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Does Spermidine Always Act as Stimulant for Enzyme Action and Yield Attributes as Diagnostic Criteria in *Vigna Mungo* Genotypes?

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ABSTRACT

An experimental approach was managed for four mash bean, *Vigna mungo* genotypes in quest of finding the kinetics of spermidine action accompanied with findings of response variations among the crop genotypes. Plant nitrate reductase activity, number of legumes plant^{-1}, number of grains plant-1, total yield plant^{-1} were evaluated against spermidine concentrations of 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM. Four genotypes i.e., MASH 80, MASH 88, MASH 97 and MASH ES-1 were grown in earthen pots filled with homogenized loamy soil. Pots by number were replicated four times for each concentration of spermidine in every genotype and were arranged completely in randomized design. Plants were sprayed thrice with the said concentrations of spermidine starting from twenty days after germination with an interval of ten days each. Nitrate Reductase Activity (NRA) was measured in leave on expiry of nine days after completion of spermidine spray while yield plant^{-1} and its contributing factors were recorded at physiological maturity of crop. Spermidine mediated stimulation in Nitrate Reductase Activity (NRA), yield plant^{-1} and yield contributory factors. The most effective concentration for all the characteristics was 1.25 mM to which all genotypes responded in a similar fashion. All the genotype exhibited sigmoidal expression pattern with an exception of MASH 80 for legumes development phenomenon. MASH 88 responded in the best way except for number of grain plant-1 for which MASH 97 was the most suitable and the least responsive of the genotypes was MASH ES-1 in all the cases studied.

KEY WORDS: SPERMIDINE, KINETICS, ENZYME, YIELD, VIGNA.

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INTRODUCTION

The polyamines (PAs) are nitrogenous compounds with low molecular weight that mediate the triggering of many developmental events of plants, (Alcázar et al. 2010; Ahmad et al., 2012; Aguria et al.2017). The PAs are protective in nature against the damaging effects of abiotic stress in tissues (Puyang et al., 2015; Pal et al., 2015; Wang et al.2019). Their presence and involvement has been reported in the key plant biological functions i.e. membrane stabilization, enzyme activation, protein synthesis, ROS scavenging, hormonal profile regulation and mineral uptake (Ahmad et al., 2012; Hu et al 2012; Puyang et al 2016; Li et al. 2016). Among the polyamines, the most known are putrescine (diamine), spermidine (both triamine and tetra-amine). These are ubiquitously existed among the plants with having crucial role in the statues of plant physiology (Fang, 2019).

Mainly three forms of these compounds have been observed in plant cells i.e. Spd (tri-amine spermidine),and SPm (tetra-amine spermine) and Put (di-amine putrescine), each of them vagrantly observed either present as free and insoluble bound form or soluble conjugated forms. With soluble forms, these form the covalently conjugated small molecules with different phenolics. Meanwhile, insoluble PAs are found to attach covalently with macromolecules in the cell with nucleic acids and polypeptides (Gill and Tuteja, 2010). The polycationic nature of these PAs can remain bounded along the cell wall, proteins, phospholipids and nucleic acids to increase their stability in the cells, (Ahmad et al. 2012).

The potential and gradient stress influences on the PAs biosynthesis has been reported by different groups (Hu et al. 2012: Puyang et al. 2016).The balanced level of polyamine is required by the cell for finely to attain good physiological state for which different organisms are potentially evolving optimum homeostatic mechanisms either through efficient catabolism, uptake and transport for biosynthesis of polyamine. For this aspect, the biosynthetic enzymes including the ODC (ornithine decarboxylase and S-adenosylmethionine decarboxylase are regulated at optimal level during transcriptional and post-transcriptional stages. In particular, these among animals and plants, the translation of S-adenosylmethionine decarboxylases are subject to negative feedback (Ivanov et al., 2010; Kovacs, 2020).

The exogenous supplement of PAs could be involved to check on rate of plant growth inhibition to minimize the ROS (remove reactive oxygen species) phenomena. Under high levels of endogenous PAs accumulation may be involved in protection of enzyme system under abiotic stressed conditions (Nahar et al. 2016 Chen et al. 2019). Exogenous provision of polyamines to plants helps them to grow and yield better. Spermidine application induced stimulatory effect and improved plant height, number of leaves, root length, biomass, seed number (size and weight of seeds per plant), oil quantity, endogenous IAA content, content of chlorophyll, reducing and non-reducing sugars, total carbohydrates and total proteins (Gul et al. 2020).

Inspite of protective role of polyamines, these are also signaling agents of the cells and tissue. Its exogenous provision to plants remains crucial in terms of dose and time of applications (Pal et al. 2019). In addition to exogenous application, endogenous level of polyamines is involved to regulate the levels of plant growth regulators from time to time. Endogenous putrescine is also closely related with IAA and gibberellin (GA), while putrescine and spermidine at high levels are not conducive for IAA and GA accumulation (Xu, 2015). The black-gram or Mash bean, *Vigna mungo* is one among the most important legume crops in Asia. This crop has high value for being used in food, fodder and /or green manure. When harvested and degraded, it is also used as a leftover additive for soil fertility, and its foremost quality is, being a cheap source of protein for diet of human beings as it is sufficiently rich in proteins (20.8–30.5%) and carbohydrates (56.5–63.7%) (Sharma et al. 2012).

The task of cultivation of mash bean is variant either mainly as pulse crop for nutrition or as green manure for the enrichment of soil fertility. Like other legumes, mash bean also develops the relationship with several symbiotic nitrogen fixing microbial cells. It has potential to fix-up 37–83 kg ha^{-1} nitrogen approximately (Mohammad et al. 2010). Keeping in view the crucial role of polyamines regarding their dose and time of application; their role in controlling the endogenous level of growth regulators, the present project was designed to evaluate the dose response cures of spermidine for Mash genotypes.

MATERIAL AND METHODS

Plant growth regulator mediated regulation of plant development is dependent upon variation in cell sensitivities and response times. Whenever an experiment is conducted on hormone-doses, it is urgent to develop different levels of the doses and to construct the curve of dose-response to the degree of growth. Hence, a pot experiment was devised to find out kinetics of enzyme action and yield parameters in four black gram [*Vigna mungo* (L.) Hepper] genotypes to evaluate the expression of various dose response curves for exogenous spermidine in term of number of legumes plant-1, number of grains $fruit^{-1}$, total yield plant-1(g) and Nitrate Reductase (EC 1.6.6.1) Activity (NRA).

Materials: Seeds of four mash genotypes i.e., MASH 80, MASH 88, MASH 97 and MASH ES-1 were obtained from Pulse Section of Ayub Agricultural Research Institute, Faisalabad Pakistan. Spermidine (N-[3-Aminopropyl]-1,4-butanediamine, (C7H19N3) obtained from Sigma Aldrich, Japan was applied as plant growth activator. The selected genotypes were collected from institute of their

origin Ayub Agricultural Research Institute, Faisalabad Pakistan and NARC (National Agricultural Research Centre) Islamabad Pakistan.

Methods and layout plan: This experiment was designed with complete randomization of treatments and genotypes to avoid unequal exposure of environmental factors. Each treatment was repeated ten times by pots and plants. Pots of 30 cm diameter were used. Each pot was filled with 10 kg sandy loam soils and lined with polyethylene bags ensuring seepage prevention. Sterilized seeds, similar in size and weight, of each genotype were germinated. After germination, 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.

Plants were irrigated with normal irrigation water. By reviewing the published data concentrations of spermidine selected for experiment were as 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mM in addition to control conditions of distilled water spray (Khoshbakht et al,2018).. 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, temperature and other environmental hazards which cause the denaturation of PGRs solution. 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. Tween-20 (0.1%) has mixed as surfactant for the spermidines foliar application (Yousefi et al. 2019).

Data recordings: Nitrate reductase activity of four plants of each treatment was determined on the expiry of ten days after last spermidine spray by using the method of Sym, (1984) method.by using spectrophotometer (Hitachi-220), while Nitrate reductase activity was estimated according to the formula:

Nitrate Reductase Activity=Graph reading × Dilution factor × O.D of sample (µmol NO_2/h/g FW)

Yield and its contributing factors were studied at the maturity of crop (80 days age). Number of legumes plant^{-1}, number of grains fruit-1and total yield plant^{-1}(g) were determined. For randomly selected four plants per treatment in each genotype.

Statistical analysis: The collected data of this experiment analyzed for ANOVA (analysis of variance) with COSTAT computer package (CoHort Software, Berkeley, CA). The DMR (Duncan's New Multiple Range) test applied at 5% level of probability (Ducan, 1955) to compare means. The individual mean difference was tested with LSD tests (0.05% significance level). The significant F obtained by using MSTAT-C Computer Statistical Programme (MSTAT Development Team, 1989).

Table 1. Number of legumes plant-1 of mash [*Vigna mungo* (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 [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 and genotypes (lower case letter) as well as among interactions (upper case letters).

Spermidine (mM)	MASH 80	MASH 88 (LSD=2.726 ;n=4)	MASH 97	MASH ES-1	TREATMENTS MEANS (LSD=1.362 ;n=16)
Distilled water	23.00±1.678 [GHI]	25.500±1.568 [DEFG]	23.500±3.502 [FGHI]	22.000±2.860 [I]	23.500 d ±2.626
0.25	22.000±1.440 (-4.347) [I]	26.000±1.718 (1.960) [CDEF]	24.000±1.440 (2.127) [FGHI]	22.000±1.940 (0.000) [I]	23.500 d ±2.262 (0.000)
0.50	22.834±1.482 (-0.721) [GHI]	25.000±2.952 (-1.960) [EFGH]	24.000±1.632 (2.127) [FGHI]	22.500±1.754 (2.272) [HI]	23.582 d ±2.090 (0.348)
0.75	26.164±1.750 (13.756) [CDEF]	27.164±1.472 (6.525) [CDE]	25.000±1.382 (6.382) [EFGH]	23.000±0.862 (4.545) [GHI]	25.332 c ±2.034 (7.795)
0.100	27.000±1.274 (17.391) [CDE]	30.000±3.568 (17.647) [AB]	27.000±1.522 (14.893) [CDE]	26.164±2.134 (18.927) [CDEF]	27.540 b ±2.560 (17.191) []
1.25	30.830±1.370 (34.043) [AB]	32.164±2.134 (36.868) [A]	28.330±1.388 (20.553) [BC]	28.664±1.884 (30.290) [BC]	29.998 a ±2.244 (27.651)
1.50	32.664±1.634 (42.017) [A]	28.164±1.666 (10.447) [BCD]	26.164±1.750 (11.336) [CDEF]	24.000±1.440 (9.090) [FGHI]	27.748 b ±3.608 (18.076)
GENOTYPES MEANS →	26.356 b ±4.130	27.712 a ±3.174 (-5.144)	25.426 b ±2.410 (3.528)	24.046 c ±2.912 (8.764)	
			(LSD=1.030 ; n=28)		

RESULTS

Number of legumes plant^{-1}: According to the results of DMR test as in Table 1, the exogenous spermidine substantially altered legume development and its action was pertinent in stimulating the enhancement of legume number. This action of spermidine was not statistically justified to all levels of its application. Foliar spray of 0.75 to 1.50mM was proved to be a significantly potent factor in their effect. Generally, maximum (27.651%) elevation in legume number was conducive to the sincere thanks of 1.25mM concentration. This trend corresponded to individual genotypic response also except for MASH 80 for which this definitive relationship could not occur and maximum effect was by 1.50mM dose. From the data, it could be inferred that some of the lower spermidine concentrations, though none significantly, but impaired with the ongoing trend for spermidine action (Figure 1). Among the genotypes, MASH 88 revealed maximum (13.856) and MASH ES-1 revealed minimum (12.023) other genotypes lying between the two.

Number of grains fruit^{-1}: Exogenous spermidine concentrations of 1.00 to 1.50mM, according to data from DMR test in Table 2 were significant in affecting the development of grain. A definitive positive proportionate relationship occurred between grain development and spermidine concentrations in all genotypes. The greatest promise, if the term may be used, in this fashion, in enhancing grain number is of 1.25mM spermidine which raised the grain number to maximum (15.591%) extent. But MASH ES-1 showed such saturation effect by 1.00 mM spermidine. The highest level of spermidine concentration showed its effect lower than this and established a sigmoidal pattern of expression for spermidine action (Figure 2). The trend of increase in grain number was, however, not reflected when 0.50mM foliar spray of spermidine was experiences on plants of MASH 80 where a slight reduction of 2.478% than control plants was observed. Among the genotypes, MASH 97 revealed maximum (8.325) and MASH ES-1 revealed minimum (7.543) other genotypes lying between the two.

Total yield plant^{-1}(g): According to data from DMR test in Table 2, it was observed that yield increased under the stimulus of spermidine and generally, in all the genotypes, spermidine effect evidently had a significant pivotal role when applied in concentration range of 0.75 to 1.50mM. Statistically no remarkable variation in yield was assessed upto 0.50mM level proving it to be a susceptable dose.

Table 2: Number of grains fruit-1 of mash [*Vigna mungo* (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 [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 and genotypes.

Spermidine (mM)	MASH 80	MASH 88 (n=4)	MASH 97 (n=4)	MASH ES-1	TREATMENTS MEANS (LSD=0.427 ;n=16)
Distilled water	7.657±0.717	7.345±0.421	7.795±1.098	7.015±0.903	7.453b±0.799
0.25	7.757±0.767 (1.305)	7.277±0.434 (0.925)	8.377±0.749 (7.466)	7.267±0.416 (3.592)	7.670b±0.722 (2.911)
0.50	7.492±.345 (-2.478)	7.522±0.544 (2.409)	8.062±0.286 (3.425)	7.082±0.268 (0.955)	7.540b±0.493 (1.167)
0.75	7.805±1.103 (1.932)	7.492±0.578 (2.001)	8.252±0.576 (5.862)	7.492±0.575 (6.799)	7.760b±0.739 (4.119)
0.100	8.650±0.534 (12.968)	8.512±0.666 (15.888)	8.492±0.420 (8.941)	8.082±0.268 (15.210)	8.434a±0.493 (13.162)
1.25	8.832±0.608 (15.345)	8.760±0.555 (19.264)	8.857±0.593 (13.624)	8.012±0.301 (14.212)	8.615a±0.595 (15.591)
1.50	8.262±0.181 (7.901)	8.647±0.274 (17.726)	8.437±0.405 (8.236)	7.852±0.978 (11.931)	8.300a±0.580 (11.364)
GENOTYPES MEANS →	8.065ab±0.762	7.936b±0.767 (1.599)	8.325a±0.773 (-3.223)	7.543c±0.648 (6.472)	7.967±0.762
	(LSD=0.3239 ; n=28)				

Plant exposure to 1.25mM concentration revealed the strongest impact of spermidine on yield in all the genotypes proving it to be optimum concentration. This gradual trend of yield enhancement was not observed under the exogenous application the highest spermidine concentration (Figure 3). Some limitations in spermidine effects reside in the 0.25mM and 0.50mM dose for MASH 80 as reduction of 3.340% and 3.118% respectively from control plants were recorded. Among the genotypes, MASH 88 was the most productive (5.542) and MASH

ES-1 was the least productive (4.502).Only MASH 97 differed statistically from rest of the genotypes.

Nitrate Reductase (EC 1.6.6.1) Activity (NRA): The data in Table 4, according to Duncan's Multiple Range test reveals, spermidine amplified Nitrate Reductase Activity (NRA) exponentially in a concentration dependent manner. Statistically significant impacts were laid down by concentration range from 0.75 to 1.50mM. The most promising and much more significant increase (27.609%) appeared to occur by 1.25mM concentration. This fact was solidified when individual genotypic response to same level of concentration was observed to be of maximum value. The highest concentrations could not have a pace with the dose dependent exponential amplification of Nitrate Reductase Activity (NRA) and deviated from expected linear expression model of spermidine action (Figure 4). Not obscure, but a negative, relationship was noted regarding 0.25mM and 0.50mM concentration application on plants of MASH ES-1 which yielded a reduction of 1.844% and 1.270% respectively from control. Of the genotypes, MASH 88 revealed maximum (9.587) and MASH ES-1 revealed minimum (8.421) value MASH 97 was similar to MASH 88 and MASH 80 behaved like MASH ES-1 in response.

Table 3. Total yield plant-1(g) of mash [*Vigna mungo* (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 [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 and genotypes.

Spermidine (mM)	MASH 80	MASH 88 (n=4)	MASH 97	MASH ES-1	TREATMENTS MEANS (LSD=0.821 ;n=16)
Distilled water	8.980±1.122	9.530±0.588	9.410±2.328	7.844±1.316	8.940 e ±1.490
0.25	8.680±0.836 (-3.340)	9.670±0.640 (1.469)	10.294±1.194 (8.472)	8.140±0.488 (3.773)	9.196 de ±1.142 (2.863)
0.50	8.700±0.178 (-3.118)	9.550±0.648 (0.209)	9.854±0.974 (3.835)	8.130±0.558 (3.646)	9.062de ±0.914 (1.364)
0.75	10.460±2.080 (16.481)	10.380±1.206 (8.919)	10.240±0.520 (7.903)	8.544±0.260 (8.924)	9.916 cd ±1.374 (10.805)
0.100	11.910±1.188 (32.628)	13.080±2.354 (37.250)	10.604±0.948 (11.738)	10.734±0.538 (36.792)	11.582 b ±1.644 (29.552)
1.25	13.870±0.674 (54.454)	14.384±1.650 (50.933)	12.770±0.728 (34.562)	11.604±0.866 (47.934)	13.156 a ±1.452 (47.158)
1.50	12.534±1.296 (39.576)	11.004±1.394 (15.466)	10.614±1.474 (11.844)	8.030±0.674 (2.371)	10.546 c ±2.012 (17.964)
GENOTYPES MEANS →	10.734a ±2.220	11.084 a ±3.700 (-3.260)	10.540 a ±1.522 (1.807)	9.004 b ±1.570 (16.117)	10.340±2.036
	(LSD=0.620 ; n=28)				

Experimental results revealed increase in yield and its attributes by foliar application of spermidine (Table 1-3). The increase in yield components may be ascribed to promotion of growth and developmental process of plants by exogenous applied spermidine. There are reports that polyamines perform vital role to improve plant growth, (Alsokari, 2011). Spermidine imparts maximal beneficial effects on the growth of plant, (Schaller et al. 2014; Ahanger et al. 2020). Improved growth in spermidine treated seedlings has been reported earlier (Nahar et al. 2016). The observed high growth rate is directed by the applied phytohormonal impact on enhancement of photosynthesis of plants. Spermidine application proved beneficial in improving photosynthesis, (Ahanger et al. 2020). With the increase in photosynthesis rate, it directly increases the plant growth rate as well as production of metabolite and high energy status ultimately, (Galili et al. 2016). Increase in photosynthesis might be due to more chlorophyll synthesis. The spermidine leads to increase the precursors or intermediates of pigments with the decrease in activities of the chlorophyllases, (Nahar et al. 2016 Ahange et al. 2018)., When the biosynthesis of photosynthetic pigments are increased than it directly influences the rate of absorption of essential mineral ions as well as regulation of transpiration and or stomatal characteristics. Both of above optimal forms lead to enhance the plant production efficiency. However, the application of spermidine enhances the stomatal conductance and improved uptake of nitrogen. Increased nitrogen uptake leads to enhance the greater Rubisco generation.

At the same time, stomatal conductance remains functional to keep balanced internal concentrations of CO_2 and water for the purpose to maintain internal temperature, (Khan et al. 2016). Thus it means that

the exogenous spermidine application inhibits the degradation of chlorophyll contents, while it enhances structural stabilization of chloroplasts (Li et al. 2016). For instance, Hu et al. (2016) have reported that on the application of exogenous spermidine application increases the D1 protein synthesis on decline of photosynthesis at chloroplast. Increase in yield contributing factors might be due to more flower differentiation and controlled flower senescence or fruit post ripening process, (Gupta et al. 2019).

The differentiation of floral buds is triggered by Polyamines (Xu, 2015). There inhibition in differentiation of floral buds due to different inhibition factors which are dropped down by application of spermidine as same is reported by Xu, (2015). It has also been observed that polyamines are found in higher abundant in flowers than other plant organs. Further supply of exogenous Polyamines significantly enhances the flower development in poorly flowering plants significantly (Applewhite et al. 2010) According to Duan et al. (2006), the reduction in concentration of polyamines is a positive signal for senescence occurrence. The application of Spd and Spm increases the polyamines in the cut flowers as well as to delay senescence with the improvement in quality (Yang and He, 2001; Cao, 2010). This delayed senescence appeared due to inhibition of ethylene biosynthesis by polyamines (Woo et al. 2013; Anwar et al. 2015). The delayed leaf senescence is associated with level of concentration of Spermidine in the tissues and reduced ROS status as reported by Sobieszczuk-Nowicka (2017). Flower senescence may be reduced due to production of antioxidants and reduction of ROS generated during stress (Ahange et al. 2018).

Table 4. Nitrate Reductase (EC 1.6.6.1) Activity (NRA) of 50 days old mash [Vigna mungo (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 [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 and genotypes (lower case letter) as well as among interactions

Spermidine (mM)	MASH 80	MASH 88 (LSD=0.819 ;n=4)	MASH 97	MASH ES-1	TREATMENTS MEANS (LSD= 0.410;n=16)
Distilled water	7.885±0.645 [JKL]	8.795±0.277 [EFGHI]	8.685±1.209 [FGHIJ]	7.48±0.579 [KL]	8.211e ±0.883
0.25	8.1±0.237 (2.726) [HIJKL]	8.865±0.374 (0.795) [EFGH]	9.16±0.660 (5.469) [DEF]	7.342±0.437 (-1.844) [L]	8.366 de ±0.835 (1.887)
0.50	8.072±0.274 (1.800) [HIJKL]	8.822±0.299 (0.306) [EFGH]	8.942±0.487 (2.959) [EFG]	7.385±0.087 (-1.270) [L]	8.305 de ±0.708 (1.144)
0.75	8.292±0.124 (5.161) [GHIJK]	9.22±0.564 (4.832) [DEF]	9.137±0.258 (5.204) [DEF]	8.227±1.005 (9.986) [GHIJK]	8.719 cd ±0.713 (6.186)
0.100	9.372±0.253 (18.858) [DEF]	10.547±1.176 (19.920) [AB]	9.292±0.474 (6.989) [DEF]	8.977±0.510 (20.013) [EFG]	9.547 b ±0.875 (16.270)
1.25	9.85±0.360 (24.920) [BCD]	11.347±0.692 (29.016) [A]	10.462±0.438 (20.460) [B]	10.252±0.533 (37.058) [BC]	10.478 a ±0.733 (27.609)
1.50	8.002±0.373 (1.483) [IJKL]	9.515±0.689 (8.186) [CDE]	9.317±0.746 (7.276) [DEF]	9.287±0.632 (24.157) [DEF]	9.030 c ±0.835 (9.974)
GENOTYPES MEANS →	8.510 b ±0.795	9.587 a ±1.096 (-1.056)	9.285 a ±0.796 (-13.846)	8.421 b ±1.182 (-10.260)	8.951±1.091
	(LSD=0.310 ; n=28)				

The application of putrescine (Nahar et al 2016) has been observed to reduce the H_2O_2 and O_2 accumulations. The exogenous application of Spermidine reduces the break-down of chlorophyll and increases the structural stabilization of chloroplast (Li et al. 2016). According to Nahar et al. (2016) the reduction in the activities of lipoxygenases is due to the applications of putrescine. the Lipoxygenase produces unsaturated molecules of hydro-oxidized fatty acid with the addition of oxygen to poly-unsaturated fatty acids. Which can lead to produce acyclic or cyclic compounds after oxidation of fatty acid for the stabilization of cell membrane (Porta and Rocha-Sosa, 2002). This stabilization of membrane is increased with spermidine applications which is involved to maintain their increased activities of antioxidant (Hu et al. 2020). Various antioxidant enzymes are performing differential roles while have different locations in the cells or tissues (Ahmad et al.2010). Nahar et al. (2016) and Li et al. (2015) reported that on application of spermidine lead to up-regulation of antioxidants.

Exogenous application improved the activity of nitrate reductase (Table 4). The nitrate reductases involved in the limitation of N metabolism thereby controlling the key pathway of N-metabolism which includes amino acid or other N-containing secondary metabolic agents (Ahanger et al. 2017). Similarly, Bashri et al. (2018) and Khalil et al. (2017) also observed elevation in the activities of nitrate

reductases with the cadaverine and Kn applications. The increase in activities of N-reductase results into improved N containing compounds biosynthesis (Khalil et al. 2017). It leads to increase the cellular protein biosynthesis and tolerance against applied stresses (Iqbal et al. 2015). The enhancement in nitrate reductase, and nitrogen rich compounds with spermidine directly involved in regulation of photosynthetic enzymes as well as other protective compounds biosynthesis, (Ahanger et al. 2020).

Furthermore, increase in K concentrations with spermidine is directly involved to increase the plant growth because of its involvement in regulation of activities of enzyme of photosynthesis, (Ahanger and Agarwal, 2017; Ahanger et al. 2017, Ahanger et al. 2020). The results revealed some deviations from the general exhibited trend in spermidine effects at high concentrations. The deviations from the augmentations for the spermidine role might be ascribed for the facts like free polyamines concentrations varies within a species also (Reggiani et al. 1992), environmental factors (Kubis, 2006), attachments of spermidine with other molecules (Gill and Tuteja, 2010).

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