Amelioration of drought stress in black matpe bean (Vigna mungo L.) by foliar application of potassium

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ABSTRACT

Growth, physiological and biochemical activities of black matpe bean grown in pot cultures were evaluated to recognize the ameliorative role of potassium against the deleterious effects of water deficit. Black matpe plants were subjected to four levels of water stress (400 ml, 200 ml, 100 ml and 50 ml). The control plants were supplied with 500 ml of water at regular intervals. Potassium was applied in form of 200 ppm KCl and KNO₃. Plants were under observation starting from 25 up to 55 days of plant growth at 10-day interval for each treatment. Water deficit stress reduced biomass, leaf area and RWC and provoked oxidative stress in plants as confirmed by considerable increase in electrolyte leakage, proline and reducing sugar content. Foliar application of KCl and KNO₃ improved all these attributes under water stress. The ameliorative effect might be maintained through decrease in proline and increase in reducing sugar content. Increased reducing sugar content in K treated plants may have a significant role in osmotic adjustment.

Key words: Biomass, Black matpe, Metabolites, RWC, Water deficit.

INTRODUCTION

India is a developing country where a large population is vegetarian and food legumes, mainly the grains or pulses are an important constituent of the diet. Pulses are cheap source and have a high nutrient content including starch, protein dietary fiber, oligosaccharides, phytochemicals and minerals (Borade et al., 1984). Vigna mungo (L.) Hepper, commonly known as black matpe or blackgram, contributes 20% to overall world pulse production and in India it is the third important pulse crop (Arubalalachandran et al., 2010). However, production of black matpe is adversely affected by various environmental stress factors, especially drought that reduce yield (Pandey et al., 2014).

Water stress is one of the most important abiotic stress that regulate plant growth and development, and limit plant production and yield of plants around the world (Mahantesh et al., 2018). Water stress is detrimental to plant growth and results in marked alteration in its morphological features such as reduction in shoot and root length, leaf area and total biomass production (Aldesuquy et al., 2012). Severe water stress reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, nutrient metabolism and growth promoters (Azadeh et al., 2014). Drought stress causes stomatal closure and reduces leaf area (Kumudini, 2010); consequently, decreasing photosynthetic activity. Thus, a common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Lisar et al., 2012).

Proline accumulation is responsible for the hydration of biopolymers surviving as a readily utilisable energy source and serving as a nitrogen source compound during periods of inhibited growth (Kala and Godara, 2011). Proline accumulation is believed to play adaptive role in plant stress tolerance (Mafakheri et al., 2010).

Reducing sugar is an important constituent and source of energy for all living organisms, plants manufacture this organic substance during photosynthesis and breakdown during respiration (Seyyednejad and Koochak, 2011). Under drought conditions, the accumulation of reducing sugar seems to be associated with drought tolerance in many plant species, reducing sugars also contribute in improving drought tolerance of peas, sugar beets and black poplars (Li et al., 2011). The accumulated sugars in the cell under stress, balances the osmotic strength of the cytosol with that of the vacuole and the external environment (Abdalla, 2011).

Plants have developed a wide range of mechanisms to resist a variety of stressed conditions. Increasing evidences suggest that mineral nutrients play a critical role in plant stress resistance (Romheld and Kirkby, 2010; Marshner, 2012). Out of all the mineral nutrients, potassium (K) plays a particularly a critical role in plant growth and metabolism, and it contributes greatly to the survival of plants that are under various biotic and abiotic stresses. Potassium (K) plays a major role in plant metabolism, growth, development, and yield (Wang et al., 2013). Foliar feeding is an effective method for correcting soil deficiencies and overcoming the soil’s inability to transfer nutrients to the plant under low

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moisture conditions (Stigler et al., 2010). It is an important tool for sustainable and productive management of crops. It is more environment friendly, immediate and target oriented than soil fertilization since nutrients can be directly delivered to plant tissues during critical stages of plant growth. Application of potassium fertilizers can increase the tolerance of plants under water stress (Zain and Ismail, 2016).

The present study aimed to evaluate and assess whether KCl and KNO₃ can ameliorate the deleterious effects of water deficit stress on black matpe through monitoring the growth, RWC, electrolytic leakage, proline and reducing sugar content.  

**MATERIALS AND METHODS**

Surface sterilized and *Rhizobium* inoculated seeds of black matpe were sown in earthenware pots containing garden soil during summer season. Hoagland’s nutrient solution was supplied to the plantlets from third day of germination at every 7-day intervals. Plants were supplied with different regimes of water (i.e. 400, 200, 100 and 50 ml) and Control plants were supplied with 500 ml of water at regular intervals. Foliar spray of KCl and KNO₃ solution (200 ppm) was given from 25 days of sowing at 15-day interval individually. Experiment was designed as Randomized complete block design (RCBD) with 3 replications.

The collection of samples was done at every 10-day and the parameters were analyzed from 25 days up to 55 days of plant growth. Oven dried plants (60±2°C) were used for estimation of biomass. Leaf area (LA) was calculated by the method of Singh (1970).

\[
LA = \text{Length} \times \text{Width} \times 0.877 \quad \text{(constant)}
\]

Relative water content was calculated as described by Schonfeld et al., (1988). Leaf samples were washed in distilled water and weighed after blot drying it with filter paper to determine the fresh weight (fw). Then leaves were soaked in distilled water for about 4h at room temperature in the diffused light. The turgid leaf samples were blotted dry and weighed to obtain the turgid weight (tw). All leaf samples were then dried at 65°C in an oven to a constant dry weight (dw). The RWC was calculated by the following formula –

\[
\text{RWC} = \frac{\text{Fresh weight (fw) - Dry weight (dw)}}{\text{Turgid weight (tw) - Dry weight (dw)}} \times 100
\]

20 leaf discs were taken in a boiling tube containing 10 ml of deionized water and Electrical conductivity (EC₀) was measured. The contents were then heated at 45-55°C for 30 min in a water bath and electrical conductivity (ECₐ) was measured. Later the contents were boiled at 100°C for 10 min and Electrical conductivity (ECₐ) was recorded again. The Electrolyte leakage was calculated by using following formula as described by Sullivan and Ross (1979).

\[
\text{Electrolyte leakage (\%)} = \frac{\text{ECₐ} - \text{EC₀}}{\text{EC₀}} \times 100
\]

Proline was estimated by the method of Bates et al., (1973). 250 mg of dried leaf tissue was homogenized in pestle-mortar with 10 ml of aqueous sulfosalicylic acid. The homogenate was filtered through Whatman No. 2 filter paper. Extraction was repeated and the filtrate was pooled. To 2.0 ml of filtrate was added 2.0 ml of acid ninhydrin (1.25g of ninhydrin in 30 ml of warm glacial acetic acid and 20 ml of 6 M phosphoric acid was mixed with agitation, the reagent being stable for 24 h when stored at 4°C) and 2.0 ml of glacial acetic acid incubated for one hour at 100°C on water bath. The tubes were transferred to an ice bath to terminate the reaction. Added 4 ml of toluene and mixed vigorously using a test tube stirrer for 15 to 20 seconds. Aspirated the chromatophore containing toluene from the aqueous phase. The chromatophore containing toluene was aspirated from the aqueous phase, thawed to room temperature and the absorbance was measured at 520 nm.

Total reducing sugar was estimated by Somogyi’s method (1952). 50 mg dried leaves of each sample were crushed in 10 ml of distilled water, the extract was filtered through 4 layers of cheese cloth and was centrifuged at 10,000 rpm for 15 minutes. To 2.0 ml of supernatant was added, 2.0 ml of Somogyi’s solution and then the mixture was incubated at 100°C for 10 minutes in water bath. After cooling 2.0 ml of Nelson’s arsenomolybdate reagent was added. The absorbance was measured at 570 nm and the amount of total reducing sugar was calculated with the standard curve prepared from glucose.

The data was analyzed statistically. Least Significant Difference (LSD) has been calculated for data where F test was found significant.

**RESULTS AND DISCUSSION**

The biomass of *Vigna* plants increased from day 25 up to day 55. The biomass was more in control plants than in stress plants with its lowest value in plants treated with 50 ml water (Fig 1). These results are in agreement with those obtained by Mahantesh et al. (2018). Reduction in plant biomass may be due to decrease in plant growth, photosynthesis and canopy structure during the water stress as reported by Shao et al. (2008). However, the biomass of plants increased on treatment with KCl and KNO₃ but the increase was prominent in KNO₃ treated plants with different water levels. This increase in biomass may be due to the fact that sufficient nutrient is available for plant growth and the plants will have good vegetative growth. Plants treated with different potassium levels showed better results under drought conditions resulting in enhanced growth and biomass production. (Zahid et al., 2013).

The leaf area decreased with the decrease in water level having the minimum value in plants treated with 50 ml water (Fig 2). Similar result was obtained by Merwad et al. (2018) in *Vigna unguiculata*. However, an increment was observed in the leaf area upon treatment with KCl and KNO₃.
but the increase was more prominent in KNO$_3$ treated plants at all water levels. Pettigrew (2008) reported that K is one of the principal plant nutrients that increases the size of individual leaves.

Relative water content declined significantly with the severity of stress with maximum decrease in plants treated with 50 ml water (severe stress) having minimum RWC (Fig 3). Foliar application of potassium increased RWC in both stress as well as control plants. KNO$_3$ treated *Vigna* plants retained more RWC at all water levels as compared to KCl treated ones. Potassium is an important macronutrient and osmoticum which helps plants to adjust to low water potential under drought stress (Bukhsh et al., 2012). Hence, under drought conditions, the accumulation of potassium increases in plant tissues that improves uptake of water from soil to plants. Potassium is accumulated in plants due to decrease in soil water potential. Consequently, the water potential in the plant root further lowers, and uptake of water continues. In conditions of water stress, potassium maintains the turgor of the plant cell and regulates stomatal functions (Waraich et al., 2011).

Electrolyte leakage was more in stressed plants as compared to control plants with maximum electrolyte leakage in severely stressed plants (50 ml water) followed by moderate and mild stress. The control plants exhibited minimum electrolyte leakage (Fig 4). These results are similar to the findings of Noori et al. (2018) in tomato cultivars and Merwad et al. (2018) in *Vigna* sp under water stress. Cell membrane stability significantly declined under drought stress (Wang and Huang 2004). However, the electrolyte leakage decreased on application of potassium at different water levels. The electrolyte leakage decreased more in KNO$_3$ treated stress plants. Premachandra et al. (1991) reported in maize plants that higher K$^+$ application...
showed greater adaptation to water stress. This improvement was mainly attributed to the role of $K^+$ in improving cell membrane stability and osmotic adjustment ability. An adequate $K^+$ supply is essential to enhance drought resistance by increasing root elongation and maintaining cell membrane stability.

A significant variation in proline content in leaves of plants treated with different water levels was observed. Proline content increased from day 25 up to day 45 followed by gradual decline up to day 55. Highest proline content was found in plants treated with 50 ml water (severe stress) followed by moderate and mild stress plants at 45 days. Proline content was less in KCl and KNO$_3$ treated plants but KNO$_3$ treated plants exhibited minimum proline content (Fig 5). This result coincides with reports of Selahvarzi et al., (2008) in ornamental turf grasses and Shooshtarian (2010) in ten species of ground cover plants.

Total reducing sugar content in the leaves increased from day 25 up to day 45 followed by gradual decrease up to day 55. The reducing sugar content was more in stress plants with maximum in plants treated with 50 ml and 100 ml water (Fig 6). This might be due to the fact that under water stress condition the breakdown of polysaccharides caused an accumulation of soluble sugars which help in maintenance of turgor (Nazarli et al., 2011). Foliar application of KCl and KNO$_3$ further increased the reducing sugar content in stress as well as control plants with a maximum increase in KNO$_3$ treated plants. Since $K$ plays crucial role in enhancing the rate of photosynthesis, so soluble sugars increase upon the application of $K$.

![Fig 3: Relative Water Content in Vigna mungo leaves at different days of plant growth under water stress alone and in combination with KCl and KNO$_3$.](image)

![Fig 4: Electrolyte Leakage in Vigna mungo leaves at different days of plant growth under water stress alone and in combination with KCl and KNO$_3$.](image)
The results of this study signify the role of K in regulating water stress response in black matpe, and suggest that KNO₃ acts as a potential growth enhancer to improve plant growth, physiological and biochemical performance. KCl and KNO₃ provoked reduction in oxidative stress in plants subjected to water deficit stress. In the present study we concluded that reducing sugar plays an important role in the osmotic adjustment in stressed plants and thus protects the membrane against oxidative stress. This protective effect led to a decrease in the proline content, which is a critical determinant under water deficit condition. These results indicate that black matpe could be possibly cultivated in moderate drought stress due to its capacity for osmotic adjustment.

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