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Does Spermidine Always Act As Stimulant: Kinetics for Enzyme Action and Yield Attributes as Diagnostic Criteria in Four [*Vigna mungo* (L.) Hepper] Genotypes

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ABSTRACT

An experimental approach was managed for four mash bean [*Vigna mungo* (L.) Hepper] genotypes in quest of finding the kinetics of spermidine action accompanied with findings of response variations among 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 1.25mM 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 97was at the most. 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

Polyamines (PAs) are group of low molecular weight nitrogenous compounds that mediate several plant developmental events (Aguria et al.2017; Ahmad et al., 2012; Alcázar et al. 2010). Polyamines (PAs) are commonly known to have protective effects on abiotic stress (Puyang et al., 2015; Pal et al., 2015; Wang et al.2019). Their involvement in key plant processes such as membrane stabilization, protein synthesis, enzyme activation, ROS scavenging, mineral uptake, and hormonal profile regulation has been reported (Ahmad et al., 2012; Hu et al 2012; Puyang et al 2016; Li et al. 2016).

Among the common occurring Polyamines are diamine putrescine, triamine spermidine, and tetra-amine spermine, existing ubiquitously in plants and playing a crucial role in plant physiological status (Fang, 2019). These compounds mainly exist in three forms in plant cells, diamine putrescine (Put), triamine spermidine (Spd), and tetraamine spermine (Spm), each of which may be present in a free, soluble conjugated or insoluble bound form. Soluble conjugated forms, such as phenolic compounds, are covalently conjugated to small molecules, whereas insoluble bound forms are covalently bound to macromolecules, such as nucleic acids and proteins (Gill and Tuteja, 2010). Due to their polycationic nature, Polyamines can interact with proteins, nucleic acids, phospholipids and cell wall components, leading to their stabilization (Ahmad et al. 2012). The differential influence of stresses on the PA metabolism has been reported (Hu et al. 2012: Puyang et al. 2016).

To adjust polyamine levels finely to the levels required by the physiological state of the cell, various organisms have evolved homeostatic mechanisms involving polyamine biosynthesis, catabolism, transport, and uptake. Among the biosynthetic enzymes, S-adenosylmethionine decarboxylase and ornithine decarboxylase (ODC) are highly regulated at the transcriptional and post-transcriptional level. In particular, both animal and plant S-adenosylmethionine decarboxylases are subject to translational negative feedback regulation (Ivanov et al., 2010; Kovacs, 2020).

The application of exogenous PAs can check the inhibition of growth, remove reactive oxygen species (ROS), increase the level of endogenous PAs, protect the activity of the enzyme system under abiotic stress (Chen et al. 2019; Nahar et al. 2016). Exogenous application of polyamines to plants helps them to grow and yield better. Spermidine application induced stimulatory effect and improved Plant height, root length, number of leaves, fresh and dry biomass, seed number and weight per plant), oil quantity, endogenous IAA content, content of chlorophyll, reducing and non-reducing sugars, total carbohydrates and total proteins (Gul et al. 2020).

Besides their protective role, polyamines also serve as signalling molecules. The exogenous application of signaling molecules is crucial in term of dose and time of application (Pal et al. 2019). In addition to exogenous application, endogenous level of polyamines is found to be closely related to regulate the plant growth regulators. Endogenous Putrescine was found to be closely related to IAA and gibberellin (GA) contents, and high levels of Putrescine and Spermidine were not conducive for the accumulation of IAA and GA (Xu, 2015).

Mash bean [*Vigna mungo* (L.) Hepper], black gram, is an important legume crop grown in Asia. It has great value as food, fodder and green manure. In addition to improving the soil fertility, it is a cheap source of protein for direct human. It has easily sufficient protein content (20.8–30.5%) and carbohydrate (56.5–63.7%) on dry weight basis (Sharma et al. 2012). Mash bean is mainly grown as a pulse crop. Sometimes it is grown as a green manure to improve soil fertility. Like other legumes, mash bean also possesses the ability for establishing a symbiotic relationship with the nitrogen fixing bacteria Mash bean can fix approximately 37–83 kg ha^{-1} of nitrogen through this symbiotic association (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 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. So in any experiment where a hormone is being used, it is important to construct a dose response curve by varying the concentration of hormone and observing the degree of response. Hence, a pot experiment was devised to find out kinetics of enzyme action and yield attributes in four mash bean [*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), Nitrate Reductase (EC 1.6.6.1) Activity (NRA). Seeds of four mash genotypes i.e., MASH 80, MASH 88, MASH 97 and MASH ES-1 were obtained from Pulse Section, Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan). Spermidine, N-[3-Aminopropyl]$^{-1}$, 4-butanediamine, ($C_7H_19N_3$) of Sigma Aldrich, Japan was used as plant growth regulators. The genotypes have their origin in Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan) and National Agricultural Research Centre (NARC) Islamabad (Pakistan).

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%) was used as a surfactant for foliar spray, Yousefi et al. 2019). 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.using spectrophotometer (Hitachi-220).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. The data were analyzed for analysis of variance using COSTAT computer package (CoHort Software, Berkeley, CA). Duncan's New Multiple Range test at 5% level of probability (Duncan, 1955) was used to compare means. Differences between individual means were tested by LSD tests at 0.05% significance level where significant F values were obtained by using MSTAT-C Computer Statistical Programme (MSTAT Development Team, 1989).

RESULTS

Number of legumes plant^{-1}: According to Duncan's Multiple Range test (Table: 1), 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 non significantly, but impaired with the ongoing trend for spermidine action (Table 1). Among the genotypes, MASH 88 revealed maximum (13.856) and MASH ES^{-1} revealed minimum (12.023) other genotypes lying between the two.

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)		

Number of grains fruit^{-1}: Exogenous spermidine concentrations of 1.00 to 1.50mM, according to Duncan's Multiple Range test (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 (Table 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): Duncan's Multiple Range test (Table: 3), showed that yield increased under the stimulus of spermidine and generally, in all the genotypes, spermidine effect evidently could have 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	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.

DISCUSSION

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 spermidine. It has been reported that Polyamines play 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 was reported earlier (Nahar et al. 2016). Increased growth following application of phytohormones results from their impact on photosynthesis. Spermidine application proved beneficial in improving photosynthesis (Ahanger et al. 2020).

Increased photosynthesis directly influences the growth and metabolite production, ultimately increasing the energy status (Galili et al. 2016). Increase in photosynthesis might be due to more chlorophyll synthesis.Spermidine application has been reported to increase the synthesis of chlorophyll intermediates and decrease chlorophyllase activity (Ahange et al. 2018; Nahar et al. 2016). The increased synthesis of photosynthetic pigments and rate of photosynthesis is directly influenced in turn by the significant impact on the uptake of key mineral ions and the regulation of stomatal characteristics.The application of Spermidine improved Nitrogen uptake and stomatal functioning. Increased Nitrogen uptake contributes to greater Rubisco generation, while stomatal functioning maintains internal CO2 concentration and water, thereby leading to temperature maintenance (Khan et al. 2016). In addition, exogenous Spermidine application reduces chlorophyll degradation and enhances the stabilization of chloroplast structures (Li et al. 2016). Hu et al. (2016) have demonstrated increased D_1 protein synthesis in chloroplasy and amelioration of the decline in photosynthesis due to the exogenous treatment of Spermidine.

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	MASH 97 (n=4)	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)				

Increase in yield contributing factors might be due to more flower differentiation and cotrolled flower senescence or fruit post ripening process (Gupta et al. 2019). Flower bud differentiation is triggered Polyamines (Xu, 2015). There are many reports that have shown that exogenous application Polyamines and Polyamine synthesis inhibitors affect flower bud differentiation. Exogenous Polyamines accelerate the process of flower bud differentiation and maintenance of flower bud (Xu, 2015). Polyamines were found to be more abundant in flowers than in any other organ, and the addition of exogenous Polyamines to poorly flowering plants are reported to significantly enhance flowering response (Applewhite et al. 2010).

A reduction in Polyamines content is signal for senescence (Duan et al. 2006). Exogenous Spd and Spm treatments can increase the Polyamines content in cut flowers, and delay their senescence and improve their quality (Yang and He, 2001; Cao, 2010). Delayed leaf senescence was found to be associated with a higher spermine level and reduced reactive oxygen species (ROS) level (Sobieszczuk-Nowicka, 2017). Polyamines appeared to delay senescence by inhibiting ethylene biosynthesis (Woo et al. 2013; Anwar et al. 2015).

Flower senescence may be reduced due to production of antioxidants and reduction of ROS generated during stress (Ahanger et al. 2018). The application of putrescine (Nahar et al. 2016) has been reported to reduce the accumulation of H_2O_2 and O_2. The exogenous application of Spermidine reduces chlorophyll degradation and enhances the stabilization of chloroplast structures (Li et al. 2016). Nahar et al. (2016) have also reported reduced lipoxygenase activity due to putrescine application. Lipoxygenase generates unsaturated fatty acid hydroperoxide by adding molecular oxygen to polyunsaturated fatty acids and can also produce excess acyclic or cyclic compounds due to fatty acid oxidation thereby stabilizing membrane (Porta and Rocha-Sosa, 2002). Such increased membrane stability due to the application of Spermidine may be due to the maintenance of increased antioxidant activity in them (Hu et al. 2020). Different antioxidant enzymes have specific roles and share different locations within cells (Ahmad et al.2010). Nahar et al. (2016) and Li et al. (2015) have also reported up-regulation of the antioxidant system due to the treatment of Spermidine.

Exogenous application improved the activity of nitrate reductase (Table 4). Nitrate reductase mediates the rate-limiting step in N metabolism, thereby regulating the key metabolic pathways including the amino acid and N-containing secondary metabolites (Ahanger et al. 2017). Bashri et al. (Bashri et al.2018) and Khalil et al. (Khalil et al. 2017) have also demonstrated increased nitrate reductase activity due to the application of cadaverine and Kn. Increased nitrate reductase activity results in improved N assimilation (Khalil et al. 2017), thereby influencing the protein synthesis and stress tolerance (Iqbal et al. 2015). Spermidine mediated enhancement in the nitrate reductase, and Nitrogen contents may have directly regulated the synthesis of photosynthetic enzymes and other protective compounds, (Ahanger et al. 2020).

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)				

Furthermore, Spermidine Increased K content (Ahanger et al. 2020) which directly influences plant growth through its involvement in the regulation of enzyme activity and photosynthesis (Ahanger and Agarwal, 2017; Ahanger and Agarwal, 2017;Ahanger et al. 2017). The results revealed some deviations from the general exhibited trend of spermidine effects at high concentration The deviation from the augmentations for spermidine role might be ascribed to facts like free polyamines concentration varies with in a species also

(Reggiani, et al. 992): environmental factors (Kubis, 2006); Attachments of spermidine to other molecules (Gill and Tuteja, 2010)

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