The increase in the activity of the titers in plants was found during the process of sprouting, seed germination and during root and shoot formation.[3,6] It is assumed that the conjugated form of polyamines is associated with flowering process.[7] In plants, putrescine is synthesized by the decarboxylation of amino acids arginine or ornithine. The process is catalyzed by enzymes arginine decarboxylase or ornithine decarboxylase, respectively.[6] The further addition of two aminopropyl groups to putrescineby enzymes spermidine synthase and spermine synthase results into the formation of spermidine and spermine, respectively.[8] The aminopropyl molecules are formed from the decarboxylation of S-adenosylmethionine by the enzyme S-adenosylmethionine decarboxylase.[4] Free polyamines level in plant cells regulated by their synthesis, transport, degradation and conjugation process. Putrescine degradation is catalyzed by diamine oxidase, a copper containing enzyme that oxidizes the diamine at the primary amino group whereas spermidine and spermine are oxidized at their secondary amino groups by a flavin - containing polyamine oxidase.[9] Polyamines can be conjugated with small molecules like proteins, antibiotics and phenolic acids like hydroxycinnamic acid.[10]

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Polyamines are responsible to performance wide range of functions in plants. It regulates growth and development and has effect on process like cell division, cell differentiation, flowering, vegetative growth and development and fruit ripening.[11] They have major regulatory effect on promoting the productivity of the plants.[12] Polyamines are anti-senesce agents, the controls the delaying of fruit softening and maintain the integrity of cell membrane.[13] Polyamines also serves as the carbon and nitrogen reserves in plants and believed as new class of growth regulators that act as second hormonal messenger.[14] Polyamines also help in the synthesis of protein and RNA.[15] Polyamines can readily bind to the negatively charged phospholipids' head groups or other anionic sites at membranes due to their positive charge and affects the stability and permeability of membranes. They are also involved in the buffering mechanism to maintain cellular pH and ion homeostasis.[16] Previous study suggests the high level of polyamines during the start of plant development as compared to maturation stages. However, this depends upon the plant species and the major polyamine content found in the plant.[17] The decline of polyamine level at the end of plant development is further act as a signal which initiates senescence and death of the plant or plant part.[18]

Polyamines are also involved in free radical scavenging, cell cycle regulation as well as signal transduction pathways and programmed cell death in plants.[19] The fine regulation of their biosynthetic and catabolic pathways as well as conjugation and transport processes ensures an accurate homeostasis of polyamines at cellular levels. Furthermore, besides their role in signal transduction pathways, Polyamines may act as sources of biologically active compounds such as hydrogen peroxide (H_2O_2) and aldehydes, generated via polyamine catabolism/inter conversion pathways.[20] In plants spermidine and spermineas well as their diamine precursor putrescine acts as modulators in signaling pathways involved in both developmental processes and responses to biotic and abiotic stresses.[21,22,23] At high intracellular levels, polyamines represent important sink of assimilated nitrogen (N) and play an important role in the carbon/nitrogen balance by modulating biochemical pathways involved in carbon metabolism.[24] H_2O_2 derived from polyamine catabolism interplays in the complex network made up of reactive oxygen species (ROS) and nitric oxide (NO).[25] Moreover, Polyamines may have a structural role incell wall assembly and thickening.[23]

Role of polyamines in regulation of cellular activities such as enzyme regulation, DNA dubling, protein synthesis and maintenance of membrane integrity is well known with many

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other biological functions in plants, further study is necessary to revile their precise action and involvement in physiological and molecular regulation. It is assumed that the more the amino groups, the stronger the physiological activity.[7] Recent studies are carried out to study exogenous effects of polyamines and their synthesis inhibitors. Transgenic methods are also intensively performed to revile the role of polyamines in plant development and their mechanism of action.[26,27] Such studies have shown that Polyamines are closely associated with plant growth, the stability of nucleic acids and membrane structure, stress resistance, and even plant survival.[28] Majority of references related to polyamines explain their physiological and biochemical effect, however molecular modulation of cellular components are least studied. However, it is clear that the major polyamines i.e. putrescine, spermidine, and spermine, are found in every plant cell in titers ranging from approximately 10 μM to mM concentration.[29] Polyamines functions with enzymes to regulate plant metabolism. In plants, polyamines occur in the free form or bound to phenolic acids, other low molecular weight compounds or macromolecules. The level of polyamines is directly controlled by various external conditions, such as light, temperature and physic-chemical agents.[30]

For present study *Vigna radiata* commonly known as moong beans or green grams is selected as a study plant. It is a leguminous plant belongs to family Fabaceae. It has potential to fix higher amount of nitrogen. Moong beans are self-pollinating plants that help in prevention of soil erosion. Moong beans are widely cultivated in India and Central Asia. It is a very rich source of protein (25%) and have high digestibility.[31] It is also a very good source of Riboflavin, Thiamine and Vitamin C.[32] Moong beans have fast germination rate and rapid growth during cultivation. Due to these abilities it is well preferred to study growth and development, physiology and other studies on plant. The present study aims to study effect of two polyamines (Spermine and Putrescine) on germination rate and growth and development and evaluation of physic-chemical parameters.

MATERIALS AND METHODS

1. Physiological parameters

1.1 Seed treatment

Fresh and healthy *Vigna* seeds were bought from a local store located in Rajkot. Before germination seeds were treated with polyamines according to method suggested by Farooq et al,

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2008[33] with slight modifications. Seeds were placed in Petri dishes (20 seeds per Petri dish) between layers of moist Whatman filter paper No. 1 wetted with three different concentrations of polyamines Spermine and Putrescine (0.01mM, 0.1mM and 01mM) and kept under dark conditions. Distilled water treatment was set as control. Four replicates of each treatment were prepared.

1.2 Seed Germination

Germination was observed daily and seeds were kept for germination for 2 days to achieve maximum sprouting.

Germination percentage was calculated using the following formula:

$$Germinationpercentage = \left(\frac{Numberofseedsgerminated}{Totalnumberofseeds}\right) x\ 100$$

1.3 Seedling emergence

Control and treated seeds were transferred on fresh Whatmann filter paper no. 1 and soaked in half strength Murashige and Skoog (MS) medium under sunlight. Photoperiod was observed as (16:8 hours) with average temperature of 27 ºC.

1.4 Leaf number

Leaf emergence was observed on day 2 after transferring the seeds into water and half strength MS medium for control and treatment, respectively. Leaf number was recorded every day.

1.5 Shoot length

Shoot length was measured with the help of scale and was recorded at every 48 hours up to 12 days from seed emergence.

2. Biochemical parameters

2.1 Protein estimation

Total soluble protein content of germinated seeds was determined according to the method described by Bradford's with modification.[34] Samples harvested for estimation were weighed and fresh weight was recorded. These samples were homogenized in 5 mL Phosphate buffer, pH 7.0.

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The crude homogenate was centrifuged at 9390xg for 20 mins at 4 ºC. The supernatant was separated and the final volume was made up to 10 mL using a phosphate buffer, pH 7.0. Out of this, 1 mL of protein extract was added to 3 mL of Bradford's reagent prepared using coomasie brilliant blue (G-250). Absorbance was measured using spectrophotometer at 595 nm using bovine serum albumin (100µg/ml) as a standard.

2.2 Chlorophyll estimation

Chlorophyll A and chlorophyll B content of leaf was determined with some modifications.[34] Samples harvested for chlorophyll A and chlorophyll B estimation were weighed and fresh weight was recorded. Samples were crushed using 100% methanol and homogenate was centrifuged at 600 xg for 10 minutes. Absorbance was measured using spectrophotometer at 666 nm for chlorophyll A content and at 653 nm for chlorophyll B content.

2.3 Statistical analysis

The homogeneity of data variance was assumed among control and treated samples was tested using Levene's test. The mean values were validated for statistical significance by one-way ANOVA. The Dunn's post hoc analysis was performed to determine significant difference between control and treatment groups at $p < 0.05$ using Past 3.26b software.[36] The figures were drawn by MS Excel 2007 software, and values in the figures and tables are the average of four replications and shown as the means and their standard deviations (SD).

3. Protein characterization using SDS-PAGE

SDS-PAGE of protein samples were performed using Laemmli, 1970.[37] Resolving gel 12 % and 5% stacking gel were used to separate the proteins at 80 volts. After run staining and destaining was performed according to method suggested by Sambrook *et al.*, 2006.[38]

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RESULTS

Physiological parameters

Seed treatment

Treated seeds were swollen and were transferred into half strength M.S. medium for further growth. Seed were kept in water for control. Seeds were kept up to 2 days.

Seed Germination

Seeds were observed for germination after 24 hours of transfer and germination percentage was calculated. After 24 hours (1 day) incubation 93 %, 89 %, 78 % and 70 % germination was recorded for distilled water control and 0.01 mM Spermine, 0.1 mM Spermine and 1 mM Spermine, respectively.

For Putrescine treatment after 24 hours (1 day) incubation germination percentage was recorded as 95 %, 89 %, 72 % and 67 % for distilled water control, 0.01 mM Putrescine, 0.1 mM Putrescine and 1 mM Putrescine, respectively.

For both the polyamines maximum germination was observed in control followed by 0.01 mM treatment concentration. It was observed that while increasing the polyamine concentration in tested range, low germination percentage was recorded after 24 hours. After further 24 hours incubation (at day 2) 100 % germination was achieved in control as well as all the treated seeds.

Seedling emergence

Germinated seeds were allowed to grow and observed for seedling emergence. First leaves were observed in seeds treated with Putrescine and spermine after 24 hours of germination, whereas first leaves in control were observed after 30 hours of germination.

Leaf number

Leaves were observed two in number and it was found to be two in number in all the seeds till the experiment was carried out up to next 10 days.

Shoot length

To check the physiological effect of polyamine treatment on *V. radiate* seeds, shoot length was measured at every 48 hours for next 10 days after seed sprouting. After sprouting cotyledons were dissociated and a set of true leaf appears on the tip of shoot (Fig. 1).

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First root appears and gradually there was increase in shoot length was observed. After 10 days of highest shoot length (14.5 cm) was observed for 0.01mM spermine treatment compared to other concentrations. There is a reduced shoot length 12.5 cm were observed for other two spermine treatments 0.1mM and 1mM, respectively. However, compared to these, highest shoot length (15.2 cm) was recorded in water control (Fig. 2).

Figure 1: Comparison of shoot length after spermine treatment at 2 days after germination.

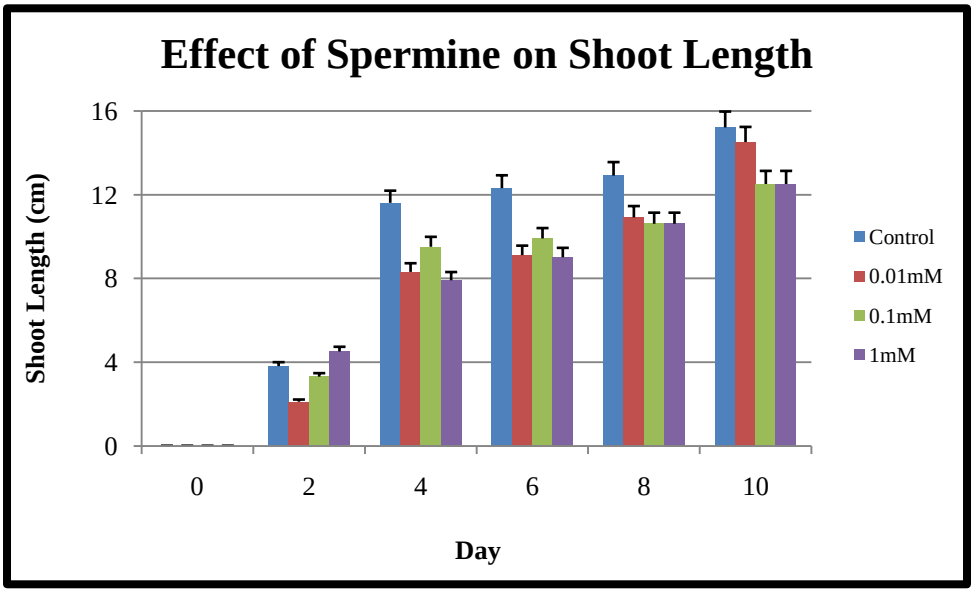

Figure 2: Effect of spermine concentrations on shoot length at various days

Shoot length was also recorded for Putrescine treatment against distilled water control. After two days of sprouting shoot length in control and all three putrescine treatments was

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recorded near to 4 cm. However, clear difference was recorded in shoot length in putrescine treatment was found at 10 days of incubation.

The shoot length was found to be 16.5 cm, 15.5 cm, 15 cm and 14.3 cm in control, 0.01 mM Putrescine, 0.1 mM Putrescine and 1 mM Putrescine treated seeds, respectively. Highest shoot length was found in control but there was no major difference in shoot length was found between seeds treated with Putrescine and distilled water control at 10 days (Fig. 3).

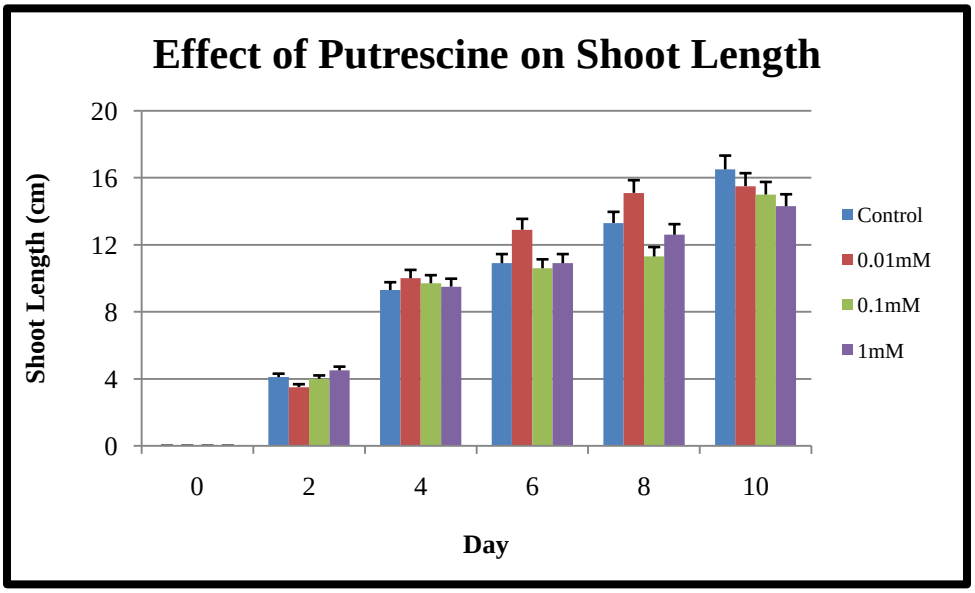

Figure 3: Effect of putrescine concentrations on shoot length at various days

The shoot length was also compared with biochemical parameters such as protein content and chlorophyll content and data was analyzed by statistical parameters.

Biochemical parameters

Protein concentration

Total protein estimation was performed using Bradford's method, where bovine serum albumin (100 µg/mL) was use as a standard. The total protein estimation was performed at every two days interval starting from sprouting and recorded up to 10^{th} day. It was observed that total protein concentration declined gradually up to 8^{th} day and there was a stability observed on 10^{th} day for both spermine (Fig. 4) and putrescine (Fig. 5) treatment.

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For spermine treatment, three treatment concentrations 0.01mM, 0.1 mM and 1 mM were tested against water control for protein concentrations. After sprouting, total protein concentration was found highest in control followed by 0.01mM, 0.1 mM and 1 mM spermine treatment. However, total protein concentration was found lower in control sample compared to spermine treatments in subsequent days (Fig. 4).

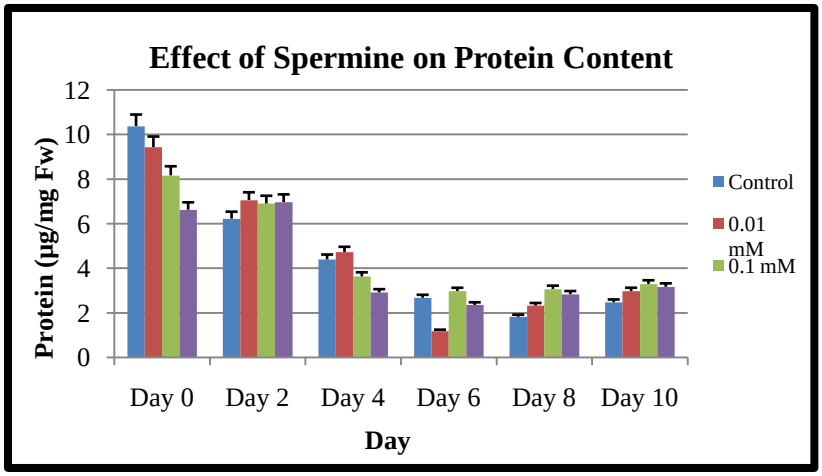

Figure 4: Effect of spermine concentrations on protein content at various days

Total protein concentration was also observed for putrescine treatments. Similar to spermine treatment three concentrations of putrescine 0.01 mM, 0.1 mM and 1 mM was tested against distilled water control. Total protein concentrations in putrescine treatments showed similar trend as spermine treatment. For putrescine treatment also protein content was found higher after sprouting which tend to decrease on subsequent days. The decline in total protein concentration in putrescine treatment was recorded up to 8th days and stability was observed on 10th day (Fig. 5).

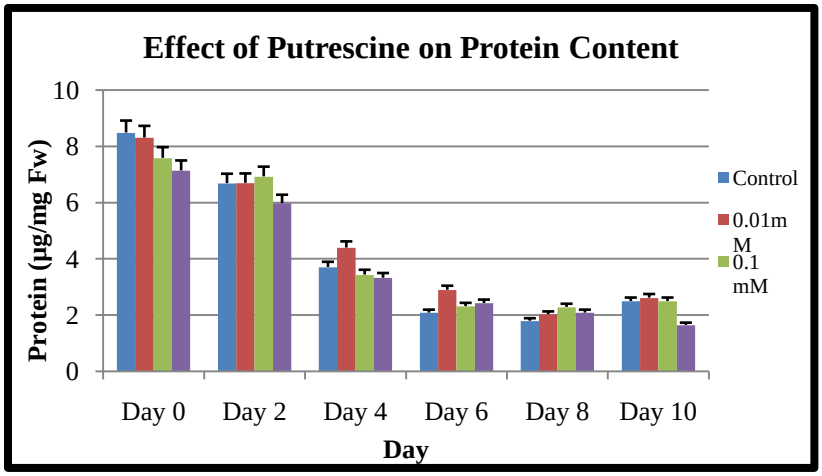

Figure 5: Effect of putrescine concentrations on protein content at various days

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Chlorophyll estimation

Chlorophyll A and B were estimated for both polyamines treatments for all three concentrations 0.01 mM, 0.1 mM and 1 mM and distilled water control. Chlorophyll A and B estimation was recorded after sprouting (Day 0) up to 10th day with 2 days of interval. Spermine treatment showed an increase in Chlorophyll A and B content with increase in spermine concentration. Chlorophyll A and B content was found increased compared to distilled water control during all the observation days. The highest chlorophyll A and B content was found for 0.1 mM spermine treatment during initial days and which became stable on 10th day (Fig. 6, 7)

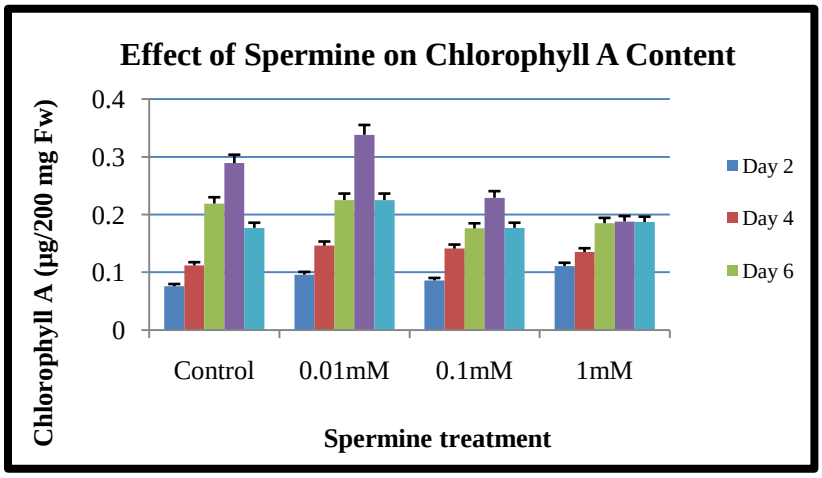

Figure 6: Effect of spermine concentrations on chlorophyll A content at various days

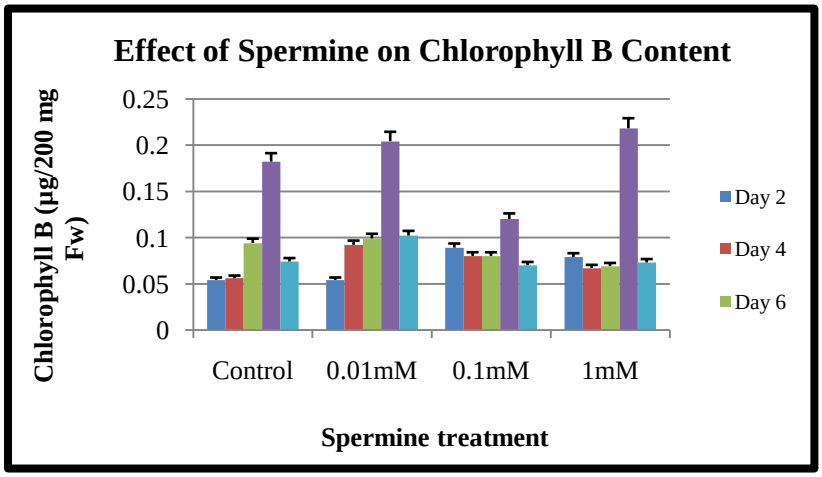

Figure 7: Effect of spermine concentrations on chlorophyll B content at various days

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Similar to spermine treatments, putrescine treatment also showed increase in chlorophyll A and B content with treatment concentration. For putrescine treatment high chlorophyll A and B content was observed for 0.1 mM treatment concentrations. For all three putrescine concentrations, chlorophyll A and B content was recorded high compared to distilled water control. Chlorophyll A and B increases after sprouting up to 8th day, however there was a stability observed on day 10th (Fig. 8, 9). The statistical analysis of Chlorophyll A and B content showed high significance $p < 0.05$.

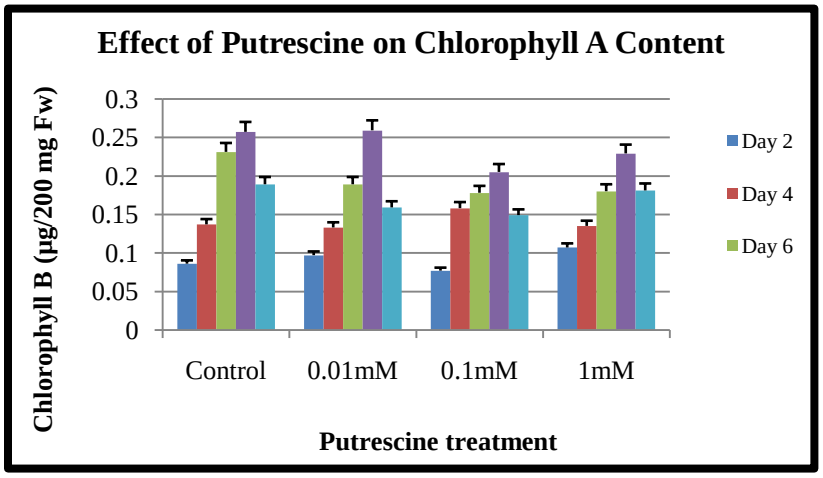

Figure 8: Effect of putrescine concentrations on chlorophyll A content at various days

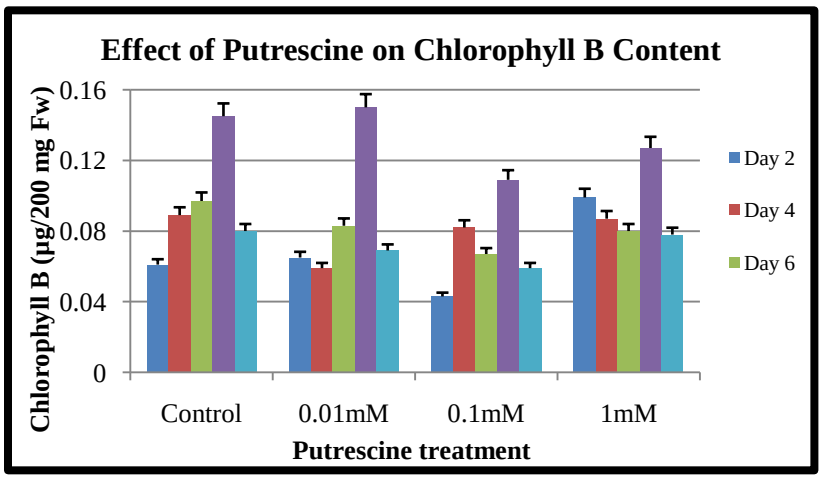

Figure 9: Effect of putrescine concentrations on chlorophyll B content at various days

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Statistical analysis

Physico-chemical parameters were statistically validated using One Way ANOVA and Dunn's Post hoc analysis. From ANOVA it was observed that there was a significant difference between and within groups (control and poyamines treated samples) at $p < 0.05$ level. From the Dunn's post hoc analysis it was observed that there was significant relationship among total protein concentration and Chlorophyll a and b content at $p < 0.05$ level (Table 1, 2, & 3). The significance was also observed between shoot length and chlorophyll a and b content. However, there was no significant relationship found between total protein content and shoot length.

Table 1: Post hoc analysis of 0.01mM treatments of spermine and putrescine against control ($p<0.05$)

	Pr. C.	Pr. S.	Pr. P.	Chl a C.	Chl a S.	Chl a P.	Chl b C.	Chl b S.	Chl b P.	Shoot L.C.	Shoot L. S.	Shoot L. P.
Pr. C.		0.984	0.995	0.033	0.052	0.035	0.004	0.010	0.004	0.761	0.956	0.759
Pr. S.	0.984		0.978	0.031	0.050	0.033	0.004	0.009	0.003	0.777	0.940	0.767
Pr. P.	0.995	0.978		0.033	0.053	0.036	0.004	0.009	0.004	0.756	0.962	0.746
Chl a C.	0.033	0.031	0.033		0.847	0.978	0.452	0.634	0.439	0.015	0.038	0.014
Chl a S.	0.052	0.050	0.053	0.847		0.868	0.344	0.503	0.334	0.025	0.059	0.024
Chl a P.	0.035	0.033	0.036	0.978	0.868		0.435	0.614	0.423	0.016	0.040	0.015
Chl b C.	0.004	0.004	0.004	0.452	0.344	0.435		0.782	0.984	0.001	0.005	0.001
Chl b S.	0.009	0.009	0.009	0.634	0.503	0.614	0.782		0.767	0.004	0.011	0.003
Chl b P.	0.004	0.003	0.004	0.439	0.334	0.423	0.984	0.767		0.001	0.004	0.001
Shoot L.C.	0.761	0.777	0.756	0.015	0.025	0.016	0.001	0.004	0.001		0.720	0.989
Shoot L.S.	0.956	0.940	0.962	0.038	0.060	0.040	0.005	0.011	0.004	0.720		0.709
Shoot L.P.	0.751	0.767	0.746	0.014	0.024	0.015	0.001	0.003	0.001	0.989	0.709	

Pr.: Protein, C: Control, S: Spermine, P: Putrescine, L: Length

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Table 2: Post hoc analysis of 0.1mM treatments of spermine and putrescine against control ($p<0.05$)

	Pr. C.	Pr. S.	Pr. P.	Chl a C.	Chl a S.	Chl a P.	Chl b C.	Chl b S.	Chl b P.	Shoot L.C.	Shoot L. S.	Shoot L. P.
Pr. C.		0.912	0.923	0.048	0.048	0.041	0.007	0.008	0.003	0.639	0.896	0.777
Pr. S.	0.912		0.836	0.061	0.061	0.053	0.009	0.012	0.005	0.562	0.809	0.694
Pr. P.	0.923	0.836		0.038	0.038	0.032	0.005	0.006	0.002	0.709	0.973	0.852
Chl a C.	0.048	0.061	0.038		1.000	0.951	0.460	0.512	0.344	0.014	0.035	0.024
Chl a S.	0.048	0.061	0.038	1.000		0.951	0.460	0.512	0.344	0.014	0.035	0.024
Chl a P.	0.041	0.053	0.032	0.951	0.951		0.499	0.553	0.377	0.012	0.030	0.020
Chl b C.	0.007	0.009	0.005	0.460	0.460	0.499		0.934	0.836	0.001	0.004	0.003
Chl b S.	0.008	0.012	0.006	0.512	0.512	0.553	0.934		0.772	0.002	0.006	0.003
Chl b P.	0.003	0.005	0.002	0.344	0.344	0.377	0.836	0.772		0.001	0.002	0.001
Shoot L.C.	0.639	0.562	0.709	0.014	0.014	0.012	0.001	0.002	0.001		0.735	0.852
Shoot L.S.	0.896	0.809	0.973	0.035	0.035	0.030	0.004	0.006	0.002	0.735		0.879
Shoot L.P.	0.777	0.694	0.852	0.024	0.024	0.020	0.003	0.003	0.001	0.852	0.879	

Pr.: Protein, C: Control, S: Spermine, P: Putrescine, L: Length

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Table 3: Post hoc analysis of 1mM treatments of spermine and putrescine against control ($p<0.05$)

	Pr. C.	Pr. S.	Pr. P.	Chl a C.	Chl a S.	Chl a P.	Chl b C.	Chl b S.	Chl b P.	Shoot L.C.	Shoot L. S.	Shoot L. P.
Pr. C.		0.858	0.989	0.036	0.046	0.040	0.004	0.005	0.007	0.699	0.907	0.756
Pr. S.	0.858		0.868	0.055	0.069	0.061	0.007	0.008	0.012	0.571	0.767	0.624
Pr. P.	0.989	0.868		0.037	0.048	0.042	0.004	0.005	0.008	0.689	0.896	0.746
Chl a C.	0.036	0.055	0.037		0.918	0.962	0.431	0.464	0.562	0.013	0.027	0.016
Chl a S.	0.046	0.069	0.048	0.918		0.956	0.373	0.404	0.494	0.017	0.035	0.021
Chl a P.	0.040	0.061	0.042	0.962	0.956		0.404	0.435	0.530	0.015	0.030	0.018
Chl b C.	0.004	0.007	0.004	0.431	0.373	0.404		0.956	0.836	0.001	0.003	0.001
Chl b S.	0.005	0.008	0.005	0.464	0.404	0.435	0.956		0.879	0.001	0.003	0.002
Chl b P.	0.007	0.012	0.008	0.562	0.494	0.530	0.836	0.879		0.002	0.005	0.003
Shoot L.C.	0.699	0.571	0.689	0.013	0.017	0.015	0.001	0.001	0.002		0.788	0.940
Shoot L.S.	0.907	0.767	0.896	0.027	0.035	0.030	0.003	0.003	0.005	0.788		0.847
Shoot L.P.	0.756	0.624	0.746	0.016	0.021	0.018	0.001	0.002	0.003	0.940	0.847	

Pr.: Protein, C: Control, S: Spermine, P: Putrescine, L: Length

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Protein characterization by SDS-PAGE

SDS-PAGE analysis of total protein was performed to observe variation in protein profile / expression in both polyamine treatments compared to distilled water control. There was not distinct band pattern was recorded after gel staining (Fig. 10). To increase the detection sensitivity the gel was also silver stained. However, poor results / resolution were achieved after silver staining (data not presented).

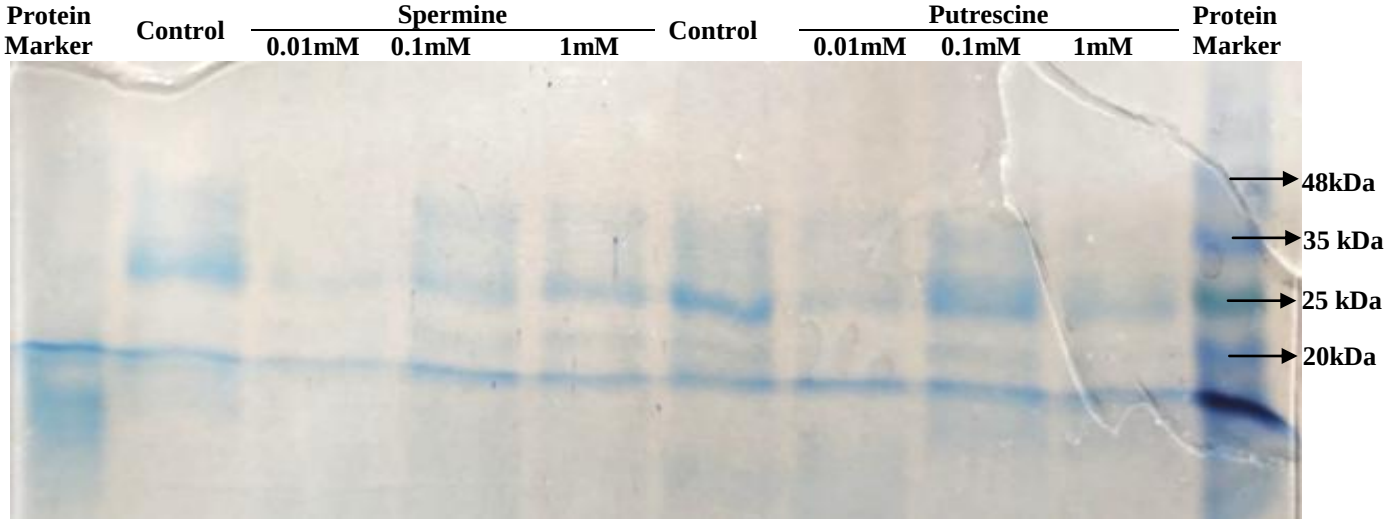

Figure 10: SDS-PAGE of protein extracted from leaf

DISCUSSION

Polyamines are important molecules that regulate several processes in plants which includes cell signaling, DNA replication and transcription and cellular division process.[39] However, the molecular interaction of polyamines with plant metabolites especially with enzymes and proteins is still under study.[40] The present study was initiated with seed treatment using various concentrations of two polyamines Putrescine and Spermine (i.e. 0.01 mM, 0.1 mM and 1 mM) along with distilled water control. Due to high viability of seeds almost 100 % germination was reported in control and treated seeds.

From seed germination study, it was observed that on the first day there were high germination percentages in low treatment concentration (0.01 mM) of spermine and putrescine. Earlier studies also suggested that low concentration of polyamines i.e. 100 µM is more preferable than the 250 µM. At low concentration polyamines can effectively modulate cellular

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process and their low concentration has least toxicity.[41] It was recorded that seedling emergence was in 0.01 mM treatment of spermine and putrescine was almost similar to control. Initially pair of leaf appears in between 24 – 48 hrs and that lasted up to 10 days of observation period.

There was a remarkable difference in shoot length and biochemical parameters (protein and chlorophyll a, b content) recorded up to 10 days. It was observed that immediately after sprouting cotyledons was detached and root development starts. Seedlings were emerging with increase in shoot length. Highest shoot length was observed in 0.01mM spermine treatment (15.4 cm) and control (16.5 cm) after 10 days (Figure 2. 3). Earlier study suggests that at high concentrations of exogenously applied polyamine has toxic effect.[42] The statistical analysis of data by One-way ANOVA and Dunn's post-hoc analysis suggest the insignificant relationship between protein content and shoot length at $p < 0.05$. Previous study by Chen D *et al*, 2019[7] and Killiny and Nehela, 2020[43] has also reported relationship between protein content and shoot length.

Along with physiological parameters two biochemical parameters i.e. total protein concentration and chlorophyll a and b content was tested to check the effect of polyamine treatment. For both the polyamines (spermine and putrescine) similar relationship was observed between and within the physic-chemical parameters (Table 1. 2, 3). Total protein concentration was measured from sprouted seeds up to 10 days at 2 days interval (Figure 4, 5). It was observed that initially protein content was found higher (8-10 µg / mg Fresh weight) in control and 0.01 mM concentrations of both polyamines which gradually decrease further up to 8^{th} day and become stable on 10^{th} day. Moreover, the chlorophyll a and b content was found low during initial days which found increased up to 8^{th} day and gradually decrease on 10^{th} day. It was observed that chlorophyll a and b content was increased at 0.01mM spermine treatment (Figure 6, 7) however, there is no concentration depended relationship found between putrescine treatment and chlorophyll a and b content (Figure 8, 9). The statistical analysis suggested that there is very significant relationship between total protein content and chlorophyll a & b content of both the polyamine treatments (Table 1, 2, & 3). The previous study suggests that polayamine prevents the loss of chlorophyll in leaf.[44] It was also observed that low concentration of polyamine had positive effect on photosynthetic activity in plant leaf[44], which further decline with protein content as senescence of leaf initiates. Previous study by Kaur-Sawhney *et al,*

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2003[40] and Besford *et al* [45] suggested that polyamines have ability to maintain thylakoid membrane by protecting negative charges.[44]

It was also proved that polyamines may enhance phytic acid degradation in mung bean seed germination.[46] Phytic acid like myo-inositol-1,2,3,4,5,6-hexakisphosphate and InsP6 are the primary storage form of phosphorus and inositol, which are preliminary required for energy metabolism.[46] It is assumed that polyamines help to make these nutrients readily available for seed developmental process. Previous studies on exogenous application of polyamines during plant stress condition resulted into reduction of sodium uptake, maintenance nutrient homeostasis, improvement of antioxidant defense.[46] It was also observed that exogenous application of polyamine results into increase of phytohormones concentrations of gibberellic acid, indole acetic acid, abscisic acid and cytokinins.[47] Further study may detail link the concentration dependent effect of polyamines with physic-chemical parameters and their relationship with metabolic changes.

For present study an attempt has been made to evaluate the changes in protein composition with the treatments of both polyamines at various concentrations. The protein profile was compared with control using SDS-PAGE (Figure 10). However, poor resolution was obtained and all the total protein bands could not be resolved. Further experimentation is required to evaluate the changes in total protein profile with polyamine treatment.

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