Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(2):1545-1553. **OPEN ACCESS**

Spermidine and kinetin mediated alteration in macronutrients accumulation of mash [*Vigna mungo* (L.) Hepper] genotypes grown under chromium stress

Ghulam Yasin*, Sadaf Khan, Iqra Tabassum, Mumtaz Ahmad and Muzammil Hussain

Department of Botany, Bahauddin Zakariya University, Multan, **Pakistan**

***Correspondence:** yasingmn_bzu@yahoo.com **Received: 26-04-2020, Revised: 26-05-2020, Accepted: 28-05-2020 e-Published: 20-06-2020**

The experiment was managed to seek the effects of spermidine and kinetin on macronutrients accumulation in organs of four mash bean [*Vignamungo* (L.) Hepper] genotypes grown under Chromium (Cr) stress. Four genotypes i.e., MASH 80, MASH 88, MASH 97 and MASH ES-1 were grown in pots which four replicates. After fifteen days of germination, Chromium was $CrCl_3$ to raise 30.0 and 60.0mg Cr kg^{-1} soil. Macronutrints (NPK) were determined in root, stem and seeds. Spermidine and Kinetin were foliarly sprayed separately with frequency of twice at age of 15 and 30 days of plants. At physiological maturity of crop macronutrients were determined. Exogenous spermidine enhanced the accumulation of nitrogen to non- significant level in root stem and seeds. Significant increase in phosphorus was observed in stem of Mash 80 and Mash 88 genotypes as 32.32% and 21.45% respectively. Significant increase in potassium concentration was noted in stem of Mash 88 (25.02%) and in mash ES-1 (29.66%). Foliar spray of Kinetin significantly increased nitrogen in root of Mash 80 (39.30%), stem (53.57%) and in seed (105.38%). Similarly kinetin significantly increased phosphorus concentration in Mash 80. This increase was 25.725 in root, 74.32% in stem and 170.52% in seeds. Significant increase in potassium was noted in root and stem of Mash 80 and Mash Es-1. In Mash 80 the increased extent was 33.31 and 33.71 for root and stem respectively. In root of Mash ES-1 the increase was 78.06% while in stem was 78.89%.

Keywords: Macronutrients, Chromium, Spermidine, Kinetin, Mash, Genotypes

INTRODUCTION

Mash bean (*Vigna mungo* (L.) Hepper), is one of the the most important pulses of the world. In Pakistan, it is amongst the least researched crops inspite of its high nutritive and economic value. Nutritive analysis of mash bean seed revealed that it contains vitamins, oil, fats, protein and carbohydrates (James, 1981). It has the capability of fixing free atmospheric N_2 for its own consumption and enriches the soil with N for next crop (Sen, 1996).

Plant mineral nutrients are essential for regulation of osmotic, membrane permeability and are structural components of some essential metabolites. Of these macronutrients, nitrogen (N), phosphorus (P) and potassium (K) are the important which are as fertilizers in modern agricultural practices (Kulcheski et al. 2015). The vital activities of plants are affected by heavy metals present in atmosphere inducing abiotic stresses (Tajti et al., 2018). Plant growth regulators are used in the mitigation of adverse effects of these stresses (Ahanger et al. 2018; Khan et al. 2015).

Polyamines are important in this regard. Chromium treatment of plant to reduced contents of polyamines. Spermidine when exogenous applied, can mitigate the adverse effects of chromium (Wang et al., 2003a; Wang and Shi, 2004). Polyamines increase uptake and transport of ions and water by roots. (Aldesuquy, 2014). Polyamines are nitrogenous compounds that have low molecular weight and are used to mediate several vital processes under stressful conditions (Agurla et al. 2017). These are involved in many plant vital activities such as membrane stability, synthesis of protein, scavenging of ROS, mineral uptake, activation of enzyme and hormonal regulation (Hu et al. 2012; Ahmad et al. 2012; Puyang et al. 2016; Li et al. 2015). Among the common polyamines are putrescine, spermidine, and spermine. These are involved in many plant developmental processes and are present ubiquitously (Ahmed et al. 2012). Polycationic nature of polyamines enables them to interact with proteins, nucleic acids and phospholipids to stabilize these molecules (Ahmad et al. 2012). The metabolism of polyamines is affected by external factors of stresses (Hu et al. 2012; Puyang et al. 2016).

Similarly cytokinins are compounds that have low molecular weight and crucial roles in plant developmental processes (Zalabaket al.2013). Cytokinins are involved in cell division regulation, germination, nutrient uptake, morphogenesis, assimilate, and signal transduction in plants (Brugiere et al. 2008; Brzobohaty et al. 1993; Vyroubalova et al. 2009). Kinetin is a synthetic cytokinin utilized in plant growth improvement under stress conditions such as metal stress (Singh and Prasad, 2014), water logging (Younis et al. 2003) and salinity (Ahanger et al. 2018). The exogenously applied kinetin up-regulates the antioxidant system (Wu et al. 2012) and enhances the metabolite accumulation (Merewitz et al. 2012). Considering the importance of mash bean and considering the ever increasing toxicity of chromium in environment, the present experiment was devised to find out the effects of spermidine and kinetin on nutrients uptake potential of crop grown in chromium contaminated soil.

MATERIALS AND METHODS

A pot experiment was devised in quest of whether kinetin and spermidine (plant growth regulators) are effectual in mitigating the adverse effects of rhizospheric metal toxicity on mash (*Vignamungo* L. Hepper).

MATERIALS

After an initial survey, soil free from effluents hazards, was selected for the experiment. Soil was air dried, ground, mixed and passed through fine sieve. Seeds of four mash genotypes varieties i-e MASH 80, MASH 88, MASH 97 and MASH ES-1 were obtained from pulse section of Ayub Agricultural Research Institute (AARI), Faisalabad (Pakistan).Chromium chlorides Sigma Aldrich, Japan were used. Kinetin, 6-Furfuryl-aminopurine ($C_{10}H_9N_5O$) and spermidine, N-[3-Aminopropyl]-1, 4-butanediamine, ($C_7H_{19}N_3$) of Sigma Aldrich, Japan were used as plant growth regulators.

METHODS AND LAYOUT PLAN

For the conduction of experiment, pots of 30 cm diameter were used. Sandy loam soil (10 kg) was filled in pots which were lined with polyethylene bags ensuring seepage prevention. Placement of pots was with completele randomization by design. To develop the rhizospheric metal toxicity, calculated amounts of chlorides of chromium was added in soil at the age of fifteen days of plants. Metals salts were applied in soil as a water solution of $CrCl_3$ (method similar to that used by Stoeva and Bineva, 2003). Seeds of similar size were selected and sterilized. Five seeds were sown in each pot. Above ground emergence of 80% seedlings was considered as germination and thinning was performed to obtain three seedlings in each pot for balanced nutrients and other resources uptake by plants. Weeds were uprooted from time to time by hand weeding and hoeing in order to avoid weed crop competition. Insects and pests were control by foliar spray of Thiodon insecticides of Hoechst (Pvt) Ltd, Pakistan. Plants were irrigated with irrigation water according to the saturation percentage of soil. Solutions of spermidine (1.0mM) and kinetin (100.0mM) 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. Tween-20 (0.1%) was added as a surfactant in solution. Plants were exposed to foliar spray of PGRs at the age of fifteen and thirty days of age with great care of avoiding falling of drops from leaf surface.

For data collection, three plants were selected from each pot. Four pots per treatment from each variety were used. Nitrogen, Phosphorus and Potassium in root, stem and seeds were recorded at maturity of crop (90 days age plants). After digestion of material, Potassium (K^+) contents were analyzed by flame photometer (Jackson, 1962). Estimation of Nitrogen %age was by kjeldahal method (Bremner, 1965). Phosphorus contents determination was done by Jackson, 1962 method. The data collected were analyzed for analysis of variance for all the parameters 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. Where significant F values were obtained in analysis, differences between individual means were tested by LSD tests at 0.05% significance level, by using MSTAT-C Computer Statistical Programme.

RESULTSAND DISCUSSION

Effects of Spermidine

Exogenous spermidine did not substantially alter Nitrogen accumulation in root of plants grown in 30mg supplemented soil (Table 1). Even in Mash 88, low amount of Nitrogen was observed than control by spermidine application. The action of spermidine for enhancing Nitrogen was not statistically justified under 60 mg chromium imposition as well. The same trend of non significant effect of spermidine was observed for stem and seed. The stem of Mash 97 and seeds of Mash ES-1 responded to spermidine in a way that Nitrogen accumulation was decreased in them.

The effectiveness of spermidine in influencing the uptake of Posphorus in root was only significant in Mash 80 which when grown in 30 mg chromium, accumulated 15.16% more Posphorus than that of control (Table 2). Remaining all genotypes under low as well as high conconteration of chromium responded to an extent of non significant degree to spermidine. Even in Mash 88 spermidine negatively influenced Posphorus accumulation. In stem of Mash 80, Mash 88 and Mash ES-1 significant increase of 32.32%, 21.45% and 43.52% respectively was recorded. In root and seed the effects of spermidine were non significant.

Spermidine action was statistically promising in influence accumulation of Potassium in stem of Mash 80,Mash 88 and Mash ES-1(Table 3). Spermidine application showed its effect to a non significant extent for accumulation of Potassium in root and seeds. Even in Mash 80, Mash 88 and mash 97 spermidine application lowered Potassium concentration in root and stem under both levels of chromium. Significant increase of Potassium in stem of Mash 80 and Mash ES-1 was 25.09% and 29.66% respectively.

Effects of kinetin

Statistical approach revealed that foliar spray of kinetin increased accumulation of Nitrogen in root stem and seed of Mash 80 while decreased in Mash 97(Table 4). A significant enhancement of 39.80%, 53.57% and 105.38% of Nitrogen accumulation was noted in root, stem and seeds respectively of Mash 80. Non-significant increase in Nitrogen was observed in other genotypes. Under high level of chromium Mash 88 stored more (33.30%) Nitrogen in root while in Mash 97 kinetin effect was reverse of this trend.

Kinetin increased the accumulation of Posphorus in root stem and seed of Mash 80 to a significant level (Table 5). The values were 25.72%, &4.32% and 170.52% for root, stem and seed respectively. Posphorus uptake was reduced in root and stem of Mash 97. Under higher level of Chromium the effect of kinetin was not significant in P accumulation even in Masdh ES-1 the concentration of Posphorus was reduced in root and seeds by kinetin application.

As regard Potassium accumulation, Mash 80 and Mash ES-1 accumulated more Potassium in their root and stem while there was reduction of Potassium uptake by kinetin in Mash 88 and Mash 97 both under low and high chromium stress (Table 6). Pottassium concentration was more influenced positively in root and stem than in seed. Overall, Phosphorus and Nitrogen accumulation was increased in seeds of plants by kinetin foliar spary. Our results (Table 1-3) regarding enhanced accumulation of potassium by polyamine application are in line with the findings of Sarjala and Kaunisto, 1993. Our findings (Table 4-6) are in accordance with those of Wierzbowska and Nowak, 1999; Chakrabarti and Mukherji, 2003 and Beheraet al. 1990 who reported enhanced uptake of Nitrogen and Phosphorus by kinetin. The increased accumulation of Potassium by kinetin application is reported also by Abutalybov and Akhundova, 1982; Hong, 1975; Oliveira et al. 1998 and Neumann et al. 1978. Similarly, Polyamine mediated nitrogen increase was also reported by Roittoet al. 2003; Balestrasseet al. 2003 and Neveset al. 2002. Hormones not only regulate plant growth and developmental process but also nutrient intake, assimilation, distribution and accumulation (Panwar et al. 1990; Nowak and Ciecko, 1991; Nowak et al., 1997; Czapla et al.2003).

Table 1: Nitrogen contents (%) of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Chromium supplemented soil (30,60mg/kg soil) and exposed to foliar spray of spermidine (1.00mM) at 15 and 30 days of age

Part	Genotype	Chromium (30 mg/kg soil)			Chromium (60 mg/kg soil)		
		no spermidine (control)	Foliar Spermidine (1.00mM)	Difference (%)	No Spermidine (Control)	Foliar Spermidine (1.00mM)	Difference (%)
Root	Mash-80	0.127±0.016a	0.190±0.041a	49.60	0.076±0.014a	0.081±0.01a	6.57
	Mash-88	0.130±0.020a	0.100±0.005a	-23.07	0.069±0.005a	0.072±0.01a	4.34
	Mash-97	0.139±0.044a	0.156±0.017a	12.23	0.061±0.009a	0.065±0.00a	6.55
	Mash ES-1	0.124±0.013a	0.136±0.015a	9.67	0.054±4.904a	0.046±0.00a	-14.81
Stem	Mash-80	0.144±0.015a	0.160±0.015a	11.11	0.098±0.010a	0.109±0.01a	11.22
	Mash-88	0.160±0.027a	0.168±0.019a	5.00	0.095±0.009a	0.115±0.01a	21.05
	Mash-97	0.350±0.050a	0.298±0.019a	-14.85	0.135.014a	0.086±0.00a	-36.29
	Mash ES-1	0.170±0.022a	0.216±0.033a	27.05	0.096±0.008a	0.090±0.01a	-8.16
Seed	Mash-80	6.712±1.167cde	7.605±1.02ce	13.30	1.962±0.317h	3.473±0.72h	77.01
	Mash-88	8.710±2.353abcd	8.908±0.366abc	2.27	2.576±0.318h	3.145±0.57h	22.08
	Mash-97	10.323±3.639a	8.817±3.677abc	-14.58	3.574±0.557gh	9.974±2.28ab	179.07
	Mash ES-1	6.031±1.919efg	4.172±0.424fgh	-30.82	6.351±1.599def	3.211±0.80h	-49.44

. [Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control Values followed by dissimilar letters, are different significantly from control.

Table 2: Phosphorus contents (%) of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Chromium supplemented soil (30,60mg/kg soil) and exposed to foliar spray of spermidine (1.00mM) at 15 and 30 days of age.

Part	Genotype	Chromium (30 mg/kg soil)			Chromium (60 mg/kg soil)		
		No Spermidine (Control)	Foliar Spermidine (1.00mM)	Difference (%)	No Spermidine (Control)	Foliar Spermidine (1.00mM)	Difference (%)
Root	Mash-80	1.071±0.084b	1.242±0.211a	15.96	0.509±0.035de	0.555±0.056d	9.03
	Mash-88	1.079±0.090ab	0.800±0.022c	-25.85	0.476±0.068def	0.432±0.033def	-9.24
	Mash-97	0.964±0.375bc	1.054±0.064b	9.33	0.393±0.041ef	0.408±0.040def	3.81
	Mash ES-1	0.876±0.066c	0.924±0.081bc	5.47	0.388±0.034ef	0.326±0.042f	-15.97
Stem	Mash-80	0.993±0.009fg	1.314±0.043cd	32.32	0.619±0.092i	0.839±0.017h	35.54
	Mash-88	1.100±0.111ef	1.336±0.040c	21.45	0.588±0.012i	0.851±0.013h	44.72
	Mash-97	2.412±0.220a	2.508±0.112a	3.98	0.876±0.015gh	0.685±0.012i	-21.80
	Mash ES-1	1.197±0.052de	1.718±0.069b	43.52	0.626±0.036i	0.683±0.019i	9.10
Seed	Mash-80	9.388±1.307bcd	12.895±1.643ab	37.35	2.739±0.257g	6.836±1.640def	149.58
	Mash-88	12.160±3.566abc	15.512±2.374a	27.56	3.692±1.153fg	6.500±1.346def	76.05
	Mash-97	14.725±5.225a	15.411±4.642a	4.65	5.013±0.683efg	07.284±1.955def	45.30
	Mash ES-1	8.987±0.745cd	9.020±1.818cd	0.36	8.674±1.173cde	7.386±1.853def	-14.84

[Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control Values followed by dissimilar letters, are different significantly from control.

Table 3: Potassium contents (%) of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Chromium supplemented soil (30,60mg/kg soil) and exposed to foliar spray of spermidine (1.00mM) at 15 and 30 days of age.

Part	Genotype	Chromium (30 mg/kg soil)			Chromium (60 mg/kg soil)		
		No Spermidine (Control)	Foliar Spermidine (1.00mM)	Difference (%)	No Spermidine (Control)	Foliar Spermidine (1.00mM)	Difference (%)
Root	Mash-80	1.475±0.317bc	2.216±0.524a	50.23	0.479±0.025e	0.550±0.081e	14.82
	Mash-88	1.300±0.127bcd	1.083±0.093d	-16.69	0.619±0.115e	0.534±0.104e	-13.73
	Mash-97	1.388±0.506bcd	1.553±0.163bc	11.88	0.466±0.072e	0.349±0.072e	-25.10
	MashES1	1.274±0.080cd	1.586±0.168b	24.48	0.486±0.024e	0.513±0.098e	5.55
Stem	Mash-80	1.526±0.143fg	1.909±0.143de	25.09	0.796±0.078i	1.033±0.088hi	29.77
	Mash-88	2.047±0.329cd	1.970±0.065de	-3.76	1.171±0.128h	1.739±0.168efg	48.50
	Mash-97	3.697±0.356a	2.971±0.424b	-19.63	1.472±0.098g	0.809±0.114i	-45.04
	MashES1	1.790±0.254def	2.321±0.223c	29.66	1.045±0.097hi	0.832±0.083i	-20.38
Seed	Mash-80	14.893±3.833cde	14.621±2.098de	-1.82	3.833±0.851i	7.870±2.579ghi	105.32
	Mash-88	23.651±8.304a	18.777±0.944bcd	-20.18	4.078±0.749i	4.506±1.373hi	10.49
	Mash-97	20.303±6.513ab	19.527±4.132abc	-3.82	3.162±0.774i	6.731±1.991ghi	112.87
	Mash E1	11.216±1.602efg	14.003±2.366def	24.84	9.270±3.567fgh	4.543±1.153hi	-50.99

[Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control Values followed by dissimilar letters, are different significantly from control.

Table 4: Nitrogen contents (%) of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Chromium supplemented soil (30,60mg/kg soil) and exposed to foliar spray ofKinetin (100.00mM) at 15 and 30 days of age

Part	Genotype	Chromium (30 mg/kg soil)			Chromium (60 mg/kg soil)		
		No Kinetin (Control	Foliar Kinetin (100mM)	Difference (%)	No Kinetin (Control)	Foliar Kinetin (100mM)	Difference (%)
Root	Mash-80	0.127±0.017a	0.177±0.029a	39.37	0.074±0.014a	0.063±0.006A	-14.86
	Mash-88	0.129±0.020a	0.131±0.020a	1.55	0.066±0.005a	0.088±0.029A	33.33
	Mash-97	0.137±0.046a	0.102±0.006a	-25.54	0.059±0.008a	0.060±0.002A	1.69
	Mash ES-1	0.125±0.012a	0.130±0.011a	4.00	0.053±0.001a	0.061±0.004A	15.09
Stem	Mash-80	0.140±0.013a	0.215±0.013a	53.57	0.092±0.004a	0.121±0.018A	31.52
	Mash-88	0.159±0.026a	0.148±0.013a	-6.91	0.094±0.009a	0.079±0.006A	-15.95
	Mash-97	0.349±0.050a	0.231±0.020a	-33.81	0.135±0.014a	0.095±0.009A	-29.62
	Mash ES-1	0.165±0.019a	0.574±0.045a	247.87	0.094±0.006a	0.097±0.009A	3.19
Seed	Mash-80	6.263±1.453cd	12.863±3.807a	105.38	1.875±0.333f	1.586±0.220F	-15.41
	Mash-88	8.710±2.353bc	8.447±2.339bc	-3.019	2.576±0.318ef	2.844±0.105eF	10.40
	Mash-97	10.971±4.899ab	12.156±0.233a	10.80	3.574±0.557def	2.846±0.101Def	-20.36
	Mash ES-1	5.900±2.097cde	8.062±1.225bc	36.64	8.918±5.969bc	7.116±0.133Bc	-20.20

[Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control Values followed by dissimilar letters, are different significantly from control.

Table 5: Phosphorus contents (%) of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Chromium supplemented soil (30,60mg/kg soil) and exposed to foliar spray ofKinetin (100.00mM) at 15 and 30 days of age.

Part	Genotype	Chromium (30 mg/kg soil)			Chromium (60 mg/kg soil)		
		No Kinetin (Control)	Foliar Kinetin (100mM)	Difference (%)	No Kinetin (Control)	Foliar Kinetin (100mM)	Difference (%)
Root	Mash-80	1.069±0.091b	1.344±0.204a	25.72	0.496±0.034ef	0.463±0.064Ef	-6.65
	Mash-88	1.077±0.090b	0.947±0.108bcd	-12.07	0.459±0.074ef	0.562±0.155e	22.44
	Mash-97	0.952±0.386bcd	0.835±0.073d	-12.28	0.380±0.034ef	0.378±0.016ef	-0.52
	Mash ES1	0.875±0.065cd	1.063±0.115bc	21.48571	0.384±0.039ef	0.326±0.041f	-15.10
Stem	Mash-80	0.970±0.030fg	1.691±0.039c	74.32	0.580±0.057ij	0.884±0.014e	52.41
	Mash-88	1.099±0.111ef	1.079±0.034de	-1.81	0.584±0.016ij	0.557±0.017h	-4.62
	Mash-97	2.411±0.221b	1.754±0.050c	-27.25	0.876±0.015hij	0.686±0.017hi	-21.68
	Mash ES1	1.161±0.016d	4.191±0.106a	260.98	0.614±0.026g	0.672±0.018g	9.44
Seed	Mash-80	8.797±1.981ef	23.798±7.087a	170.52	2.609±0.193g	3.247±0.416g	24.45
	Mash-88	12.161±3.566cde	17.391±7.203bc	43.00	3.692±1.153fg	2.198±0.139g	-40.46
	Mash-97	15.633±6.905bcd	13.987±3.203cd	-10.52	5.013±0.683fg	5.275±1.011fg	5.22
	Mash ES1	8.685±0.603ef	17.632±2.555b	103.01	11.751±6.341de	2.715±0.299g	-76.89

[Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control Values followed by dissimilar letters, are different significantly from control.

Table 6: Potassium contents (%) of 90 days old mash [*Vigna mungo* (L.) Hepper] grown in Chromium supplemented soil (30,60mg/kg soil) and exposed to foliar spray ofKinetin (100.00mM) at 15 and 30 days of age

Part	Genotype	Chromium (30 mg/kg soil)			Chromium (60 mg/kg soil)		
		No Kinetin (Control)	Foliar Kinetin (100mM)	Difference (%)	No Kinetin (Control)	Foliar Kinetin (100mM)	Difference (%)
Root	Mash-80	1.474±0.329cd	1.965±0.199b	33.31	0.468±0.030ef	0.572±0.057ef	22.22
	Mash-88	1.298±0.124d	1.497±0.244cd	15.33	0.596±0.104ef	0.741±0.195e	24.32
	Mash-97	1.371±0.523d	1.678±0.242bc	22.39	0.451±0.070ef	0.434±0.054f	-3.76
	Mash ES-1	1.274±0.080d	2.280±0.200a	78.96	0.480±0.035ef	0.628±0.146ef	30.83
Stem	Mash-80	1.489±0.134ef	1.991±0.120d	33.71	0.752±0.108hi	0.615±0.101i	-18.21
	Mash-88	2.046±0.329d	1.556±0.119e	-23.94	1.165±0.137fg	0.983±0.093ghi	-15.62
	Mash-97	3.697±0.356b	2.759±0.194c	-25.37	1.472±0.098ef	0.946±0.066ghi	-35.73
	Mash ES-1	1.734±0.201de	3.101±0.824a	78.83	1.024±0.084gh	1.044±0.082gh	1.95
Seed	Mash-80	13.956±4.294bcd	19.397±4.694ab	38.98	3.676±0.946ef	2.952±0.444f	-27.85
	Mash-88	23.651±8.304a	21.402±8.253a	-9.50	4.078±0.749ef	2.293±0.266f	-43.77
	Mash-97	21.526±8.828a	11.688±3.376cd	-45.70	3.162±0.774f	1.686±0.062f	-46.67
	Mash ES-1	10.838±1.496de	18.824±2.573abc	73.68	13.120±9.474bcd	10.521±0.342de	237.21

. [Values represent means ± SE]. Values of %age difference represent increase (+)/decrease (-) over control Values followed by dissimilar letters, are different significantly from control.

According to Nowak and Ciecko (1991) increased concentration of minerals elements in the above ground parts of plants by applying synthetic hormones are caused by extensive growth of the root and enlargement of hair on root (Svenson, 1991; Meuwly and Pilet, 1991; Ali et al. 2008). This enalrgent of hair increases intake of ground water and nutrients. Foliar application of kinetin mitigates the Potassium uptake reduction by regulating the stomatal functioning [Ahmed et al. 2016].Stomatal opening is regulated by guard cell turgidity (Assmann and Shimazaki 1999 and Lemtiri-Chlieh et al. 2000) or increased stomatal pore area. This may be attributed to the combined effects of kinetin: (Barceloet al.1986). Kinetin induced enhanced K^+ accumulation may be due to root growth (Sharp and Davies, 1979 and Saab et al.1990). Hence, transported ions from the root are accumulated in above ground parts. Polyamines not only regulate ions concentration, but also their transportation as well. Polyamines modulate a number of ion channels (Johnson, 1996). The channel activities are regulated both at whole cell level or single channel (Bruggemann et al. 1998). Polyamines regulate ion channels such as KIR (inwardly rectifying K^+), NMDA (N-methyl-D-aspartate) and Ca^{2+} channels (Williams, 1997, Nichols and Lopatin, 1997 and Johnson, 1996). In higher plants polyamines control vacuolar cation channel also. Polyamines control ion channels by binding to membrane component or channel protein (Johnson, 1996).

CONCLUSION

Exogenous application of spermidine and kinetin enhanced the accumulation of macronutrients.

CONFLICT OF INTEREST

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

ACKNOWLEGEMENT

The authors acknowledge the help of laboratory staff.

AUTHOR CONTRIBUTIONS

GY and IT designed and performleed the experiments and also wrote the manuscript. MA and MH performed data analysis. SK eviewed the manuscript. All authors read and approved the final version.

REFERENCES

Abutalybov, M., Gand, T.S. & Akhundova.(1982). Cytokinin participation in regulation of potassium ion activity in root epidermal cells.Sov. Plant Physiol. 29: 395-402.

Agurla, S., Gayatri, G. & Raghavendra, A.S.

(2017). Polyamines increase nitric oxide and reactive oxygen species in guard cells of Arabidopsis thaliana during stomatal closure. Protoplasma. 255, 153–162.

Ahanger, M.A. & Agarwal, R.M. (2017). Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (*Triticum aestivum* L.) as influenced by potassium supplementation. Plant Physiol. Biochem. 115, 449–460.

Ahanger, M.A., Alyemeni, M.N., Wijaya, L., Alamri, S.A., Alam, P., Ashraf, M. & Ahmad, P. (2018).Potential of exogenously sourced kinetin in protecting *Solanum lycopersicum* from NaCl-induced oxidative stress through up-regulation of the antioxidant system, ascorbate-glutathione cycle and glyoxalase system.PLoS ONE, 13.

Ahanger, M.A., Ashraf, M., Bajguz, A. & Ahmad, P. (2018). Brassinosteroids Regulate Growth in Plants Under Stressful Environments and Crosstalk with Other Potential Phytohormones. J. Plant Growth Regul. 1007–1024.

Ahmad, P., Abdel Latef, A.A., Hashem, A., Abd_Allah, E.F., Gucel, S. & Tran, L.S.P. (2016). Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. Fron. Plant Sci. 7, 347.

Ahmad, P., Ahanger, M.A., Alyemeni, M.N., Wijaya, L. & Alam, P. (2018). Exogenous application of nitric oxide modulates osmolyte metabolism, antioxidants, enzymes of ascorbate-glutathione cycle and promotes growth under cadmium stress in tomato. Protoplasma. 255, 79–93.

Ahmad, P., Kumar, A., Gupta, A., Hu, X., Azooz, M.M. & Sharma, S. (2012). Polyamines: Role in plants under abiotic stress. In Crop Production for Agricultural Improvement, Springer: Dordrecht, The Netherlands, pp. 491–512.

Ahmad, P., Sarwat, M., Bhat, N.A., Wani, M.R., Kazi, A.G. &Tran, L.S.P. (2015).Alleviation of Cadmium Toxicity in *Brassica juncea* L. (Czern. & Coss.) by Calcium Application Involves Various Physiological and Biochemical Strategies.PLoSONE . 10.

Alcazar, R., Altabella, T., Marco, F., Bortolotti, C., Reymond, M., Koncz, C., Carrasco, P & Tiburcio, A.F. (2010). Polyamines: Molecules with regulatory functions in plant abiotic stress tolerance. Planta. 231, 1237–1249.

Aldesuquy, H., Haroun, S., Abo-Hamed, S., Whab, A. & El-Saied. (2014). Involvement of spermine and spermidine in the control of productivity and biochemical aspects of yielded grains of wheat plants irrigated with waste water. Egypt. J. Basic App. Sci.1, 16-28.

Aldesuquy, H., Baka, Z. & Mickky, B. (2014). Kinetin and spermine mediated induction of salt tolerance in wheat plants: Leaf area, photosynthesis and chloroplast ultrastructure of flag leaf at ear emergence. Egypt. J. Basic App. Sci. 1, 77–87.

Ali, B., Hayat, S., Hasan, S. & Ahmad, A. (2008).A comparative effect of IAA and 4-Cl-IAA on growth, nodulation and nitrogen fixation in *Vigna radiata* (L.). Acta Physiol. Plant. 30, 35–41.

Alyemeni, M.N., Ahanger, M.A., Wijaya, L., Alam, P., Bhardwaj, R. & Ahmad, P. (2018). Selenium mitigates cadmium-induced oxidative stress in tomato (*Solanum lycopersicum* L.) plants by modulating chlorophyll fluorescence, osmolyte accumulation, and antioxidant system. Protoplasma. 255, 459–469.

Assmann, S.M. & Shimazak, K.I. (1999). The multisensory guard cell.Stomatal responses to blue light and abscisic acid. Plant Physiol. 119, 337-361.

Balestrasse, K.B., Benavides, M.P., Gallego, S.M. &Tomaro, M.L. (2003). Effect of cadmium stress on nitrogen metabolism in nodules and roots of soybean plants.Function. Plant Biol. 30, 57-64.

Behera, P.K., Sarangi, C.S., Mishra, D. & Patra, H.K. (1990).Effect of abscisic acid and kinetin on enzyme activities during petal senescence in cut carnation flowers.Isr. J. Bot. 39, 229-238.

Bremner, J.M. (1965). Total nitrogen and inorganic forms of nitrogen.In. Methods of soil analysis. Ed. C. A. Black.Vol 2. American Society of Agronomy, Madison, Wisonsin.pp: 1449-1237.

Bruggemann, U.I., Pottosin, I.I. & Schonknecht, G. (1998). Cytoplasmic polyamines block the fast-activating vacuolar cation channel. Plant J. 16, 101–105.

Brugiere, N., Humbert, S., Rizzo, N., Bohn, J. & Habben, J.E. (2008). A member of the maize isopentenyltransferase gene family, *Zea mays* isopentenyl transferase 2 (ZmIPT2), encodes a cytokinin biosynthetic enzyme expressed during kernel development. Plant Mol. Biol. 67, 215–229.

Brzobohaty, B., Moore, I., Kristoffersen, P., Bako, L., Campos, N., Schell, J. & Palme, K. (1993). Release of active cytokinin by a beta-glucosidase localized to the maize root meristem. Sci. 262, 1051–1054.

Chakrabarti, N & Mukherji, S. (2003). Effect of phytohormone pretreatment on nitrogen metabolism in *Vignaradiata* under salt stress.Biol. Plant. 46, 63-66.

Czapla, J., Nogalska, A. & Stasiulewicz, L. (2003).Synthetic auxin effect on the yield and the mineral in soybeans. Acta Scien.Polon.Agricul. 2, 123–131.

Duncan, D.B. (1955). Multiple Range and Multiple F-Test. Biomet.11, 1-42.

Groppa, M.D., Ianuzzo, M.P., Rosales, E.P., Vázquez, S.C. & Benavides, M.P.(2012). Cadmium modulates NADPH oxidase activity and expression in sunflower leaves. Biol. Plant. 56, 167–171.

Hu, X., Zhang, Y., Shi, Y., Zhang, Z., Zou, Z., Zhang, H. & Zhao, J. (2012). Effect of exogenous spermidine on polyamine content and metabolism in tomato exposed to salinity–alkalinity mixed stress. Plant Physiol. Bioch. 57, 200–209.

Jackson, M.L. (1962). Soil Chemical analysis. Constable and company, Ltd. England.

Johnson, T. D. (1996). Modulation of channel function by polyamines.Trends Biochem. Sci. 17, 22–27

Khan, M.I.R., Asgher, M. & Khan, N.A. (2014). Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vignaradiata* L.). Plant Physiol. Biochem. 80, 67–74.

Khan, M.I.R., Fatma, M., Per, T.S., Anjum, N.A. & Khan, N.A. (2015). Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front. Plant Sci. 6,462.

Kulcheski, F.R., Correa, R., Gomes, I.A., de Lima, J. & Margis, R. (2015).NPK macronutrients and microRNA homeostasis. Front. Plant Sci. https://doi.org/10.3389/fpls.2015.00451

Lemtiri-Chlieh, F., MacRobbie, E.A.C. & Brearley, C.A. (2000). Inositol hexabisphosphate is a physiological signal regulating the K^+inward rectifying conductance in guard cells. Procedings of National Academy of Science, USA. 97, 8687-8692.

Li, J., Hu, L., Zhang, L., Pan, X. & Hu, X. (2015). Exogenous spermidine is enhancing tomato tolerance to salinity–alkalinity stress by regulating chloroplast antioxidant system and chlorophyll metabolism.BMC Plant Biol. 15, 303.

Merewitz, E.B., Du, H., Yu, W., Liu, Y., Gianfagna, T. & Huang, B. (2012). Elevated cytokinin content in ipt transgenic creeping bentgrass promotes drought tolerance through regulating metabolite accumulation. J. Exp. Bot. 63, 1315–1328.

Meuwly, P. & Pilet, P. (1991). Local treatment with indole-3-acetic acid induces differential growth responses in *Zea mays* L. root. Planta. 185, 58–64.

Neumann, K.H., Pertzsch, C., Moos, G. & Krommelbein, G. (1978). Characterization of the nutrition of carrot tissue cultures: photosynthesis, carbon and nitrogen nutrition, mineral uptake.Zeitschrift fur Pflanzenernahrung und Bodenkunde.141, 299-311.

Neves, C., Santos, H. Vilas-Boas, L. & Amancio, S. (2002). Involvement of free and conjugated polyamines and free amino acids in the adventitious rooting of micropropagated cork oak and grapevine shoots. Plant Physiol.Biochem. 40, 1071-108.

Nichols, C.G. & Lopatin, A.N. (1997).Inward rectifier potassium channels. Ann. Reviw Plant Physiol. 59, 171–191.

Nowak, G. & Ciecko, Z. (1991).Effect of GA_3 on the yield and value of forage turnip.Zesz.Nauk.AR Krakow 262, 325–33.

Nowak, G.A., Klasa, A., Wierzbowska, J. & Rotkiewicz, M. (1997). Yield and macronutrient content in faba bean plants in the conditions of use retardant and plant hormones. Biul. IHAR 201, 289–294.

Oliveira, R. F., L. Pace and C. A. Rosolem.1998. Common beans yield and nutritional status as affected by a growth regulator. Científica (Jaboticabal), 26, 203-212.

Panwar, J.D.S., Abbas, S. & Raum, S. (1990). Effects of benzol-amino purine and girdling site on photosynthesis translocation and nitrogen fixation in Y-shaped mungbean. Ind. J. Plant Physiol. 33, 16–20.

Per, T.S., Khan, N.A., Masood, A. & Fatma, M. (2016). Methyl Jasmonate Alleviates Cadmium-Induced Photosynthetic Damages through Increased S-Assimilation and Glutathione Production in Mustard. Front. Plant Sci. 7,1933.

Puyang, X., An, M., Xu, L., Han, L. & Zhang, X. (2016). Protective effect of exogenous spermidineon ion and polyamine metabolism in Kentucky bluegrass under salinity stress. Horticulture, Environ Biotechnol. 57, 11–19.]

Qian, H., Li, J., Sun, L., Chen, W., Sheng, G.D., Liu, W. & Fu, Z. (2009). Combined effect of copper and cadmium on Chlorella vulgaris growth and photosynthesis-related gene transcription.Aquatic Toxicol. 94, 56–61.

Roitto, M., Markkola,A., Julkunen-Tiitto,R., Sarjala,T., Rautio, P., Kuikka, K. &Tuomi,J. (2003). Defoliation-induced responses in peroxidases, phenolics and polyamines in Scots pine (*Pinus sylvestris* L.) needles. J. Chem. Ecol. 29, 1905-1918.

Saab, I.N., Sharp,R.E., Pritchard, J. & Voetberg,G. (1990). Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physiol. 93, 1329–1336.

Sarjala, T., Haggman, H. & Aronen, T. (1997). Effect of exogenous polyamines and inhibitors of polyamine biosynthesis on growth and free polyamine contents of embryogenic Scots pine callus. J. Plant Physiol. 150, 597-602.

Sharp, R.E. & Davies, W.J. (1979).Solute regulation and growth by roots and shoots in water-stressed maize plants.Planta. 147, 43–49.

Singh, S. & Prasad, S.M. (2014). Growth, photosynthesis and oxidative responses of Solanummelongena L. seedlings to cadmium stress: Mechanism of toxicity amelioration by kinetin. Sci. Hort. 176, 1–10.

Stoeva, N. & Bineva, T. (2003).Oxidative changes and photosynthesis in oat plants grown in as-contaminated soil. Bulg. J. Plant Physiol. 29, 87–95.

Svenson, S.E. (1991). Rooting and lateral shoot elongation of Verbena following benzylaminopurine application.Hort. Sci. 26, 391–392.

Tajti, J., Janda, T., Majlath, I., Szalai, G. & Pal M. (2018).Comparative study on the effects of putrescine and spermidine pre-treatment on Cadmium stress in wheat.Ecotox. Environ. Safety. 148, 546–554.

Vyroubalova, S., Vaclavikova, K., Tureckova, V., Novak, O., Smehilova, M., Hluska, T., Ohnoutkova, L., Frebort, I. &Galuszka, P. (2009). Characterization of New Maize Genes Putatively Involved in Cytokinin Metabolism and Their Expression during Osmotic Stress in Relation to Cytokinin Levels. Plant Physiol. 151, 433–447.

Wang, X. & Shi, G. (2004).Effect of exogenous spermidine on anti-Hg^{2+} stress ability of Amaranth. J. Plant Physiol.Mol. Biol. 30, 69–74.

Wang, X., Shi, G. & Xu, Q. (2003).Toxic effects of Cr^{6+} on *nymphoidespeltatum*mitigated by exogenous polyamine.Acta Sci. Circum. 23, 689–693

Wierzbowska, J. & Nowak, G.A. (1999).The effect of cytokinin and auxin on yield and quality of spring wheat grain depending on NPK rates.Part 2.Quality of grain and germination ability.BiuletynInstytutuHodowli i Aklimaty zacji Roslin, 208, 35-42.

Williams, K. (1997). Interactions of polyamines with ion channels.Biochem. J. 325, 289–297

Wu, X., Zhu, Z., Li, X. & Zha, D. (2015). Effects of cytokinin on photosynthetic gas exchange, chlorophyll fluorescence parameters and antioxidative system in seedlings of eggplant (*Solanum melongena* L.) under salinity stress. Acta Physiol. Plant. 34, 2105–2114.

Younis, M.E., El-Shahaby, O.A., NematAlla, M.N. & El-Bastawisy, Z.M.(2003). Kinetin alleviates the influence of waterlogging and salinity on growth and affects the production of plant growth regulators in *Vigna sinensis* and *Zea mays*. Agron. 23, 277–285.

Zalabak, D., Pospisilova, H., Smehilova, M., Mrizova, K., Frebort, I. & Galuszka, P. (2013). Genetic engineering of cytokinin metabolism: Prospective way to improve agricultural traits of crop plants. Biotechnol.Advan. 31, 97–117.