Effect of zinc application on water content and photosynthetic pigments of mungbean (Vigna radiata L.) under water 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 relative water content (RWC) and photosynthetic pigments in mungbean under moisture 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 plants that were subjected to water stress from flowering stage decreased the RWC, total chlorophyll and carotenoid contents by 10.0, 7.5 and 12.6 per cent, respectively, over control plants. Among zinc treatments, Zinc foliar spray @ 500 ppm at 30 DAS increased the RWC, total chlorophyll and carotenoid contents by 8.1, 10.2 and 6.1 per cent respectively, over untreated plants. It can be concluded that the use of zinc spray @ 500 ppm at 30 DAS counteracted the negative effects of water stress on mungbean by maintaining high water potential and by actively synthesizing the photosynthetic pigments.

Keywords: Relative water content, chlorophyll, carotenoid content, zinc and water stress

Introduction
Mungbean is an excellent source of high quality protein in vegetarian diet of Indian population and is particularly preferred for invalids and infants for its easy digestibility and non flatulence protein. It is cultivated in India since ancient times. The productivity of mungbean is very low, because it is grown in marginal and sub marginal lands with low fertilizer under rainfed conditions. It is frequently grown where soil moisture is a limiting factor for successful crop production. It is cultivated throughout grown in an area of about 0.27 million hectares, with a production of 0.18 million tonnes and with an average productivity of 651 kg ha⁻¹ (Ministry of Agriculture, 2016-17).

Zinc 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., 2013) [2]. It plays a significant role in regulating stomatal opening and closing and ionic balance in crops and reduces the detrimental effects of drought (Moghadam et al., 2013) [3]. The application of zinc under drought conditions would influence crop yield and quality. Therefore, the present investigation was undertaken to study the effect of seed pre-treatment and foliar application of zinc on relative water content, chlorophyll and carotenoid contents in mungbean plants under water stress.

Material and methods
The experiment was conducted during Rabi, 2017-18 at Agricultural college farm, Bapatla. The experiment was laid out in a Split Plot Design, replicated thrice with a plot size of 12

~ 183 ~
square meter and the row spacing of 30 cm and intra row spacing of 10 cm. Sowing was done by dibbling and recommended dose of fertilizers were applied and other package of practices were followed to raise a healthy crop. Prophylactic measures were adopted against pests and diseases.

**Relative water content**

Relative water content of leaf samples collected for different treatments was determined by following the method described by Slatyer and Mcilroy (1967) [11]. Fully expanded and matured leaves were excised from the plants and the fresh weight was recorded immediately. The leaves were then immersed in water for attaining maximum turgidity and then gently blotted before taking turgid weight. Afterwards, leaves were oven dried at 80°C for two days and dry weight of leaves was recorded. The recorded values were substituted in the following formula to determine the relative water content.

\[
RWC(\%) = \left(1 - \frac{W_f - W_d}{W_t - W_d}\right) \times 100
\]

Where

- \(W_t\) = Weight of fresh leaves
- \(W_f\) = Weight of turgid leaves
- \(W_d\) = Weight of oven dried leaves

**Chlorophyll and carotenoid contents**

Total chlorophyll content in leaves was estimated calorimetrically by dimethyl sulphoxide (DMSO) method as described by Hiscox and Stam (1979) [3]. A sample of 300 mg of sliced leaf material was taken and immersed in stoppered test tubes containing 10 ml of pure dimethyl sulphoxide (DMSO). Then test tubes were kept in hot air oven maintained at 60-65°C for 3 hours. The optical densities of the supernatant liquid were taken at 480, 510 and 652 nm separately using Spectrophotometer Systronics (Model 105) and then total chlorophyll and carotenoid contents were calculated by the following formulae (Arnon, 1949) [3].

\[
\text{Carotenoids (mg g}^{-1}\text{)} = 7.6 \times 1000 \times \frac{V}{D \times 480 - 1.49 \times D \times 510 - 34.5 \times 1000 \times W}
\]

\[
\text{Total chlorophyll (mg g}^{-1}\text{)} = \frac{652 \times 1000}{34.5 \times 1000 \times W}
\]

Where

- \(D\) = Optical density
- \(V\) = Final volume of DMSO
- \(W\) = Fresh weight of sample taken

**Results and Discussion**

**Relative Water Content (%)**

Relative water content (RWC) is an important characteristic feature that affects plant water relations. The data pertaining to the effect of water stress and zinc treatments on the relative water content (RWC) of mungbean leaves recorded at 15 days interval from 15 to 60 DAS were presented in Table 1. Significant difference was observed between main treatments after imposition of water stress i.e. from flowering. At 45 and 60 DAS, relative water content in leaves of mungbean plants significantly decreased with drought stress from flowering stage \((M_1 = 71.04, 63.66\%), respectively\) compared to control i.e. irrigated plants \((M_0 = 72.98, 70.62\%), respectively\). Water stress from flowering stage decreased the RWC by 2.7 and 9.9 per cent at 45 and 60 DAS, respectively, compared to normal irrigation. The obtained results in the present study were in accordance with those reported by Sofy, (2015) [12] in wheat, who reported that with delay in irrigation interval, relative water content significantly decreased. Similar trend was also observed in bean plants under water stress by Yadavi et al. (2014) [13]. Plants grown under water stress conditions decrease the intracellular water by increasing the concentration of osmotic compounds to absorb water from the soil powerfully. It seems, there is a direct relationship between the soil moisture content and relative water content of leaf, so that reduction in soil moisture and increasing water stress reduce relative water content of leaf (Khan et al., 2007) [8].

Significant differences were noticed among the sub treatments at 15, 30, 45 and 60 DAS. At 15 and 30 DAS, seed pre-treatment with zinc @ 0.075% before sowing recorded higher RWC \((S_2 = 80.52, 79.16\%), respectively\) compared to seed pre-treatment with zinc @ 0.05% before sowing \((S_1 = 76.91, 75.38\%, respectively\) and other treatments. All other treatments except \(S_p\) were at par with each other. The treatment \(S_y\) was superior over other treatments and inferior to \(S_x\). Shahri et al. (2012) [10] stated that, foliar application of zinc sulphate @ 1.0% two weeks before and two weeks after flowering had more leaf relative moisture in sunflower. At 45 and 60 DAS, the highest RWC was recorded by foliar spray of zinc @ 500 ppm at 30 DAS \((S_y = 73.55, 70.00\%, respectively\), followed by zinc foliar spray @ 400 ppm \((S_x = 72.97\%)\) at 45 DAS, and seed pre-treatment with zinc @ 0.075% before sowing \((S_z = 69.09\%)\) at 60 DAS. The lowest RWC was recorded by untreated plants i.e. no zinc treatment \((S_0 = 70.41, 64.73\%)\) at 45 and 60 DAS, respectively. Foliar application of zinc @ 500 ppm at 30 DAS increased the leaf RWC by 4.5 and 8.1 per cent, 400 ppm zinc spray by 3.6 and 5.2 per cent and seed pre-treatment with zinc @ 0.075% before sowing by 3.1 and 6.7 per cent, at 45 and 60 DAS, respectively, over control plants. Zinc foliar application @ 0.3% increased the RWC of leaves in wheat (Yavas and Unay, 2016) [16].

Among the interactions, significant difference was observed at 45 DAS only. The highest RWC was obtained with foliar application of zinc @ 500 ppm at 30 DAS to the control plants i.e. normal irrigation \((M_{12} = 74.61\%), whereas the lowest RWC was recorded by the plants that were subjected to water stress from flowering stage with no zinc treatment \((M_{10} = 69.46\%)\) at 45 DAS. The plants that were stressed from flowering stage sprayed with zinc @ 500 ppm recorded significantly higher RWC \((M_{15} = 72.49\%)\) compared to normal irrigated plants with no zinc application \((M_{01} = 71.35\%). Akbari et al. (2013) [3] also reported that, the RWC in cumin leaves decreased under water stress and the high amount of RWC was recorded in Zn + Fe foliar application in both irrigated and non-irrigated conditions. In the present study, foliar spray of zinc @ 500 ppm at 30 DAS exhibited superior performance by recording high RWC both under irrigated as well as water stress conditions. Hence, zinc application mitigated the adverse effects of drought stress in mungbean. The application of zinc under drought conditions influences crop yield and quality. It plays a significant role in regulating stomata and ionic balance in crops, thereby
reducing the detrimental effects of drought (Moghadam et al., 2013) [8].

**Total Chlorophyll (mg g⁻¹)**

Total chlorophyll content of mungbean leaves was affected by water stress and zinc treatments and data was furnished in Table 2. Significant differences were observed among the main treatments after imposition of water stress. At 45 and 60 DAS, the plants that were stressed from flowering stage recorded lesser total chlorophyll content (M₁ - 1.463 and 1.569 mg g⁻¹, respectively) compared to unstressed plants i.e. control (M₀ - 1.593 and 1.697 mg g⁻¹, respectively). Compared to the control plants (i.e. no stress), the total chlorophyll content was reduced by 7.5 per cent in the plants that were stressed from flowering stage. Thalooth et al. (2016) stated that, withholding one irrigation at any growth stage (i.e. at vegetative, flowering and pod formation) decreased the content of chlorophyll a and b and carotenoids in the leaves of mungbean plants. Such decrease in chlorophyll content in the leaves of mungbean plants may be attributed to the high rate of chlorophyll degradation more than its biosynthesis under water stress conditions.

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 total chlorophyll content (S₂ - 0.715 and 1.287 mg g⁻¹, respectively) compared to seed pre - treatment with zinc @ 0.05% (S₁ - 0.692 and 1.192 mg g⁻¹, respectively). The treatment S₁ recorded higher total chlorophyll compared to other treatments, but inferior to S₂ treatment. The remaining treatments were on par with each other, and lesser total chlorophyll content was recorded by S₀ (i.e. no zinc treatment – 0.644 and 1.107 mg g⁻¹, respectively). Zinc is essential for the biosynthesis of carbonic anhydrase enzyme required for chlorophyll biosynthesis (Rehman et al., 2012) [9]. Zinc application in maize improved photosynthetic rate, chlorophyll synthesis, metabolism of nitrogen and resistance to abiotic and biotic stresses (Yosefi et al., 2011) [17].

At 45 and 60 DAS, the highest total chlorophyll content was recorded by foliar application of zinc @ 500 ppm at 30 DAS (S₃ - 1.599 and 1.705 mg g⁻¹, respectively), whereas the lowest total chlorophyll content was recorded by the treatment where zinc was not applied (S₀ - 1.442 and 1.547 mg g⁻¹, respectively) which was at par with S₆ (i.e. foliar spray of water – 1.450 and 1.556 mg g⁻¹, respectively). Seed pre - treatment with zinc @ 0.075% before sowing (S₂ - 1.571 and 1.676 mg g⁻¹) recorded the total chlorophyll content which is in parity with the foliar spray of zinc @ 400 ppm (S₄ - 1.572 and 1.674 mg g⁻¹), at 45 and 60 DAS, respectively, and both the treatments came in the second order pertaining to total chlorophyll content. In the present study, foliar spray of zinc @ 500 ppm, seed pre - treatment with zinc @ 0.075% and zinc spray @ 400 ppm increased the total chlorophyll content by 10.2, 8.3 and 8.2 per cent, respectively over control (i.e. no zinc treatment). In the current study, increased total chlorophyll content is due to zinc, which acts as a structural and catalytic component of proteins, enzymes and as a co-factor for normal development of pigment biosynthesis (Balashouri, 1995) [9].

**Carotenoid Content (mg g⁻¹)**

The data pertaining to the influence of water stress and zinc treatments on carotenoid content of mungbean leaves were presented in Table 3. Significant difference was observed among the main treatments after imposition of water stress. At 45 and 60 DAS, the plants which were exposed to water stress from flowering stage recorded lesser carotenoid content (M₁ - 0.420 and 0.410 mg g⁻¹, respectively) compared to unstressed plants i.e. control (M₀ - 0.455 and 0.469 mg g⁻¹, respectively).

The carotenoid content decreased by 12.6 per cent in the plants that were subjected to water stress from flowering stage, compared to control plants (i.e. no stress) at 60 DAS. Thalooth et al. (2006) [13] stated that withholding one irrigation at any growth stage (i.e. at vegetative, flowering and pod formation) decreased the content of chlorophyll a and b and carotenoids in the leaves of mungbean plants. Such decrease in chlorophyll content in the leaves of mungbean plants may be attributed to the high rate of chlorophyll degradation more than its biosynthesis under water stress conditions.

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 carotenoid content (S₂ – 0.202 and 0.298 mg g⁻¹, respectively) compared to seed pre - treatment with zinc @ 0.05% (S₁ – 0.192 and 0.292 mg g⁻¹, respectively). The treatment S₁ recorded higher carotenoid content compared to other treatments, but inferior over S₂ treatment. The remaining treatments were on par with each other at 15 and 30 DAS. At 45 DAS, the highest carotenoid content was recorded by foliar application of zinc @ 500 ppm (S₃ – 0.444 mg g⁻¹) followed by seed pre - soaking with zinc @ 0.075% before sowing (S₂ – 0.444 mg g⁻¹) and zinc spray @ 400 ppm (S₄ – 0.442 mg g⁻¹), whereas the lowest carotenoid content was recorded by the treatment where zinc was not applied (S₀ – 0.426 mg g⁻¹). Foliar spray of water recorded significantly higher carotenoid content (S₃ – 0.431 mg g⁻¹) compared to control (S₀), but lesser carotenoid content when compared to other zinc treatments.

At 60 DAS, the highest carotenoid content was recorded by foliar application of zinc @ 500 ppm at 30 DAS (S₃ – 0.451 mg g⁻¹), which was at par with seed pre - soaking with zinc @ 0.075% before sowing (S₂ – 0.449 mg g⁻¹), followed by zinc spray @ 400 ppm (S₄ – 0.445 mg g⁻¹) which was on a par with seed pre - soaking with zinc @ 0.05% (S₁ – 0.442 mg g⁻¹), whereas the lowest carotenoid content was recorded by untreated control (S₀ – 0.425 mg g⁻¹) and it is on a par with water spray (S₀ – 0.429 mg g⁻¹). In the present study, foliar spray of zinc @ 500 ppm and seed pre - treatment with zinc @ 0.075% exhibited superior performance in increasing the carotenoid content by 6.1 and 5.6 per cent, respectively over control (i.e. no zinc treatment). Similar increase in carotenoid content in mungbean leaves was also reported earlier by Thalooth et al. (2006) [13].

### Table 1: Effect of zinc on relative water content (RWC) (%) of mungbean under water stress

| Treatments                  | 15 DAS            | 30 DAS            | 45 DAS            | 60 DAS            |
|-----------------------------|-------------------|-------------------|-------------------|-------------------|
|                            | Mo | Mi | Mean | Mo | Mi | Mean | Mo | Mi | Mean | Mo | Mi | Mean |
| S₀: No Zinc application     | 73.77 | 73.18 | 73.47 | 72.85 | 71.74 | 72.30 | 71.35 | 69.46 | 70.41 | 68.45 | 61.02 | 64.73 |
| S₁: Seed treatment with Zinc @ 0.05% before sowing | 77.19 | 76.63 | 76.91 | 74.67 | 76.09 | 75.38 | 72.61 | 70.61 | 71.61 | 70.28 | 65.42 | 66.85 |
| S₂: Seed treatment with Zinc @ 0.075% before sowing | 80.26 | 80.78 | 80.52 | 78.31 | 80.00 | 79.16 | 73.80 | 71.34 | 72.57 | 72.48 | 65.70 | 66.09 |
| S₃: Foliar spray of Zinc @ 300 ppm at 30 DAS | 73.70 | 73.95 | 73.83 | 70.89 | 72.99 | 71.94 | 72.76 | 70.73 | 71.75 | 69.48 | 62.70 | 66.09 |

~ 185 ~
S2: Foliar spray of Zinc @ 400 ppm at 30 DAS
S3: Foliar spray of Zinc @ 500 ppm at 30 DAS
Sc: Foliar spray of water at 30 DAS

Table 2: Effect of zinc on total chlorophyll content (mg g⁻¹) of mungbean leaves under water stress

| Treatments | 15 DAS | 30 DAS | 45 DAS | 60 DAS |
|------------|--------|--------|--------|--------|
| Mo | Mi | Mean | Mo | Mi | Mean | Mo | Mi | Mean | Mo | Mi | Mean |
| S2: No Zinc application | 0.641 | 0.646 | 0.644 | 1.093 | 1.121 | 1.107 | 1.513 | 1.371 | 1.442 | 1.618 | 1.477 | 1.547 |
| S2: Foliar spray of Zinc @ 0.05% before sowing | 0.629 | 0.692 | 0.692 | 1.188 | 1.199 | 1.192 | 1.592 | 1.458 | 1.525 | 1.694 | 1.569 | 1.631 |
| S2: Foliar spray of Zinc @ 0.075% before sowing | 0.713 | 0.716 | 0.715 | 1.294 | 1.279 | 1.287 | 1.638 | 1.504 | 1.571 | 1.743 | 1.608 | 1.676 |
| S2: Foliar spray of Zinc @ 300 ppm at 30 DAS | 0.635 | 0.660 | 0.647 | 1.100 | 1.118 | 1.109 | 1.596 | 1.477 | 1.537 | 1.700 | 1.583 | 1.642 |
| S2: Foliar spray of Zinc @ 400 ppm at 30 DAS | 0.642 | 0.663 | 0.653 | 1.101 | 1.122 | 1.111 | 1.631 | 1.513 | 1.572 | 1.735 | 1.613 | 1.674 |
| S2: Foliar spray of Zinc @ 500 ppm at 30 DAS | 0.638 | 0.648 | 0.648 | 1.097 | 1.120 | 1.108 | 1.663 | 1.535 | 1.599 | 1.767 | 1.643 | 1.705 |
| Sc: Foliar spray of water at 30 DAS | 0.661 | 0.648 | 0.654 | 1.116 | 1.125 | 1.121 | 1.517 | 1.382 | 1.450 | 1.621 | 1.491 | 1.556 |

Table 3: Effect of zinc on carotenoid content (mg g⁻¹) of mungbean leaves under water stress

| Treatments | 15 DAS | 30 DAS | 45 DAS | 60 DAS |
|------------|--------|--------|--------|--------|
| Mo | Mi | Mean | Mo | Mi | Mean | Mo | Mi | Mean | Mo | Mi | Mean |
| S2: No Zinc application | 0.183 | 0.179 | 0.181 | 0.278 | 0.277 | 0.278 | 0.439 | 0.413 | 0.426 | 0.435 | 0.395 | 0.425 |
| S2: Seed treatment with Zinc @ 0.05% before sowing | 0.193 | 0.191 | 0.192 | 0.293 | 0.290 | 0.292 | 0.454 | 0.418 | 0.436 | 0.472 | 0.413 | 0.442 |
| S2: Seed treatment with Zinc @ 0.075% before sowing | 0.201 | 0.203 | 0.202 | 0.300 | 0.296 | 0.298 | 0.464 | 0.425 | 0.444 | 0.478 | 0.421 | 0.449 |
| S2: Foliar spray of Zinc @ 300 ppm at 30 DAS | 0.182 | 0.181 | 0.182 | 0.280 | 0.278 | 0.279 | 0.455 | 0.415 | 0.435 | 0.465 | 0.407 | 0.436 |
| S2: Foliar spray of Zinc @ 400 ppm at 30 DAS | 0.185 | 0.180 | 0.183 | 0.279 | 0.279 | 0.279 | 0.460 | 0.424 | 0.442 | 0.475 | 0.414 | 0.445 |
| S2: Foliar spray of Zinc @ 500 ppm at 30 DAS | 0.176 | 0.181 | 0.179 | 0.280 | 0.274 | 0.277 | 0.466 | 0.429 | 0.448 | 0.481 | 0.421 | 0.451 |
| Sc: Foliar spray of water at 30 DAS | 0.181 | 0.182 | 0.182 | 0.280 | 0.276 | 0.278 | 0.447 | 0.414 | 0.431 | 0.458 | 0.399 | 0.429 |

Fig 1: Effect of zinc on relative water content (RWC) (%) of mungbean under water stress
Conclusion
The current study revealed that seed pretreatment and foliar application of zinc on mungbean plants have counteracted the negative effects of water stress on relative water content, chlorophyll and carotenoid contents. Foliar spray of zinc @ 500 ppm at 30 DAS have shown better results compared to other treatments in mitigating the deleterious effects of water stress on mungbean from flowering stage.

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