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Dose response curves of spermidine for water relations and photosynthetic pigments in mash bean [*Vigna mungo* (L.) Hepper]

Ghulam Yasin[1*], Ikramul Haq[2], Ali Noman[3], Khalid Hussain[4], Sundus Mumtaz[1] and Gull-e-Nokhaiz[1]

[1]Department of Botany, Bahauddin Zakariya University, Multan. **Pakistan**
[2]Institute of Biotechnology and Genetic Engineering (IBGE) University of Sindh, Jamshoro **Pakistan**
[3]Department of Botany, Govt. College University Faisalabad **Pakistan**
[4]Department of Botany, University of Gujrat, Gujrat. **Pakistan**

***Correspondence: yasingmn_bzu@yahoo.com** **Received 06-05-2020, Revised: 29-05-2020, Accepted: 30-05-2020 e-Published: 09-06-2020**

The experiment was managed for four mash bean [*Vigna mungo* (L.) Hepper] genotypes to evaluate the spermidine efficacy index for determination of its dose response curves accompanied with emphasize on genotypic variations by their response. Spermidine concentrations of 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM were tested for leaf water potential, osmotic potential, chlorophyll a, chlorophyll b and total carotenoids contents of MASH 80, MASH 88, MASH 97 and MASH ES-1. Seeds were grown in pots which were replicated four times for each concentration of spermidine in every genotype and were sit in completely randomized fashion. Plants were sprayed thrice with the said concentrations of spermidine starting from twenty days after germination with an interval of ten days each. Data were collected for pigments concentration and water relations attribute on expiry of ten days after completion of spermidine spray. The action of exogenous spermidine was significantly effective in stimulating photosynthetic pigments and water relation attributes. The lowest significant effective dose for each studied characteristic varied. MASH ES-1 and MASH 97 for chlorophyll a concentration while MASH 88 and MASH ES-1 for water potential exhibited linear expression model. All other measurements revealed a sigmoidal dose response curves. Genotype MASH ES-1 responded at the most in term of pigments concentration and water potential while MASH 80 responded to the least extent regarding most of the characteristics.

Keywords: carotenoids, chlorophyll, spermidine, osmotic potential, vigna, water potential

INTRODUCTION

Plant growth regulators has been widely applied on plants under in-vitro to in-vivo practices. The correct growth activators directly interfere with plant's hormonal system. Such bio-agents are symbolized as plant growth activators, which may be inhibited or blocked by specific inhibitors of its own biosynthesis and its receptor. Typically, the growth regulator displays their phytotoxic affects in the local to transient ways when applied externally (Wilhelm 2015).

A controversy remained among the views about plant growth regulator nature of polyamines. The concentration of polyamines in cell versus to its effective dose does not include these compounds among the plant growth regulator's list (Evans and Malmberg 1989;Galston and Kaur-Sawhney 1995). Many researchers reported the polyamines (polyamines) including the spermidine (SPD), spermine (SPM), and their obligate

precursor putrescine (PUT) them among the growth regulators of plants (Galston and Kaur-Sawhney 1990; Tiburcio et al.1993; Scoccianti et al. 2000 and Tassoni et al. 2000). Very little information is available about the polyamine's mode of action (Walden et al. 1997), while a variety of physiological processes are modulated ranging from cell multiplication and or its differentiation under plant stressed conditions positively. During the last few years, interest to use polyamine has been increasing tremendously due to its magical effects in plants growth and now they are especially used to improve the plant developmental processes in many important food crops (Chi et al.1994; Bajaj and Rajam 1996; Rajam 1997; Urszula 2014). They are synthesized in plants during stress conditions and help in various plant developmental processes (Benavides 1997; Dayadevi et al. 1994; Nag et al. 2001).

Polyamines can regulate water potential of plant by regulating stomatal opening (Galston and Sawhney 1990). The spermidine have role in regulation of stomatal movements, in general this function could also be represented common by other polyamines. For stomatal regulation, changes in turgor pressure in guard cell are regulated by many ionic channels and pumps (Ward et al. 1995). Interaction of polyamines with Ca^{2+} channels (Williams 1997) leads to ionic balance maintenance in control of water balance for regulation of growth and developmental processes (Aziz et al. 1999). Also the polyamines have been reported to promote osmotic adjustment, which helps plant to maintain turgor under stress conditions (Islam et al. 2003).

Chlorophyll concentration determines the extent of important biological phenomenon of photosynthesis which ultimately has a direct relation with plant growth and development. Chlorophylls are important plant pigments for plant primary productivity regulated through photosynthesis. Polyamines are possibly involved in the increased rate of photosynthesis through higher growth rates and high leaf chlorophyll content (Borrell et al. 1997). Besides chlorophylls, carotenoids including other coloring pigments responsible for the bright colors of various fruits and vegetables are synthesized. Polyamines application has an increasing effect on the level of carotenoids (Nassar et al. 2003).

There are many reported facts which account for variation in dose response of polyamines. Sensitivity of plant to polyamine varies depending upon many aspects. Like as various cell especially located at apexes are differentially sensitive from the applied concentrations of polyamines. Such raising or lowering the polyamine's concentrations may diversly affect the relative cell division as well as cell differentiation rates in different groups of cells or even may alter morphogenetic expression patterns (Bernier et al. 1993). Dual action of polyamine as regulator of cell death (apoptosis) and cell growth leaves an ambiguity for dose specificity (Schipper et al. 2000). Under extreme conditions, application in high concentrations of exogenous polyamine can causes cell death (Brunton et al. 1991). Whenever to conduct an experiment, it is very essential to pin out the optimal dose of polyamines. For that dose response curve is required to construct with hormonal concentration and degree of response of group of cells. Evaluation of water relations of plants for its screening against a particular external factor, are considered to be a satisfactory criteria (Schonfeld et al. 1988) to find out the threshold level of factor reducing 10% growth of plant (Edwards and Asher 1982). However, the results of most of such type of studies do not agree with each other. The reasons behind this are the experimental differences, climatic conditions, soil type, variation in nature and life cycle of crops, genotypic differences etc.

Black gram [*Vignamungo* (L.)Hepper], a self-pollinated grain legume crop is cultivated as one among the most important pulse crops widely (Nag et al., 2006). It's very cheap protein source one human for the distant areas with economic value. Based on bio-chemical analysis, seeds of mash bean contain 1-2% fats, 2.1% oil, 20-24% protein, carbohydrates and traces of vitamin A and B (James 1981). By considering the importance of mash bean [*Vignamungo* (L.)Hepper] and the variation in spermidine dose responses; the present study was designed to find out dose response curves for various exogenously applied spermidine concentrations.

MATERIALS AND METHODS

Plant growth regulator mediated regulation of plant development is dependent upon variation in cell sensitivities and its response times. Whenever an experiment is conducted on hormonal applications there a dose response curve must be constructed by keeping differential hormonal concentration against degree of plant growth responses. Hence, an experiment was devised to find out spermidine efficacy for chlorophyll a, chlorophyll b, carotenoids contents, leaf water potential and leaf osmotic potential of four

[*Vignamungo*(L.)Hepper] genotypes to evaluate the expression of various dose response curves for exogenous spermidine

2.1 Materials

Seeds of four mash genotypes i-e MASH 80, MASH 88, MASH 97 and MASH ES-1 were used in the experiment. The seed of these genotypes were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan). The origin of these genotypes are Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan) and National Agricultural Research Centre (NARC),Islamabad (Pakistan). Spermidine, N-[3-Aminopropyl]-1,4-butanediamine,($C_7H_{19}N_3$) of Sigma Aldrich, Japan was used as plant growth regulators.

2.2 Methods and layout plan

Experiment was designed with complete randomization of treatments and genotypes to avoid unequal exposure of environmental factors. Each dose was repeated 4-times in pots experiment. For the conduction of experiment, pots of 30 cm diameter were used. Each earthern-pot filled with 10 kg soil (sandy loam) which were lined with polyethylene bags ensuring seepage prevention. Pots were arranged in completely randomized design. Sterilized seeds, with similar morphology (size &weight) of selected genotype cultivated and germinated. Weeds were uprooted from time to time by hand weeding and hoeing in order to avoid weed crop competition. Thinning was performed to maintain one seedling in each pot in order to avoid the imbalanced uptake of nutrients by plants. Insects and pests were control by foliar spray of Thiodon insecticides of Hoechst (Pvt) Ltd, Pakistan. After reviewing the published data, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM concentration of spermidine were selected in addition to control conditions of distilled water spray. Solutions of spermidine in respective concentrations were prepared in estimated (pre determined by trial method) amount of water by taking the great care of their half life. Plants were exposed to first spray of PGRs after twenty days of germination repeated twice after each fifteen days with a great care of avoiding falling of drops of solution from leaf surface. The tween-20 used as surfactant with 0.1 concentration for foliar spray.

2.3 Data recordings

Chlorophyll (a,b),and Total Carotenoids Contents (mg g-1 leaf F. wt)

Pigments contents, after ten days of last spray, were measured by applying procedure and formula reported by Arnon (1949). Fresh leaves were extracted with acetone (80%). The OD was taken at 645nm and 663nm for chlorophyll a,b and at 480nm for carotenoids on spectrophotometer (Hitachi Model-U 2001,Japan). Chlorophyll contents were calculated according to the Lichtenthaler (1987) formulae and carotenoids contents were calculated after Davis, (1976).

Chl a(mg g^{-1})=[12.7(OD663)-2.69(OD645)]× V/1000×W.

Chl b(mg g^{-1})=[22.9(OD645)-4.68(OD663)]× V/1000×W.

Carotenoids(mg g^{-1})=[Acar/EM] × 1000.

Where

Acar=OD480+0.114(OD663)-0.638(OD645); EM(100%)=2500; OD=Optical density; V=Volume of sample; W=Weight of sample.

Leaf water potential [Ψw; -MPa]

Fully expanded youngest leaf was utilized for predawn leaf water potential (Ψw) measurements using a pressure chamber (ARIMAD 2-Japan) after ten days of spermidine spray. Water potential Ψw was measured at noon from 11:00am to 01:00pm (Fischer et al. 1977) as Ψw remains stable at this time period.

Osmotic potential of Leaf [Ψs; -MPa]

Fresh leaf was folded in aluminum foil and frozen @ -20^{o}C. After 7-days, it was thawed. The cell sap was extracted by using disposable syringe. This extracted cell sap was subjected for the measurement of leaf osmotic potential with osmometer (Wescor, 5500).

2.4 Statistical analysis

The data collected were analyzed for ANOVA (analysis of variance) for data of all selected parameters with COSTAT computer based package (CoHort Software,Berkeley,CA). To compare means, Duncan's New Multiple Range (DNMR) test @ 5% level of probability was used (Duncan 1955). Significant F values were tested by LSD tests at 0.05% significance level, by using MSTAT-C Computer Statistical Programme (MSTAT Development Team, 1989)

RESULTS

Chlorophyll a contents (mg g^{-1} leaf F. wt)

Statistical analysis of Duncan's Multiple Range test (Table: 1) depicts that increasing concentration of spermidine appeared to be responsible for gradual increase in chlorophyll a contents the significant being by the effects of 1.00 to 1.50mM concentrations. Although not statistically justified, but to a considerable extent, the variations in chlorophyll contents were dose dependent in various genotypes. Of the four

genotypes, three revealed sigmoidal pattern and others one revealed linear pattern for spermidine effects. Maximum effect in MASH 80 and MASH 88 was by 1.25mM but in MASH 97 and MASH ES-1 same was by 1.50mM concentration. However, the observations were excluded irregularly from the on going trends by the application of some lower concentrations thereby decreasing chlorophyll a contents than control plants (Figure 1). Among the genotypes, MASH 88 revealed maximum (1.0103) and MASH ES-1 revealed minimum (1.002) and all the genotypes differed to statistically non significant extent.

Chlorophyll b contents (mg g^{-1} leaf F. wt)

Statistical approach by Duncan's Multiple Range test (Table: 2) shows that an exponential relationship occurred between chlorophyll b contents and applied spermidine. Spermidine established a statistically marked degree of induction for chlorophyll b increase at all levels of its concentrations except 0.50mM. As implies the mean performance data, maximum increase in chlorophyll b contents (50.000%) was by 1.00 mM and 1.25 while the highest level of spermidine concentration was less effective than this level revealing a sigmoidal curve for spermidine action (Figure 2). Maximum effect on MASH 80 and MASH 97 was by 1.00 mM while on MASH 88 and MASH ES-1 was by 1.25mM. All the genotypes differed statistically to a non-significant extent. The genotype, MASH 88 revealed maximum (0.078) and MASH 80 revealed minimum (0.070) values.

Table 1: Chlorophyll a Contents (mg g^{-1} leaf F. wt) of 50 days old mash [*Vignamungo*(L.) Hepper] exposed to three shoot system sprays of spermidine concentrations (0, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM) at 20 to 40 days of age

Spermidine (mM)	MASH 80	MASH 88	MASH 97	MASH ES-1	TREATMENTS MEANS (LSD=0.037 ;n=16)
	(n=4)				
Distilled water	0.972±0.051	0.997±0.040	0.976±0.031	0.949±0.054	0.973b±0.044
0.25	0.985±0.077 (1.319)	0.962±0.087 (-3.510)	0.969±0.080 (-0.717)	0.970±0.080 (2.212)	0.971b±0.073 (-0.205)
0.50	1.004±0.050 (3.292)	1.011±0.062 (1.404)	0.993±0.049 (2.236)	0.991±0.046 (4.425)	1.000ab±0.047 (2.774)
0.75	0.995±0.063 (2.366)	1.014±0.017 (1.705)	1.023±0.031 (4.815)	1.005±0.025 (5.900)	1.009ab±0.036 (3.699)
0.100	1.012±0.027 (4.115)	1.015±0.032 (1.805)	1.013±0.025 (3.790)	1.020±0.020 (7.480)	1.015a±0.024 (4.316)
1.25	1.048±0.066 (7.818)	1.057±0.067 (6.018)	1.002±0.065 (2.663)	1.039±0.069 (9.483)	1.039a±0.062 (6.783)
1.50	1.040±0.040 (6.995)	1.033±0.038 (3.610)	1.033±0.038 (5.840)	1.040±0.041 (9.589)	1.036a±0.035 (6.478)
GENOTYPES MEANS →	1.008±0.056	1.013±0.055 (-0.490)	1.003±0.049 (0.496)	1.002±0.056 (0.545)	1.006±0.053
	(LSD=0.028 ; n=28)				

[Values represent means ± SE].Values in parentheses represent % age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments.

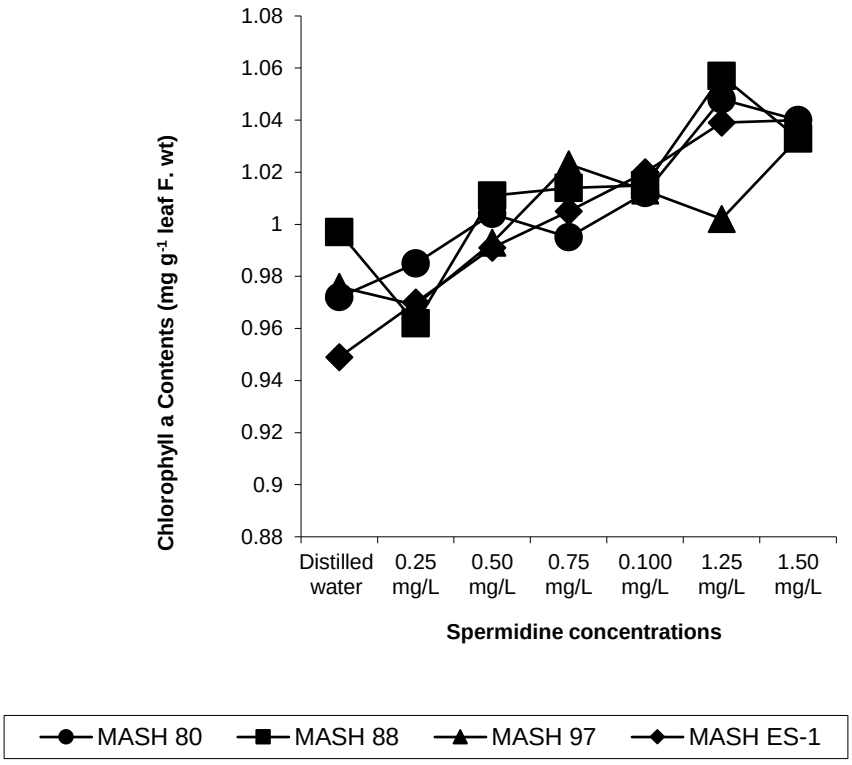

Figure 1: chlorophyll a contents (mg g^{-1} leaf f. Wt) of 50 days old mash [*Vignamungo*(L.) Hepper] exposed to three shoot system sprays of spermidine at 20 to 40 days of age

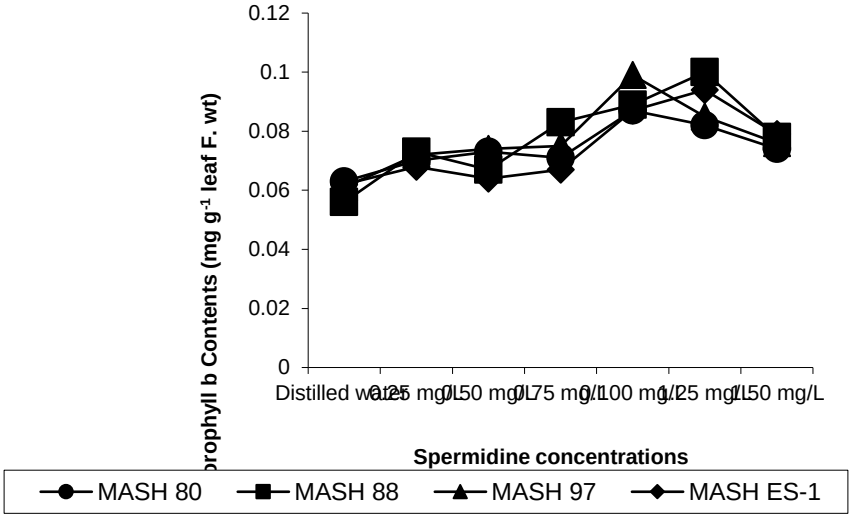

Figure 2: chlorophyll b contents (mg g^{-1} leaf f. Wt) of 50 days old mash [*Vignamungo*(L.) Hepper] exposed to three shoot system sprays of spermidine at 20 to 40 days of age

Table 2: Chlorophyll b Contents (mg g^{-1} leaf F. wt) of 50 days old mash [*Vignamungo*(L.) Hepper] exposed to three shoot system sprays of spermidine concentrations (0, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM) at 20 to 40 days of age

Spermidine (mM)	MASH 80	MASH 88	MASH 97	MASH ES-1	TREATMENTS MEANS (LSD=0.009 ;n=16)
	(n=4)				
Distilled water	0.063±0.015	0.056±0.016	0.061±0.003	0.062±0.010	0.060c±0.011
0.25	0.070±0.018 (11.111)	0.073±0.024 (30.357)	0.072±0.013 (18.032)	0.068±0.021 (9.677)	0.071b±0.017 (18.335)
0.50	0.073±0.021 (15.873)	0.067±0.010 (19.642)	0.074±0.004 (21.311)	0.064±0.007 (3.225)	0.069bc±0.011 (15.000)
0.75	0.071±0.015 (12.698)	0.083±0.026 (48.218)	0.075±0.008 (22.950)	0.067±0.015 (6.200)	0.074b±0.016 (23.333)
0.100	0.087±0.006 (38.095)	0.089±0.001 (58.928)	0.099±0.011 (62.295)	0.087±0.005 (40.322)	0.090a±0.008 (50.000)
1.25	0.082±0.006 (30.158)	0.100±0.014 (78.571)	0.085±0.004 (39.344)	0.094±0.008 (51.612)	0.090a±0.011 (50.000)
1.50	0.074±0.008 (17.460)	0.078±0.007 (39.285)	0.076±0.003 (24.590)	0.079±0.003 (27.419)	0.077b±0.005 (28.333)
GENOTYPES MEANS	0.0748±0.014	0.078±0.019 (-11.428)	0.077±0.013 (-10.000)	0.075±0.015 (-7.142)	0.076±0.015
	(LSD=0.006 ; n=28)				

[Values represent means ± SE].Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments.

Table 3: Total Carotenoids Contents (mg g^{-1} leaf F. wt) of 50 days old mash [*Vignamungo*(L.) Hepper] exposed to three shoot system sprays of spermidine concentrations (0, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM) at 20 to 40 days of age

Spermidine (mM)	MASH 80	MASH 88	MASH 97	MASH ES-1	TREATMENTS MEANS (LSD=0.008 ;n=16)
	(n=4)				
Distilled water	0.050±0.010	0.050±0.012	0.049±0.001	0.050±0.011	0.050b±0.010
0.25	0.056±0.013 (12.000)	0.046±0.009 (-8.000)	0.055±0.017 (12.244)	0.055±0.018 (10.000)	0.053 b±0.014 (6.000)
0.50	0.065±0.002 (30.000)	0.057±0.005 (14.000)	0.047±0.006 (-4.081)	0.050±0.005 (0.000)	0.054b±0.008 (8.000)
0.75	0.054±0.008 (8.000)	0.066±0.006 (32.000)	0.053±0.010 (8.163)	0.055±0.013 (10.000)	0.057ab±0.010 (14.000)
0.100	0.060±0.004 (20.000)	0.053±0.013 (6.000)	0.070±0.009 (42.857)	0.079±0.013 (58.000)	0.058ab±0.012 (16.000)
1.25	0.052±0.013 (38.540)	0.073±0.018 (46.000)	0.068±0.012 (38.776)	0.067±0.018 (34.000)	0.065a±0.016 (30.000)
1.50	0.061±0.008 (22.000)	0.062±0.003 (24.000)	0.070±0.011 (42.857)	0.066±0.011 (32.000)	0.065a±0.009 (30.000)
GENOTYPES MEANS →	0.057±0.010	0.058±0.013 (-1.750)	0.059±0.014 (-3.508)	0.056±0.014 (1.754)	0.057±0.012
	(LSD=0.006 ; n=28)				

[Values represent means ± SE].Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments.

Table 4: Leaf water potential [Ψw; -MPa] of 50 days old mash [*Vignamungo*(L.)Hepper] exposed to three shoot system sprays of spermidine concentrations (0, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM) at 20 to 40 days of age

Spermidine (mM)	MASH 80	MASH 88	MASH 97	MASH ES-1	TREATMENTS MEANS (LSD=0.060 ;n=16)
	(n=4)				
Distilled water	0.666±0.041	0.621±0.067	0.531±0.082	0.663±0.162	0.620 d ±0.105
0.25	0.681±0.045 (2.252)	0.607±0.055 (-2.254)	0.582±0.032 (9.604)	0.725±0.082 (9.351)	0.649 cd ±0.078 (4.677)
0.50	0.671±0.057 (0.750)	0.784±0.041 (26.247)	0.650±0.125 (22.410)	0.666±0.041 (0.452)	0.693 bc ±0.086 (11.774)
0.75	0.732±0.082 (9.909)	0.736±0.143 (18.518)	0.644±0.100 (21.280)	0.749±0.011 (12.971)	0.715 b ±0.96 (15.322)
0.100	1.094±0.140 (64.260)	0.844±0.084 (35.909)	0.771±0.054 (45.197)	0.797±0.123 (20.211)	0.839 a ±0.116 (35.322)
1.25	0.937±0.054 (40.690)	0.828±0.062 (33.333)	0.773±0.016 (45.574)	0.840±0.083 (26.696)	0.844 a ±0.093 (36.129)
1.50	0.898±0.075 (34.834)	0.970±0.057 (56.199)	0.772±0.102 (45.386)	0.866±0.045 (30.618)	0.876 a ±0.098 (41.290)
GENOTYPES MEANS →	0.790 a ±0.140 (-2.597)	0.770 a ±0.139	0.675 a ±0.123 (12.337)	0.758 b ±0.111 (1.558)	0.748±0.134
	(LSD=0.045 ; n=28)				

[Values represent means ± SE].Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments.

Table 5: Leaf osmotic potential [Ψs; -MPa] of 50 days old mash [*Vignamungo*(L.)Hepper] exposed to three shoot system sprays of spermidine concentrations (0, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM) at 20 to 40 days of age

Spermidine (mM)	MASH 80	MASH 88	MASH 97	MASH ES-1	TREATMENTS MEANS (LSD=0.054 ;n=16)
	(n=4)				
Distilled water	0.992±0.067	1.004±0.041	1.03±0.162	0.899±0.082	0.981 c ±0.102
0.25	0.978±0.055 (-1.411)	1.019±0.045 (1.494)	1.077±0.78 (4.563)	0.920±0.049 (2.335)	0.999 bc ±0.79 (1.834)
0.50	0.98±0.015 (-1.209)	1.009±0.047 (0.498)	1.040±0.071 (0.970)	1.039±0.034 (15.572)	1.017 bc ±0.049 (3.669)
0.75	0.997±0.013 (0.504)	1.007±0.090 (0.298)	1.052±0.043 (2.135)	0.945±0.046 (5.116)	1.000 bc ±0.063 (1.936)
0.100	0.956±0.097 (-3.629)	1.076±0.072 (7.171)	1.095±0.42 (6.310)	1.097±0.030 (22.024)	1.056 ab ±0.084 (7.645)
1.25	1.059±0.170 (6.754)	1.077±0.076 (7.270)	1.112±0.057 (7.961)	1.066±0.098 (18.576)	1.079 a ±0.099 (9.989)
1.50	1.082±0.043 (9.072)	1.11±0.091 (10.557)	1.128±0.139 (9.514)	0.98±0.022 (9.010)	1.077 a ±0.098 (9.785)
GENOTYPES MEANS →	1.006 bc ±0.085	1.044 ab ±0.074 (-0.037)	1.076 a ±0.091 (-6.958)	0.992 c ±0.088 (1.391)	1.030±0.090

[Values represent means ± SE].Values in parentheses represent %age increase (+)/decrease (-) over untreated of row#1 or over MASH 80 for genotypes means. Values followed by dissimilar letters, are different at P = 0.05 among means of treatments.

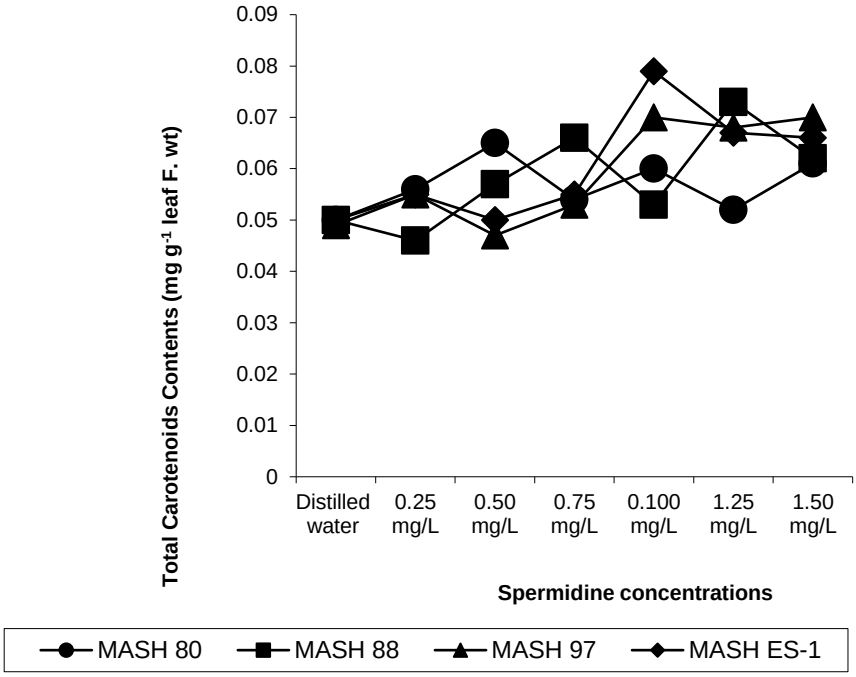

Figure 3: Total carotenoids contents (mg g^{-1} leaf f. Wt) of 50 days old mash [*Vignamungo*(L.) Hepper] exposed to three shoot system sprays of spermidine at 20 to 40 days of age

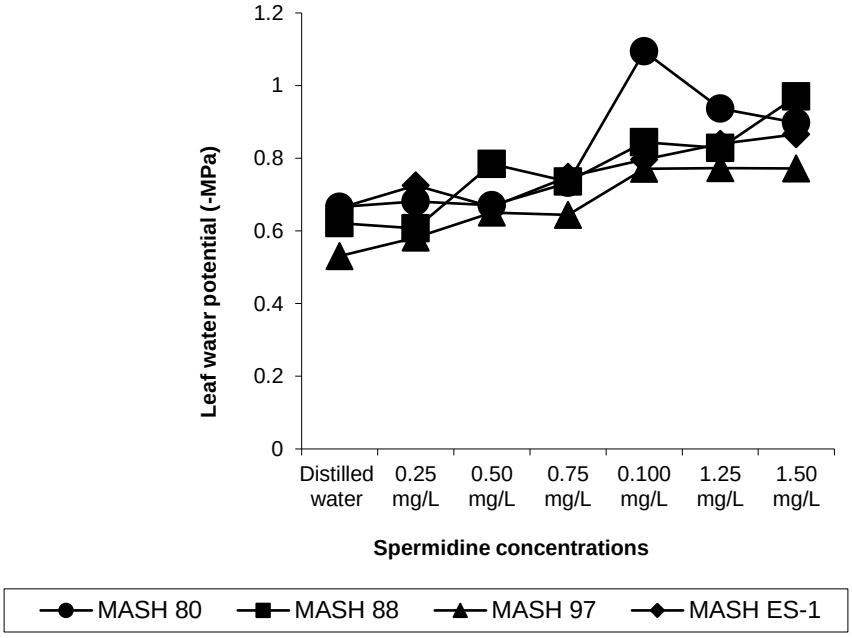

Figure 4: Leaf water potential of 50 days old mash [*Vignamungo*(L.) Hepper] exposed to three shoot system sprays of spermidine at 20 to 40 days of age

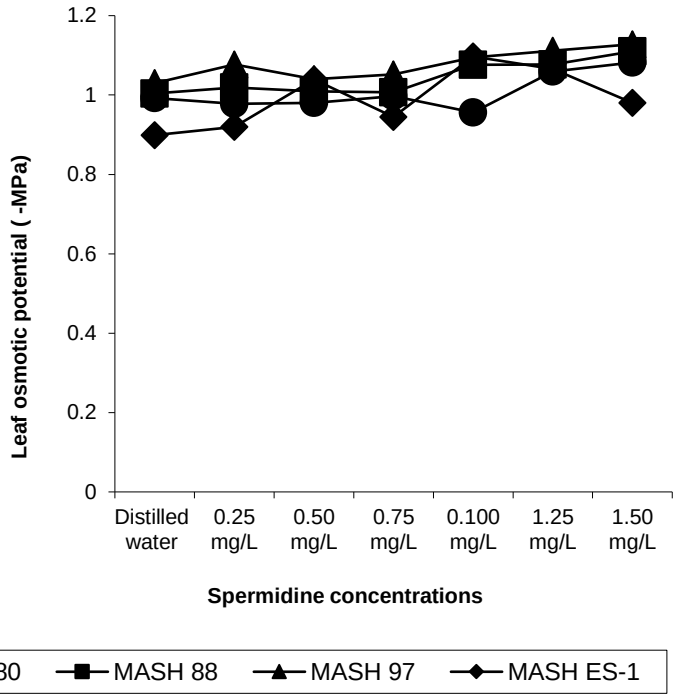

Figure 5: Leaf osmotic potential of 50 days old mash [*Vignamungo*(L.) Hepper] exposed to three shoot system sprays of spermidine at 20 to 40 days of age

Total Carotenoids contents (mg g^{-1} leaf F. wt)

According to statistical data presented in Table: 3, foliar spray of spermidine accelerated the contents of total carotenoids. Application of 1.25mM and 1.50mM spermidine exerted statistically important function in induction of carotenoids increase. Generally, maximum (30.000%) increase was documented by the application of 1.25mM and 1.50mM spermidine and minimum (6.000%) by 0.25mM level. Saturation effect in MASH 80 and MASH 88 was by 1.25mM while in MASH 97 and MASH ES-1 by 1.00 mM 1.50mM also in MASH 97.this reflects that in term of carotenoids contents spermidine have both linear and sigmoidal curves for its actions (Figure 3). Some exclusions also were randomly noted from the ongoing trend of carotenoids increase with escalating level of spermidine. All the genotypes differed to statistically non-significant extent. The genotypes, MASH 97 revealed maximum (0.059) and MASH ES-1 revealed minimum (0.056)

Leaf water potential [Ψw; -MPa]

Exogenous spray of spermidine, exponentially and significantly amplified water potential (Table: 5). An exception to this was observed when the plants of 0.25mM treatment failed to maintain the vagueness and revealed no significant induction of spermidine. Maximum effect was in diversified manner. In MASH 88 and MASH ES-1, it was by 1.50mM. In MASH 80 by 1.00 mM and in MASH 97 by 1.25mM concentrations revealing both sigmoidal as well as linear expression curves (Figure 4). Spermidine role deviated from the logical expectation of water potential rise when applied in concentration of 0.25mM in plants of MASH 88 reflecting a 2.254% reduction from control plants. Among the genotypes, MASH 80 revealed maximum (0.790) and MASH 97 revealed minimum (0.675) value. Only MASH ES-1 differed statistically from rest of the genotypes.

Leaf osmotic potential [Ψs; -MPa]

Spermidine concentrations, according to Duncan's Multiple Range tests (Table: 5), established a significant stimulation for osmotic potential of plants when sprayed at concentrations of 1.00 to 1.50mM and this induction was maximum (9.989%) by 1.25mM dose and minimum (1.834%) by 0.25mM level. Maximum effect in all the genotypes was by 1.50mM but in MASH ES-1 was by 1.00 mM concentration. Of

the four genotypes, MASH ES-1 exhibited a sigmoidal curve of its response to spermidine in term of osmotic potential increment (Figure 5). The augmentation of increase in osmotic potential by spermidine could not have a pace in plants of MASH 88 when sprayed with lower concentrations. Among the genotypes, MASH 97 revealed maximum (1.076) and MASH ES-1 revealed minimum (0.992) value while the rest of the genotypes revealed intermediate response.

DISCUSSION

In this experiment, foliar application of spermidine increased chlorophyll contents. Induction of chlorophyll increase by spermidine might be due to prevention of the losses chlorophyll with thylakoid membrane stabilization. A positive correlation between prevention of chlorophyll loss and preservation of thylakoid membrane structure has been reported with polyamines and other plant growth regulators (Anderson and Rowan 1966; Biswal and Mohanty 1976; Dennis et al. 1967). Chlorophyll loss and membrane stability are related processes during leaf senescence. At the time of leaf senescence, the increases in proteinase activity have observed (Martin and Thimann 1972; Peterson and Huffakar 1975). Proteinase destabilizes the thylakoid membrane and process might be responsible for chlorophyll loss. Since both structural and functional integrity of chloroplast membranes are affected with inorganic cations (Argyroudi et al 1977; Arntzen and Ditto 1976; Murakami et al. 1975; Smillie et al.1976), meanwhile, the anionic binding sites on thylakoid membranes are already reported by various groups (Nakatani et al. 1978; Prochaska and Gross 1977). The cationic binding with the negatively charged loci on the membranes could be preserving the morphology of thylakoids and chlorophyll. As the polyamines are cationic in nature which may also synthesized within plant cells occasionally (Cohen and Zalik 1978). The mechanistic regulations and intracellular distribution of polyamines are yet have unassumed role over structural and functional properties of chloroplasts.

In the experiment, foliar application of spermidine increased carotenoids contents. An increase in pigments, like carotenoids, has also been reported earlier by exogenous application of plant growth regulator (Gowdu and Nayudu 1989). Similarly, green forage of barley and pea, when treated with plant growth regulators, had been reported to have greater contents of carotenoids than control (Averyna et al. 1989). Little is known about the mechanisms of the enzymes involved in carotenoids biosynthesis (Bartley and Scolnik 1995) in plants. However, it is known that the biosynthetic precursor proteins, after translational process, are transferred to plastids which is a site of carotenoids biosynthesis. The mRNA level for earlier steps in carotenoids biosynthesis is dependent on the plant developmental stage and signals from environmental conditions (Bartley and Scolnik 1995). Plant growth regulator has been reported to stabilize the transcription which might be a reason for carotenoids contents stability (Thomas et al. 1992). Carotenoids degradation might be controlled by thylakoid membrane stability. Gadallah (1995) found that application of plant growth regulator increased the stability of membranes. Spermidine and spermine are reported to stabilize the DNA through bridging the major and minor DNA grooves (Matthews 1993). On the basis of structural studies, it is indicated that polyamines have individual interacts rather than multiple DNA sites (Tabor and Tabor, 1985). Polyamines also lead to trigger expressions of growth regulatory loci i.e. c-myc (Hampel et al. 199; Celano et al. 1992).

The results of this experimental revealed that spermidine enhanced water potential of leaf. An improvement in leaf water potentials by PGRs has been published by many groups (Lefevre and Lutts 2000; Islam et al. 2003). Polyamine mediated enhanced water potential water might be due to increased water uptake by root owing to an increased surface area of root for greater water absorption. The exogenous polyamines applications on many plant species shows the positive plant growth promotion (Krizek et al. 1997). They can act like to hormonal activity on cell division as its rate was reported to increase at G_2-cell phase and even prior to M-phase (Bettuzzi et al. 1999). Polyamines enhance growth by increasing chlorophylls (a, b) and carotenoids contents and ultimately photosynthesis (Zheleva et al. 1994).

Another possible mechanism for root growth promotion which accounts for increased water uptake is membrane stability. Polyamines acts as a bridge element among the membrane as well as cytoskeletal-network (Wyse and Butterfield 1988), and impart on membranal rigidity (Munro and Sauerbier 1973 and Tabor 1960). Polyamine regulate stomatal aperture for maintenance of water potential. Polyamine levels induce stomatal closure by modeling stomatal aperture (Galston and Kaur- Sawhney 1990). The regulation of stomatal movements by spermidine is considered

as the general function of almost all polyamines in plants. Even the changes turgor of guard cell turgor is controlled by various ionic channels and or pumps to instigate the stomatal movements (Raschke et al. 1988; Hedrich and Schroeder 1989; Ward et al. 1995). Interactions of polyamines with Ca^{2+} channels (Williams 1997; Nichols and Lopatin 1997 and Johnson 1996) leading to maintain the cellular ionic balance in plant growth and development has also been reported (Aziz et al. 1999).

Deviants for sigmoidal curves

The deviation from logical augmentations of linear correlation in spermidine role is ascribed to various aspects (Figure 1-5). Polyamines concentration and specificity is thought to be critical on many plant developmental processes i.e. cell division rate, root growth, floral initiation, somatic embryogenesis and development of fruit in various plant species (Evans and Malmberg 1989; Galston and Kaur-Sawhney 1990). Another reason might be that metabolism of polyamines in plants changes and the intensity and direction of these changes depend on the genotype of the plant as well as on the type, concentration and duration of the effect of the stress factor if present (Bouchereau et al. 1999; Kubis 2006). Attachments of spermidine to a specific protein (Cohen et al. 1982) leading to post-translationally modification of protein might change the effective concentration of polyamine with generation of a protein with a different morphogenetic role. Variation in efficiency of spermidine might be dependent on presence and stability of transglutaminase as spermidine binding to protein is mediated by transglutaminase (Williams et al. 1980).

In addition to transglutaminase, other enzymes with polyamine-binding activity have been reported in plant tissue (Icekson and Apelbaum, 1987; Serafini et al, 1988). The data of current study don't have direct evidences about the spermidine binding to cell proteins which is mediated via transglutaminase, but possibility is consistent.

Polyamines treatment prevents chlorophyll loss and preserves thylakoid membrane structure this might be through their interaction with membranous negatively charged loci (Nakatani et al, 1978; Prochaska and Gross, 1977), while sometimes this structural integrity could be maintained or accompanied by inactivation of thylakoid. For explanatory evidences are explainable with analysis of differential proteolysis of thylakoids which are required for stability and functioning of chlorophyll. There increases in proteinase activity occurs at the time of leaf senescence (Martin and Thimann, 1972; Peterson and Huffakar, 1975). Another possible reason for that application of high dose polyamines may not have positive effects on plant as accumulation of free cellular spermidine could be a symbol of plant growth and development. The polyamines in the conjugated forms are the valuable source under plant stressed conditions for their active forms (Tonon et al. 2007).

CONCLUSION

The optimum dose of spermidine for studied characters was proven at the rate of 1.25 mM.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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We acknowledge the cooperation of lab staff who cooperated in a number of ways for conduction of experiment. Dose response curve was sigmoidal for spermidine action.

AUTHOR CONTRIBUTIONS

GY and IH designed experiment; AN and KH worked for manuscript writing; SM and GN performed statistical analysis and proof reading of manuscript.

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