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Response of black gram (*Vigna mungo*) to potassium under water stress condition

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ABSTRACT

To evaluate the impact of limited water availability and potassium fertilization on growth, antioxidative systems and pigment content in urad bean, plants were grown in pot culture during 2016-2017. The treatments were based on a randomized complete block design (RCBD) with three replications. Plants were supplied with four moisture regimes (400 ml, 200ml, 100ml and 50ml of water). Control plants were supplied with 500ml of water. Potassium was applied in the form of 200ppm KCl and KNO_3. Plants were observed from 25 upto 55 days of plant growth at 10-day interval for different treatments. Decrements in growth parameters (viz. shoot length, number of leaves and fresh weight) and chlorophyll stability index were observed due to water stress, and maximum decrement was observed when plants were supplied with 50 ml water. While antioxidant enzymes (viz. POD and CAT) and carotenoid content increased with increasing level of stress (50 ml water) as compared to control. Growth parameters and CSI increased while antioxidant enzyme activity and carotenoid content decreased upon foliar treatment with KCl and KNO_3, due to maintenance of favorable internal tissue moisture. However, the effect of KNO_3 was more prominent than that of KCl.

Keywords: Water stress, growth, antioxidant enzymes, pigment content, potassium.

INTRODUCTION

Pulses are recognized as a major source of vegetable protein with minerals and vitamins. They play a vital role in maintaining soil fertility by fixing atmospheric nitrogen. Black gram (*Vigna mungo* L. Hepper) is an important pulse crop which belongs to family Papilionaceae. This pulse legume has its origin in Indian subcontinent and is extensively cultivated all over India. The plants are used as green manure as it produces 66-70 kg/acre nitrogen. *Vigna* seeds are rich in protein (approx. 25%) and are used in various ways in Indian cuisine. Water is the basic need for the proper functioning of protoplasm of cell. Water stress affects the water status in plant. Plants are exposed to several abiotic stresses such as drought and salinity which has adverse effect on growth and development of plants. It is a recognized fact that the different physiological and metabolic processes in plant are affected by scarcity of water. Due to drought, there is a significant reduction in growth, chlorophyll, and water contents and different fluorescence parameters are changed (Ahmad *et al.* 2017). Functioning of stomata in photosynthesis and transpiration such as maintenance of plant turgor, photophosphorylation, and activation of enzyme and transportation of photo-assimilates is controlled by K^+. Down-regulation of photosynthetic carbon metabolism in turn leads to generation of excess excitation energy and formation of reactive oxygen species that induce damages to photosystems (Hajiboland, 2014). Potassium is a macro-nutrient and plays vital role in plant growth and development. Potassium has significant role in cell expansion, maintains the turgor pressure of plants, assists in osmoregulation of cell, helps in opening and closing of stomata and activates more than 60 enzymes. Increasing evidences indicate that plants under drought stress have a larger internal requirement for K^+. Under drought stress more K^+ is required for maintenance of photosynthetic CO_2 fixation, protection of chloroplasts from oxidative damage, impairment of associated disturbances in carbohydrate metabolism, regulation of stomatal opening and water relations (Cakmak, 2005). Improvement of K nutritional status of plants seems to be of great importance for sustaining high yields under water-deficit conditions (Bahrami and Hajiboland 2017). Drought stress tolerance requires the activation of complex metabolic activities including antioxidative pathways, especially Reactive Oxygen Species (ROS) and favor plant growth (Grzebisz *et al.* 2013). KNO_3

scavenging systems within the cells which can contribute to continued growth under drought stress. Considering the importance of black gram as a source of high protein, the present study was designed and implemented to determine the effects of foliar application of potassium on various physiological traits of this plant under drought stress.

MATERIALS AND METHODS

Seeds of urad bean plants were surface sterilized and inoculated with *Rhizobium phaseoli* and then germinated in growth chamber under controlled conditions (Temp. 25 ± 2ºC). Three days old plantlets were transplanted to earthen-ware pots containing garden soil. The pots were supplied with Hoagland's nutrient solution at weekly basis. Plants were treated with various regimes of water (400, 200, 100 and 50 ml). Control plants were supplied with 500 ml of water. Foliar application of KCl and KNO_3 (200 ppm) were given from 25 days of sowing at 15-day interval. Randomized complete block design (RCBD) was used with 3 replicates. The 6 samples were collected at every 10 day and various growth parameters such as shoot length, number of leaves, fresh weight, antioxidant enzymes such as catalase and peroxidase, chlorophyll stability index and carotenoid content were analyzed from 25 days up to 55 days of plant growth. The Catalase [CAT, E.C.1.11.1.6] activity and Peroxidase [POD, E.C.1.11.1.7] activity was determined in the primary leaves by the modified method of Chance and Maehly (1955) and Shannon *et al*., (1966) respectively. The enzyme activity was assayed by the *in-vivo* method. 200 mg of fresh leaves were cut into narrow strips and placed in 10 ml capacity vials containing 3 ml phosphate buffer (pH 6.8). The leaf strips were frozen for 3 h at -4℃ followed by thawing. For Catalase activity, the reaction was initiated by adding the 1.0 ml enzyme extract to 2.0 ml of 2.5 mM H_2O_2 for 10 min at 37℃ in an incubator. The reaction was stopped by adding 1.0 ml of 1% Titanic sulphate (in 2.5% H_2SO_4 w/v) and the mixture was centrifuged at 10,000 rpm for 15 minutes. For estimation of Peroxidase activity, the reaction was initiated by adding 1.0 ml enzyme extract to the assay mixture at 30℃. The assay mixture contained 1.0 ml of 15 mM pyrogallol, 1.0 ml of 50 mM H_2O_2 and 5.0 ml distilled water . This reaction mixture was then incubated for 15 minutes at 25℃ and incubation reaction was stopped by adding 0.5 ml of 5% H_2SO_4. The amount of color formed was determined by measuring the absorbance at 410 nm for CAT activity and the intensity of yellow colour was measured at 420 nm for POD activity. Chlorophyll and carotenoid content was measured in primary leaves by the method of Arnon (1949). Chlorophyll index was calculated as-

$$CSI = \frac{Chlorophyll\ content\ in\ treated\ plants}{Chlorophyll\ content\ in\ control\ plant}$$

Carotenoid content was estimated using following formula-

$$Carotenoid\ content = OD_{440} \times \frac{V}{196 \times W}$$

Where, V = Final volume of chlorophyll extract in 80% acetone.

W = Fresh weight of leaves in mg.

A two-way analysis of variance (ANOVA) of data for all attributes was calculated using Graph Pad Prism software. LSD (least significant difference) was used to compare the mean values of all treatments where F-test was found significant. LSD has been shown in figures using error bars.

RESULTS AND DISCUSSION

Growth parameters viz. shoot length, number of leaves and fresh weight (Fig.1) of plants decreased at rainfed condition at all the stages in variable moisture regimes as compared to control. These increased with foliar application of KCl and KNO_3 and increment was more in KNO_3 treated plants. Srivastava and Srivastava (2015) in *Stevia* reported an increase in plant height under drought upon foliar application with KNO_3. Our findings match with those of Shah *et al.* (2017) working on wheat plants. Ali *et al* (2014) also observed similar result in which fresh weight and shoot length of *Brassica napus* plants were negatively affected by water stress and positively with K application. It may be due to improvement of a plant's access to K which increases water uptake by the root cells, which in turn increases their osmotic potential and thereby allow extension growth. This in turn promotes access to other mineral elements (including nitrogen) and water, which also provides nitrate in addition to potassium, hence, promotion in growth was more in KNO_3

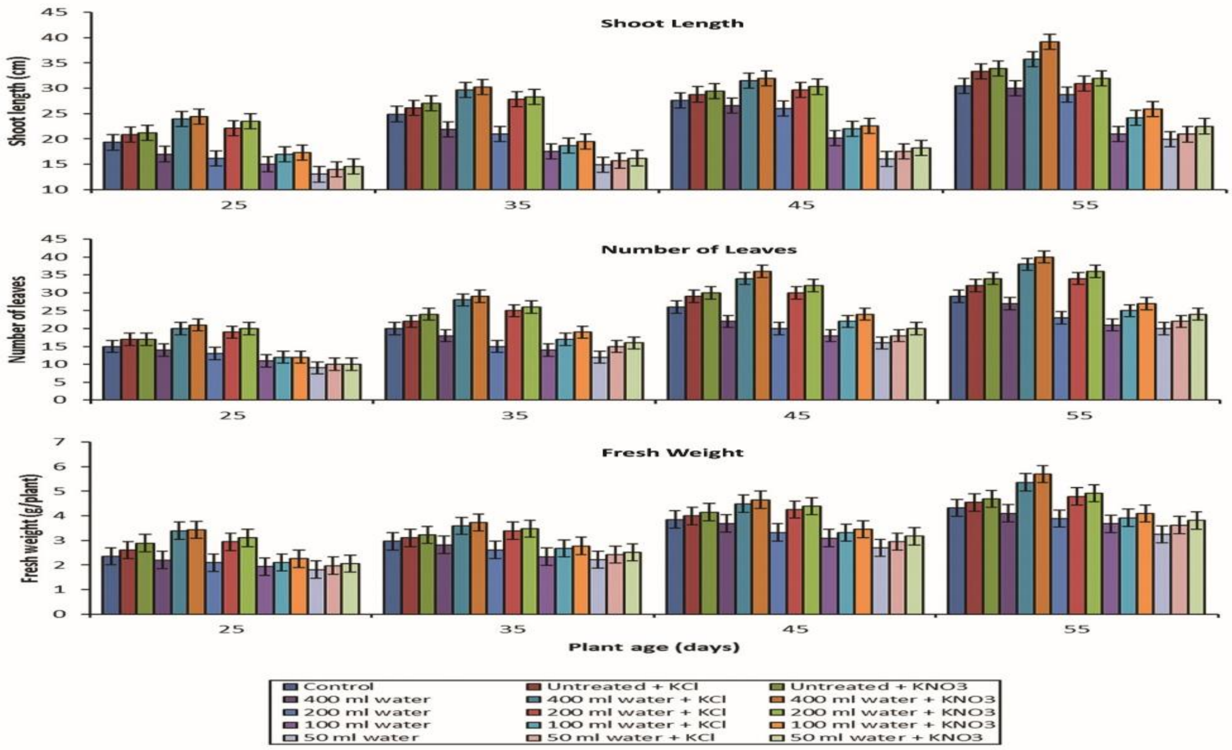

Fig. 1: Vigna mungo: Shoot length, number of leaves and fresh weight at different days of plant growth under water stress alone and in combination with KCl and KNO_3

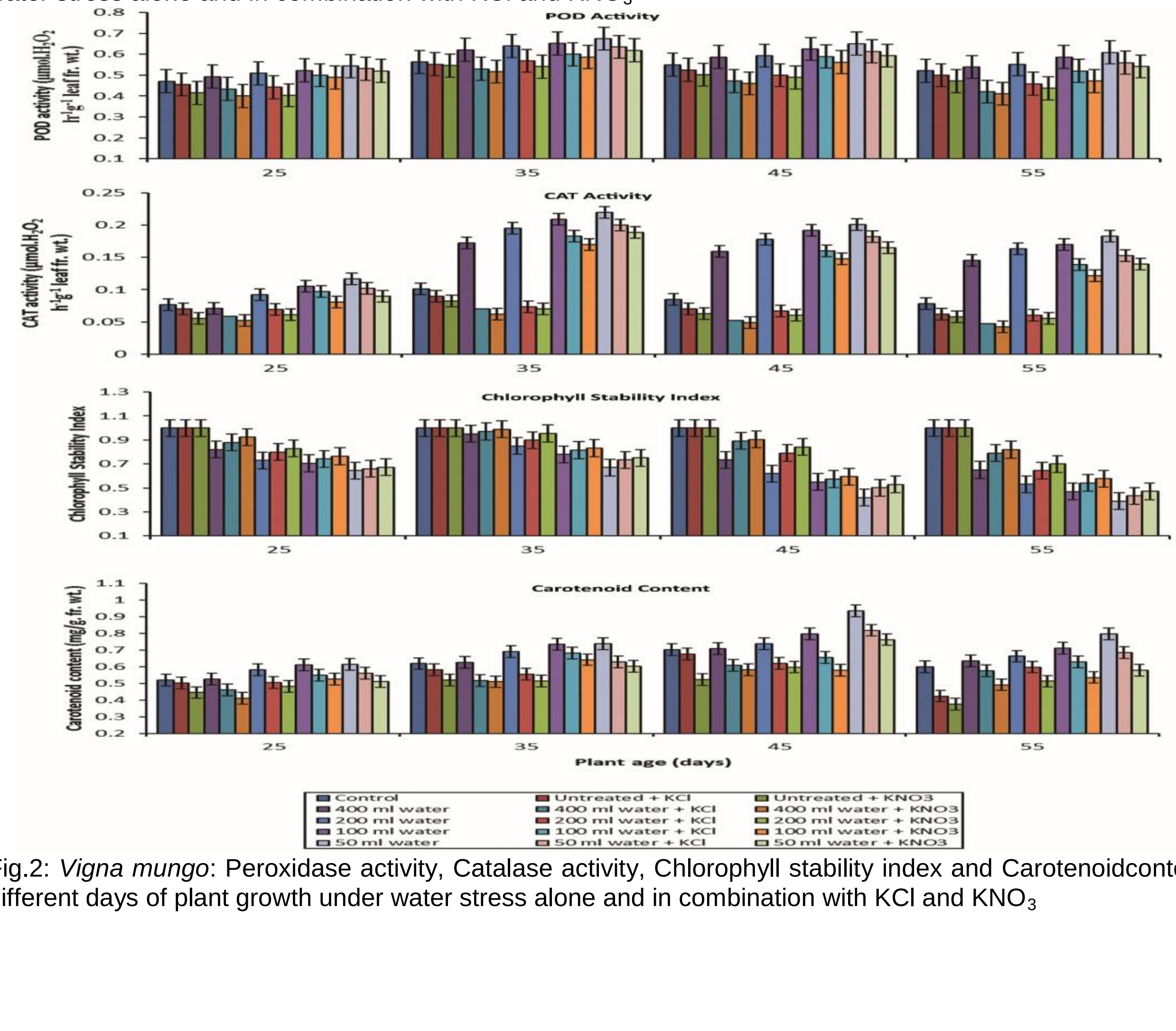

Fig.2: *Vigna mungo*: Peroxidase activity, Catalase activity, Chlorophyll stability index and Carotenoidcontent at different days of plant growth under water stress alone and in combination with KCl and KNO_3

treated plants as compared to KCl. This view is supported by a report of Son *et al* (2012). A significant variation was observed in antioxidant enzymes viz. peroxidase (POD) and catalase (CAT) in the leaves of water stressed plants. Enzyme activity showed a gradual increase from 25 days to 35 days followed by gradual decline. The enzyme activity was less in plants treated with KCl and KNO_3 in combination with water stress, due to maintenance of favorable internal tissue moisture (Fig 2). Similar results were obtained by Zain and Ismail (2016) in rice plants where the application of potassium reduced the CAT activity and indicate low oxidative stress of the plants. Zain *et al* (2014) have reported that in rice plants, CAT and POD activity increases under the different water stress levels to resist the impact of water stress. However, under the influence of K the CAT and POD activity decreases. The lower activity of antioxidant enzymes upon K application might be an indicator of removal of stressful condition. This indicates that defense mechanism was highest when K was applied on water stressed plant (Pyngrope *et al.* 2013). CSI gradually increased from 25 days to 35 days followed by gradual decline till 55 days. CSI decreased significantly as level of drought stress increased. The CSI was more in plants treated with KCl and KNO_3 in combination with water stress. Prominent increase in the CSI in KNO_3 treated stress plants was observed. Decrease in CSI under drought in cotton genotypes were observed by Anathi *et al* (2013). Decrease in photosynthetic pigments due to water stress could be attributed to drought induced deterioration of thylakoid membranes causing substantial damage to photosynthetic pigments (Anjum *et al*. 2011). Treatment of the black gram plants with potassium improved chlorophyll concentration over the untreated control regardless of water stress treatment (Sharma *et al*., 2017). Carotenoid content gradually increased from 25 days upto 55 days with increasing level of stress. Plants treated with KCl and KNO_3 showed comparatively less increase in carotenoid content (Fig. 2). Abid *et al* (2018) reported an increase in enzymatic and non-enzymatic antioxidant (carotenoid) under drought. Carotenoids are accumulated in most of the plants during stress as they are lipophilic secondary metabolites, derived from the isoprenoid pathway. Improvement of stressful condition upon foliar treatment of K might be most probable reason of decrease in carotenoid content.

The results of the present study suggest that foliar application of potassium improved growth parameters, antioxidant enzyme activity and pigment content enabling plants to perform better under drought conditions. K foliar application on the optimal level can ameliorate the extreme losses imparted by the water stress by improving pigment content and antioxidative systems (enzymatic and non-enzymatic) which consequently improves growth of the plants even under limited water availability.

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