



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 1511-1514
Received: 02-01-2019
Accepted: 05-02-2019

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Zinc induced osmoregulation and antioxidant defence system in mungbean (*Vigna radiata* L.) under drought stress

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Abstract

A field experiment was conducted during *rabi* season of 2017-18 at agricultural college farm, Bapatla to investigate the effect of seed pretreatment and foliar application of zinc on osmoregulation and antioxidant defence system in mungbean under drought stress, in a split plot design with three replications. The treatments comprised of two main treatments *viz.*, no stress *i.e.* control (M₀) and stress from flowering stage (*i.e.* from 30 DAS) up to harvest (M₁) and seven sub-treatments *viz.*, no zinc application (S₀), seed treatment with 0.05% and 0.075% ZnSO₄ solutions for 5 hrs before sowing (S₁ and S₂), foliar spray of 300, 400 and 500 ppm ZnSO₄ at 30 DAS (S₃, S₄ and S₅) and water spray at 30 DAS (S₆). The results of the study revealed that Leaf proline and super oxide dismutase (SOD) contents were enhanced by 10.3 and 30.0 per cent, respectively, in the plants that were subjected to water stress from flowering stage compared to control plants, which is an indication of plants adaption to abiotic stress conditions. Among zinc treatments, foliar spray of zinc @ 500 ppm at 30 DAS and seed pre-treatment with zinc @ 0.075% before sowing increased the proline and SOD activities by 10.8 and 9.0; 21.3 and 13.5 per cent, respectively, over untreated (control) plants at 60 DAS. It can be concluded that the use of zinc spray @ 500 ppm at 30 DAS counteracted the deleterious effects of water stress on mungbean by osmoregulation and antioxidant defence mechanism.

Keywords: Mungbean, proline, SOD, Zinc

Introduction

Mungbean (*Vigna radiata* L.) is an important pulse crop in arid and semi-arid regions, cultivated for edible green pods and dry seeds having high nutritive value and considered as a good source of protein for both humans and animals. Its seed contains 24.2% protein, 1.3% fat and 60.4% carbohydrate. The average yield of mungbean is quite low which requires the attention of the crop experts. Among the various factors influencing the growth and development of mungbean, drought stress is the one which occurs more frequently during the crop growth and causes severe damages at the cellular, tissue, organs level and even the whole plant. It leads to the increased production of reactive oxygen species (ROS) and leads to crop losses by deregulating plant defence systems, modifying plant physiological, biochemical, (Upadhyaya and Panda, 2004; Upadhyaya *et al.*, 2008) [16, 17] and molecular processes during vegetative and reproductive phases. Among many plant defence systems against environmental stresses, antioxidative defences, osmotic adjustment (Mahajan and Tuteja, 2005) [8] and gene expression are major mechanisms that help the plant to tolerate drought stress. Antioxidant enzymes such as catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) act through detoxification of ROS from plant cells and maintain the balance between plant antioxidative defence and the oxidative generation (Gill and Tuteja, 2010) [6]. Maiti *et al.* (2000) [9] reported that proline accumulation is a mechanism for plants adaptation to abiotic stress conditions.

Zinc (Zn) is an essential micronutrient which is involved in many physiological functions such as auxin biosynthesis, activation of dehydrogenase enzymes and stabilization of ribosomal fractions (Aghatise and Tayo, 1994) [1], protein and carbohydrate synthesis (Yadavi *et al.*, 2014) [20]. Application of Zn causes a reduction in activity of membrane-bound NADPH oxidase producing ROS, while the activities of SOD (superoxide dismutase), POD (peroxidase), and CAT (catalase) enhance (Hong and Ji-yun, 2007) [7]. It is essential for the biosynthesis of the carbonic anhydrase enzyme required for chlorophyll biosynthesis (Xi-Wen *et al.*, 2011 and Rehman *et al.*, 2012) [19, 14], and also as a key constituent of alcohol dehydrogenase and superoxide dismutase (Welch *et al.*, 1982) [18]. It plays a significant role in regulating the stomatal opening and closing and ionic balance in crops and reduces the

detrimental effects of drought (Moghadam *et al.*, 2013 and Monjezi *et al.*, 2013) ^[11, 12], and also has protective effects on oxidative damage caused by ROS in response to stresses (Akbari *et al.*, 2013) ^[2].

Material and Methods

Green gram seeds of LGG 460 variety were sown on November 2017 at agricultural college farm, Bapatla. The average temperature during the crop period varied from 30.53°C and 18.16°C. The total amount of rainfall received during the crop growth period was 23.8 mm in four rainy days. Weekly mean relative humidity ranged from 90.29% to 81.43%. The experiment was laid out in split plot design with three replications keeping no stress *i.e.* control (M₀) and stress from flowering stage (*i.e.* from 30 DAS) up to harvest (M₁) as main plots and seven sub-treatments *viz.*, no zinc application (S₀), seed treatment with 0.05% ZnSO₄ solution for 5 hrs before sowing (S₁), seed treatment with 0.075% ZnSO₄ solution for 5 hrs before sowing (S₂), foliar spray of 300 ppm ZnSO₄ at 30 DAS (S₃), foliar spray of 400 ppm ZnSO₄ at 30 DAS (S₄), foliar spray of 500 ppm ZnSO₄ at 30 DAS (S₅) and water spray at 30 DAS (S₆). Nitrogen and phosphorus fertilizers were applied as per the recommendation. Irrigation was given for control (M₀) as per the irrigation schedule up to harvest, and irrigation was withhold for M₁ treatment from the flowering stage (*i.e.* from 30 DAS) up to harvest. Foliar spray of ZnSO₄ was done at the flowering stage (*i.e.* at 30 DAS).

Proline assay

Proline content was estimated as per Bates *et al.*, 1973 ^[3]. The proline concentration in samples was determined according to the standard curve plotted with known concentrations of L-proline and calculated on a fresh weight basis. Proline content was calculated by the formula Bates *et al.* (1973) ^[3]

$$\text{Proline } (\mu\text{g g}^{-1} \text{ fresh weight}) = \frac{\text{OD} \times 36.231 \times V}{Y \times W}$$

OD = Optical density at 520 nm

36.231 = Factor

V = final volume of extract

Y = volume of an aliquot taken

W = weight of plant material

Antioxidant enzyme activity assay

Superoxide dismutase (SOD, EC: 1.15.1.1) was assayed following the method as described by Dhindsa *et al.*, 1981 ^[5]. The absorbance of samples along with blank 'B' is measured at 560 nm against the blank 'A'. Then the difference of per cent reduction in the colour between blank 'B' and the sample is worked out. 50% reduction in the colour is considered as one unit of enzyme activity and the activity is expressed in units of the enzyme per mg protein per hour.

$$\text{SOD activity (unit mg}^{-1} \text{ protein h}^{-1}) = \frac{\% \text{ reduction in colour between blank and Sample} \times \text{dilution factor} \times 60}{50 \times \text{incubation time} \times \text{mg protein in sample}}$$

Results and Discussion

Total rainfall of 23.8 mm was received in four rainy days during the first week of sowing. Later, there was no rainfall received, and the crop was exposed to water stress from flowering to maturity stage (*i.e.* treatment M₁). Control was

maintained by providing irrigation as per the irrigation schedule (*i.e.* no stress). The soil moisture measured at different depths at the time of sowing was 26.23 and 26.36 per cent at 15-30 cm depth, and 24.24 and 24.28 per cent at 30-45 cm depth in M₀ and M₁ treatments, respectively. At 15 DAS, the soil moisture was 25.97 and 26.05 per cent at 15-30 cm depth, and 22.71 and 22.78 per cent at 30-45 cm depth in M₀ and M₁ treatments, respectively. At 30 DAS, the soil moisture was 25.39 and 24.46 per cent at 15-30 cm depth, and 22.05 and 21.69 per cent at 30-45 cm depth in M₀ and M₁ treatments, respectively. The soil moisture depleted from 24.46 to 20.39 per cent at 15-30 cm and 21.69 to 17.59 per cent at 30-45 cm depth from 30 to 45 DAS, and 20.39 to 17.28 per cent at 15-30 cm and 17.59 to 14.15 per cent at 30-45 cm depth from 45 to 60 DAS in the plots which received no irrigation from flowering stage (*i.e.* M₁ treatment).

Proline Content ($\mu\text{g g}^{-1}$ fresh weight)

The data pertaining to proline content in mungbean leaves as affected by water stress and zinc treatments were furnished in Table 1. At 15 and 30 DAS, no significant difference was noted among the main treatments and interactions. Among the sub-treatments, significant differences were noted from 15 DAS onwards.

After the imposition of water stress, significant differences were noted among the main treatments. At 45 and 60 DAS, higher proline content was recorded in the plants that were subjected to water stress from the flowering stage (M₁-176.20 and 199.52 $\mu\text{g g}^{-1}$ fresh weight [fr. wt.] respectively) compared with the unstressed plants *i.e.* control (M₀-150.58 and 180.86 $\mu\text{g g}^{-1}$ fr. wt., respectively). The proline content increased by 10.3 per cent in the plants that were subjected to stress from flowering stage compared with the control plants (*i.e.* no stress) at 60 DAS. The obtained results are in agreement with the findings of Maiti *et al.* (2000) ^[9], who reported that proline accumulation is a mechanism for plants adaption to abiotic stress conditions. Other roles for proline include stabilization of macromolecules, a sink for excess reductant and a store of carbon and nitrogen for use after relief of water deficit.

Significant differences were recorded among the sub-treatments at 15, 30, 45 and 60 DAS. At 15 and 30 DAS, higher proline content was recorded by pre-soaking of seeds with zinc @ 0.075% before sowing (S₂-84.60 and 119.01 $\mu\text{g g}^{-1}$ fr. wt., respectively) compared to seed pretreatment with zinc @ 0.05% (S₁-81.33 and 114.86 $\mu\text{g g}^{-1}$ fr. wt., respectively). The treatment S₁ recorded higher proline content compared to other zinc treatments and lesser compared to S₂ treatment. The remaining treatments were on par with each other, and lesser proline content was recorded by S₆ (*i.e.* foliar spray of water 75.49 and 109.94 $\mu\text{g g}^{-1}$ fr. wt., respectively). At 45 and 60 DAS, foliar application of zinc @ 500 ppm at 30 DAS (S₅-172.93 and 201.68 $\mu\text{g g}^{-1}$ fr. wt., respectively) recorded the highest proline content, followed by seed pre-soaking with zinc @ 0.075% before sowing (S₂-169.18 and 198.48 $\mu\text{g g}^{-1}$ fr. wt., respectively) and zinc spray @ 400 ppm (S₄-167.35 and 193.24 $\mu\text{g g}^{-1}$ fr. wt., respectively), whereas the lowest proline content was recorded by the treatment S₆ (water spray-153.91 and 179.78 $\mu\text{g g}^{-1}$ fr. wt., respectively). S₀ (no zinc application-156.07 and 182.07 $\mu\text{g g}^{-1}$ fr. wt., respectively) recorded significantly higher proline content compared to a water spray (S₆), but lesser proline content when compared to other zinc treatments. In the present study, foliar spray of zinc @ 500 ppm at 30 DAS, seed pre-treatment with zinc @ 0.075%

before sowing and zinc spray @ 400 ppm at 30 DAS increased the proline content by 10.8, 9.0 and 6.1 per cent, respectively over untreated plants, at 60 DAS.

At 45 and 60 DAS, a significant difference was observed among the interactions. The highest proline content in mungbean leaves was recorded by the plants that were stressed from the flowering stage with a foliar spray of zinc @ 500 ppm (M_1S_5 -213.15 $\mu\text{g g}^{-1}$ fr. wt.), whereas the lowest proline content was recorded by the unstressed plants with water spray (M_0S_6 -171.53 $\mu\text{g g}^{-1}$ fr. wt.). Sofy, (2015) [15] reported that foliar application of zinc (75 ppm) at 30 and 75 DAS to the wheat plants that were irrigated once in every 14 and 28 days interval significantly increased the proline content in leaves. In the current study, irrigation withholding from flowering to maturity stage and with zinc foliar application increased the leaf proline content in mungbean.

Superoxide Dismutase (SOD) Activity

The data pertaining to SOD activity in mungbean leaves as affected by water stress and zinc treatments are presented in Table 2. At 15 and 30 DAS, no significant difference was noted among the main treatments and interactions. Among the sub-treatments, significant differences were noted from 15 DAS onwards.

Among the main treatments, significant differences were observed only after the imposition of water stress. Higher SOD activity was recorded in the plants that were subjected to water stress from the flowering stage (M_1 -27.09 and 36.66 units mg^{-1} protein h^{-1}) compared with the unstressed plants i.e. control (M_0 -24.02 and 28.21 units mg^{-1} protein h^{-1}) at 45 and 60 DAS, respectively. In the current study, irrigation withholding from the flowering stage up to maturity increased the SOD activity in mungbean leaves from 21.07 to 36.66 units mg^{-1} protein h^{-1} . The SOD activity was enhanced by 30.0 per cent in the plants that were subjected to stress from flowering stage compared with the control plants (i.e. no stress) at 60 DAS. It was proved that drought stress increases the production of reactive oxygen species (ROS) (Mittler, 2002). To scavenge these ROS, plants either synthesize different antioxidant compounds or activate antioxidant enzymes. Plants can detoxify ROS by upregulating antioxidant enzymes, such as SOD, CAT and POX as well as some non - enzymatic antioxidant compounds. It is evident that high levels of antioxidants are related to plant water deficit tolerance. The combined action of SOD and CAT converts the toxic O_2^- and H_2O_2 to water and molecular oxygen, averting the cellular damage under unfavourable conditions such as drought stress (Reddy *et al.*, 2000 and Chaitanya *et al.*, 2002) [13,4].

Among the sub-treatments, significant differences were observed at 15, 30, 45 and 60 DAS. At 15 and 30 DAS, pre-soaking of seeds with zinc @ 0.075% before sowing recorded higher SOD activity (S_2 -19.24 and 22.50 units mg^{-1} protein h^{-1} , respectively) compared to seed pre-treatment with zinc @ 0.05% (S_1 -18.73 and 21.63 units mg^{-1} protein h^{-1} , respectively) and other treatments. The remaining treatments were on par with each other, and lesser SOD activity was recorded by S_6 (i.e. foliar spray of water-17.64 and 20.43 units mg^{-1} protein h^{-1} , respectively). At 45 DAS, the highest SOD activity was recorded by foliar application of zinc @ 500 ppm at 30 DAS (S_5 -28.70 units mg^{-1} protein h^{-1}), followed by seed pre-soaking with zinc @ 0.075% before sowing (S_2 -27.18 units mg^{-1} protein h^{-1}) and foliar application of zinc @ 400 ppm at 30 DAS (S_4 -26.37 units mg^{-1} protein h^{-1}). The lowest SOD activity was recorded by the treatment S_6 (water spray 23.04 units mg^{-1} protein h^{-1}). S_0 (no zinc treatment 23.49 units mg^{-1} protein h^{-1}) recorded significantly higher SOD activity compared to a water spray (S_6), but lesser SOD activity when compared to other zinc treatments. At 60 DAS, foliar application of zinc @ 500 ppm at 30 DAS (S_5 -36.10 units mg^{-1} protein h^{-1}) recorded significantly higher SOD activity compared to control (i.e. untreated) and other zinc treatments. Zinc spray @ 400 ppm at 30 DAS (S_4 -34.38 units mg^{-1} protein h^{-1}) and seed pre-treatment with zinc @ 0.075% before sowing (S_2 -33.83 units mg^{-1} protein h^{-1}) came in the second order pertaining to SOD activity. The lowest SOD activity was recorded by the treatment S_6 (i.e. water spray 28.94 units mg^{-1} protein h^{-1}). S_0 (no zinc treatment 29.81 units mg^{-1} protein h^{-1}) recorded significantly higher SOD activity compared to a water spray (S_6), but lesser SOD activity when compared to other zinc treatments. In the present study, foliar spray of zinc @ 500 and 400 ppm at 30 DAS and seed pretreatment with zinc @ 0.075% before sowing increased the SOD activity by 21.1, 15.3 and 13.5 per cent, respectively over control (i.e. no zinc application), at 60 DAS. Zafar *et al.* (2014) [21] also observed, higher SOD activity was obtained with zinc foliar application in sunflower compared with untreated control. Our results are in agreement with the findings of Zafar *et al.* (2014) [21].

Among the interactions, a significant difference was observed at 45 and 60 DAS. The highest SOD activity in mungbean leaves was recorded by the plants that were sprayed with zinc @ 500 ppm at 30 DAS in water stress condition (M_1S_5 -41.77 units mg^{-1} protein h^{-1}). The lowest SOD activity was recorded by the unstressed plants with a water spray (M_0S_6 -26.02 units mg^{-1} protein h^{-1}). Zafar *et al.* (2014) [21] stated that zinc foliar application increased the antioxidant enzyme activity (viz., SOD and CAT) in drought stress condition in sunflower.

Table 1: Effect of zinc on proline content ($\mu\text{g g}^{-1}$ fresh weight) of mungbean leaves under water stress

Treatments	15 DAS			30 DAS			45 DAS			60 DAS		
	M_0	M_1	Mean	M_0	M_1	Mean	M_0	M_1	Mean	M_0	M_1	Mean
S_0 : No Zinc application	75.81	75.99	75.90	109.98	110.19	110.09	143.61	168.52	156.07	173.62	190.52	182.07
S_1 : Seed treatment with Zinc @ 0.05% before sowing	81.91	80.76	81.33	115.31	114.41	114.86	151.13	175.90	163.51	181.30	198.04	189.67
S_2 : Seed treatment with Zinc @ 0.075% before sowing	84.65	84.55	84.60	119.37	118.64	119.01	155.71	182.64	169.18	187.04	209.92	198.48
S_3 : Foliar spray of Zinc @ 300 ppm at 30 DAS	76.40	76.13	76.26	110.13	110.49	110.31	147.89	173.70	160.80	178.09	194.69	186.39
S_4 : Foliar spray of Zinc @ 400 ppm at 30 DAS	76.06	75.93	75.99	110.32	110.62	110.47	153.81	180.89	167.35	184.21	202.26	193.24
S_5 : Foliar spray of Zinc @ 500 ppm at 30 DAS	75.54	75.50	75.52	110.53	110.57	110.55	159.29	186.56	172.93	190.21	213.15	201.68
S_6 : Foliar spray of water at 30 DAS	75.43	75.55	75.49	110.30	109.58	109.94	142.65	165.17	153.91	171.53	188.03	179.78
Mean	77.97	77.77		112.28	112.07		150.58	176.20		180.86	199.52	
	SEm \pm	CD	CV (%)	SEm \pm	CD	CV (%)	SEm \pm	CD	CV (%)	SEm \pm	CD	CV (%)
Main	0.17	NS	2.98	0.09	NS	2.36	0.11	0.69	3.32	0.110	0.67	3.27
Sub	0.40	1.17	1.26	0.50	1.46	1.09	0.27	0.80	2.41	0.258	0.75	2.33
Interactions	0.57	NS		0.71	NS		0.39	1.13		0.365	1.06	

Table 2: Effect of zinc on superoxide dismutase (SOD) (unit mg⁻¹ protein h⁻¹) of mungbean leaves under water stress

TREATMENTS	15 DAS			30 DAS			45 DAS			60 DAS		
	M ₀	M ₁	Mean	M ₀	M ₁	Mean	M ₀	M ₁	Mean	M ₀	M ₁	Mean
S ₀ : No Zinc application	17.67	17.88	17.77	20.53	20.71	20.50	22.33	24.66	23.49	26.89	32.74	29.81
S ₁ : Seed treatment with Zinc @ 0.05% before sowing	18.77	18.68	18.73	21.64	21.61	21.63	23.90	26.86	25.38	28.14	35.90	32.02
S ₂ : Seed treatment with Zinc @ 0.075% before sowing	19.29	19.20	19.24	22.41	22.58	22.50	25.71	28.65	27.18	29.20	38.45	33.83
S ₃ : Foliar spray of Zinc @ 300 ppm at 30 DAS	17.90	17.73	17.82	20.72	20.59	20.65	22.93	26.53	24.73	27.86	36.14	32.00
S ₄ : Foliar spray of Zinc @ 400 ppm at 30 DAS	17.75	17.55	17.65	20.56	20.74	20.57	24.59	28.14	26.37	28.99	39.76	34.38
S ₅ : Foliar spray of Zinc @ 500 ppm at 30 DAS	17.80	17.92	17.86	20.40	20.64	20.45	26.65	30.75	28.70	30.42	41.77	36.10
S ₆ : Foliar spray of water at 30 DAS	17.61	17.67	17.64	20.43	20.59	20.43	22.01	24.06	23.04	26.02	31.86	28.94
Mean	18.11	18.09		20.96	21.07		24.02	27.09		28.21	36.66	
	SEm±	CD	CV (%)	SEm±	CD	CV (%)	SEm±	CD	CV (%)	SEm±	CD	CV (%)
Main	0.04	NS	2.91	0.09	NS	2.06	0.01	0.04	3.12	0.060	0.36	3.85
Sub	0.13	0.39	1.80	0.12	0.36	1.43	0.11	0.33	2.08	0.101	0.30	2.77
Interactions	0.19	NS		0.17	NS		0.16	0.47		0.143	0.42	

Conclusion

In the present study, foliar spray of zinc @ 500 ppm at 30 DAS increased both the proline content and SOD activity, thereby effectively regulating the osmosis and antioxidant defence system. Hence, the use of zinc spray in mungbean plants grown under water stress condition from flowering stage counteracted the deleterious effects of stress on growth and development of plants.

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