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**Research Article** 



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# Effect of Water Stress on Morphological, Physiological and Biochemical Parameters in Seedlings of *Vigna mungo* (L.) Hepper

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#### Abstract

Abiotic stresses, including drought, chilling, heat, and flooding, are prevalent in nature and can substantially diminish crop yields. Stresses involving water deficit may arise from drought conditions, saline soils, or low temperature. Measuring the water status of the plant is important for determining the impact of the environmental condition. In the present investigations, effect of water deficit has been studied on morphological, physiological and biochemical parameters in seedlings of *Vigna mungo* (L.) Hepper. Most of the parameters *i.e.* shoot growth, root growth, leaf area, relative leaf water content (RLWC), photosynthetic pigments (chl *a*, *b*, carotenoids) exhibited a decline with an increase in water stress treatment except the catalase activity which displayed increasing trend. Stress-induced changes in metabolism and development can be attributed to altered patterns of gene expression.

Keywords: ABA, abiotic stress, drought, RLWC, ROS

## Introduction

Under both natural and agricultural situations, plants are often subjected to environmental stresses. Stress plays an important role in determining how soil and climate restrict the distribution of plant species. Plants grow and reproduce in hostile environments containing large numbers of abiotic chemical and physical variables, which differ both with time and geographical location. Fluctuations of these abiotic factors generally have negative biochemical and physiological impact on plants. Responses to abiotic stress depend on the extremity and time duration of the stress, developmental stage, tissue type, and interactions between multiple stresses. Experiencing stress typically promotes alterations in gene expression and metabolism, and reactions are frequently centred on altered patterns of secondary metabolites. The primary abiotic environmental factors that affect plant growth are water, temperature, light, available water, soil aeration, soil salinity and heavy metals. Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and the natural status of the environment. Increased salinization of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year

2050 (Bray et al., 2000).Water is one of the most important environmental factors regulating plant growth and development. Drought is a major stress that disrupts metabolic processes and constraints plant growth and development and thus limiting the crop productivity (Carrow, 1996: Crasta & Cox 1996: Dean et al., 1996; Faver et al., 1996; Pustovoitova et al., 1996; Chaves et al., 2003). The negative effects of drought include reduced plant growth (Delgado et al., 1992; Ohashi et al., 2000), photosynthesis (Boyer, 1970; Ogen & Öquist, 1985), cell growth (Bohnert et al., 1995; Nonami et al., 1997) and hormone production (Munns & Gramer, 1996). Toscano and Romano (2021) studied the impact of drought stress treatments on the growth of Zinnia. Different morphological parameters (dry biomass, leaf number, root to shoot ratio) and the relative water content (RWC) were reduced in severe drought stress treatment (25% field capacity). Catalase (CAT), Guaiacol peroxidase (GPX), and superoxide dismutase (SOD) activity significantly increased in 50% and 25% field capacity. Impact of drought stress was also reported on morphological, physiological, and biochemical characteristics in apple, pear and many other fruit trees (Bolat et al., 2014; Zarafshar et al., 2014). Globally 20% of irrigated land and 2.1% of dry land agriculture suffers from the water stress due to salt problem (FAO, 2000).

Plants have evolved mechanisms that allow them to perceive the incoming stresses and rapidly regulate their physiology and metabolism to cope with them (Gholami et al., 2012; Zhang et al., 2006). Plants can respond and adapt to water stress by altering their cellular metabolism and generating and transmitting signals for various defence mechanisms. Survival under this stressful condition depends on the plant's ability to cope up with the environment and initiate various physiological and chemical changes (Bohnert & Jensen, 1996). Soil salinity leading to water stress increases the rate of reactive oxygen species (ROS) via enhanced leakage of electron to oxygen in the chloroplasts and mitochondria (El-baky et al., 2003). Plants with better antioxidant machinery, either constitutive or induced, survive better in these conditions resisting this oxidative damage (Young and Jung, 1999). Various types of genes are induced by water deficit, including those that may protect the

plant from abiotic stresses like LEA proteins and heat shock proteins (HSPs).

Vigna mungo (L.) Hepper, popularly known as Urad bean or black gram, a member of family Fabaceae, is a nutritious legume largely grown in warmer regions of South and South East Asia. In India, Urad bean is one of the highly prized pulses grown in both Kharif and Rabi seasons and is mainly cultivated for its edible seeds and cattle fodder. Urad bean being one of the very important summer crops mostly affected by drought, has been selected for investigations. Relative leaf water content (RLWC) is the most appropriate measure of the plant water status. Other parameters being taken care of in present studies are root, shoot and leaf growth along with estimation of photosynthetic pigments such as chlorophyll a, b and carotenoids. Water stress also affects activity of natural antioxidant scavengers like catalase (CAT) which has also been measured in the present investigations.

### Health benefits of Urad bean

- The high fiber, low glycemic index properties of this food are supposedly able to modulate lipid homeostasis in people with a highsaturated-fat diet.
- The high fiber, low glycemic index properties of this food reputedly help to maintain blood glucose control in people with diabetes mellitus.
- Reputedly, diets high in black lentils have some benefit in controlling body weight since they are claimed to have satiety effects, thus limiting overall food consumption.
- Black lentils inhibit -amylase. -amylases are known to delay carbohydrate absorption and to reduce peak postprandial plasma glucose concentration.

# Materials and Methods

The present investigation was conducted to study the impact of water stress on some of the morphological, physiological and biochemical parameters in seedlings of Urad bean (*Vigna mungo*). Seeds of Uradbean were sown in field in natural conditions. 20-day old seedlings were used for experimentation. Control plants were maintained with regular watering, whereas water stress was provided by dewatering one set of

plants at regular intervals of 5, 7,9,11, and 13 days. All the experiments were performed in three replicates. Graphs were generated by using MS-Excel version 10. All the chemicals used were of analytical grade (AR). Stomata were also studied by preparing peel mounts at different time intervals and clicked by image projection camera attached to compound microscope (Nikon).

*Measurement of root and shoot length*: Seedlings were excised from the soil at different time intervals. Shoot and root length was measured starting exactly above and below the root-shoot junction respectively.

*Measurement of leaf area*: Leaves were detached and leaf area was measured by placing them on the graph paper, drawing an outline and counting the respective number of squares.

*Measurement of Relative leaf water content (RLWC)*: The relative leaf water content was determined in the fully expanded topmost leaf of the main shoot. The fresh weight of the sample leaves was recorded and the leaves were immersed in distilled water in a Petri dish. After 2 h, the leaves were removed, the surface water was blotted-off and the turgid weight was recorded. Samples were then dried in an oven at 70°C to constant weight.

Relative leaf water content was calculated using the following formula (Turner, 1981):

RLWC (%) =  $[(F.W - D.W) / (T.W - D.W)] \times 100$ 

Where: F.W., Fresh weight; D.W., Dry weight; T.W., Turgid weight

*Measurement of chlorophyll a, b and carotenoids*: 0.5g of small cut pieces of fresh leaf material was taken into a clean mortar and ground with the addition of 20 ml of 80% acetone for 5 min. The resulting extract was filtered using suction by Buchner funnel containing a layer of Whatman no.1 filter paper. Grinding of the pulp is repeated with 15ml of 80% acetone for another 5 min. Final volume of the filtrate was adjusted to 5 ml by adding sufficient 80% acetone. The absorbance of the extract was determined at 663nm, 645nm, 440nm using spectrophotometer (Yang et al., 1998).

The contents of chl*a*, *b* and carotenoids were calculated using the following formulae:

Chlorophyll $a(mg/1) = 9.78xA_{663}-0.99xA_{645}$ Chlorophyll $b(mg/1) = 21.4xA_{645}-4.65xA_{663}$ Carotenoids(mg/1) = 4.69xA\_{440}-0.268x (20.2xA\_{645} + 8.02xA\_{663})

Measurement of Catalase activity: CAT (EC 1.11.1.6) activity was measured as decline in absorbance at 240 nm due to the disappearance of  $H_2O_2$  (Cakmak & Marschner, 1992). The reaction mixture (2 mL) contained 25 mM phosphate buffer (pH 7.0), 10 mM  $H_2O_2$ , and 0.2 mL of enzyme extract. Enzyme activity was calculated using an extinction coefficient of 39.4 mM<sup>-1</sup>cm<sup>-1</sup>. One unit of specific enzyme activity determined the amount necessary to decompose 1 µmol of  $H_2O_2$  min<sup>-1</sup> mg<sup>-1</sup> protein at 25 °C.

# **Results and Discussion**

In the first set of experiment, the effect of water stress at 5, 7, 9, 11, and 13 days was studied on morphological parameters *viz.* shoot length, root length and leaf area (Fig. 1, 2 and 3). Both shoot length and leaf area decreased significantly as compared to their respective controls at different time intervals of stress. Maximum stress becomes evident after 9<sup>th</sup> day as a sharp decrease has been observed in shoot length and leaf area (Fig. 1 and 3). Development of optimal leaf area plays a pivotal role in photosynthesis and dry matter yield. Our results are consistent with the studies done by Zhang et al., (2004) on another legume soybean where water stress reduced leaf growth and in turn, the leaf area.

However, root length was not affected severely as compared to the control (Fig. 2), this is in corroboration with the earlier reports showing more ramification of the root system to procure more water during drought. A prolific root system can confer the advantage to support accelerated plant growth during the early crop growth stage and extract water from shallow soil layers that is otherwise easily lost by evaporation in legumes (Johansen et al., 1992). An increased root growth due to water stress has also been reported in sunflower (Tahir et al., 2002) and *Catharanthus roseus* (Jaleel et al., 2008).

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Fig. 1: Effect of water stress on shoot length (cm) of seedlings of Urad bean (*Vigna mungo*) at different time intervals (5, 7, 9, 11 and 13 days).



Fig. 2: Effect of water stress on root length (cm) of seedlings of Urad bean (*Vigna mungo*) at different time intervals (5, 7, 9, 11 and 13 days).

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Fig. 3: Effect of water stress on leaf area (cm<sup>2</sup>) of seedlings of Urad bean (*Vigna mungo*) at different time intervals (5, 7, 9, 11 and 13 days).

Relative leaf water content (RLWC) is the most appropriate measure of the water status in plants. Moreover, decline in RLWC is generally correlated with changes in plant nutrition, carbon dioxide balance and water relations (Levitt, 1980). The reductions in water uptake and transpiration are usually associated with reduction in the water content of the shoots and stomatal aperture, thereby indicating the development of water stress in the leaves (Gerakis et al., 1975; White et al., 2000). Similarly in the present studies, it was observed that the relative leaf water content in Urad bean declined drastically after 9<sup>th</sup> day of water stress. This may occur as a result of drought or osmotic limitation of water availability due to salinity. However, in control plants, percentage of RLWC tends to increase with the time interval (Fig. 4), thus suggesting a connection between root as well as shoot growth and water content of leaves. These results are in consistency with the work done by Pirzad et al., (2011) demonstrating the effect of water stress on relative leaf water content. Further the studies of Najihah et al., (2019) in oil palm seedlings also showed that severe water stress decreased vegetative plant growth, leaf water potential, relative water content, leaf moisture content, stomatal conductance, transpiration rate, net photosynthesis, and water use efficiency (WUE).



Fig. 4: Effect of water stress on percentage of relative leaf water content (RLWC) of seedlings of Urad bean (*Vigna mungo*) at different time intervals (5, 7, 9, 11 and 13 days).

Plants usually respond to water stress by closing their stomata to compensate for the water loss through transpiration. Toscano et al., (2016) studied in ornamental shrubs- *Eugenia* and *Photinia*- that stomatal regulation was the main physiological strategy to reduce water losses during drought period. Jaleel *et al.*, (2008) also observed decrease in cell enlargement and growth, and closure of stomata following water deficit besides decrease in various

morphological and biochemical parameters. Similar observations regarding stomatal closure have been recorded in the present studies too. In Urad bean, at 0 day all stomata were found to be open but with an increase in number of days after being subjected to water stress, they tend to close. On the contrary, in control seedlings more or less all stomata were found to be open by 13<sup>th</sup> day (Fig. 5 A, B and C).





#### Fig. 5: Peel mounts of Urad bean (*Vigna mungo*) showing stomata (400 X).

- A. Peel mount on 0 day
- B. Peel mount of control seedlings on 13<sup>th</sup> day
- C. Peel mount of seedlings under water stress on 13<sup>th</sup> day.

It was observed that in the leaf peel mount, stomatal pores were completely open on 0 day (Fig. 5 A) whereas after  $13^{th}$  day of stress, they were found to be closed (Fig. 5 C). Though, no variation was reported in leaf peel mount of control seedlings even after  $13^{th}$  day of the experiment (Fig. 5 B). Stomatal closure is triggered by reducing water potential in the leaf mesophyll cells and seems to involve abscisic acid (ABA). ABA levels begin to increase markedly in leaf

tissues and roots followed by closure of stomata and decrease in transpiration rate. ABA disrupts the proton pump operating in the plasma membrane of guard cells and as a consequence, stimulates  $K^+$  efflux from them, leading to loss of turgor and stomatal closure. Hence, ABA appears to be involved as part of a rootto-leaf signal chain that initiates stomatal closure when soil begins to dry out.

Drought stress leads to overproduction of reactive oxygen species (ROS) in plants which inactivate enzymes, damage important cellular components, and initiate destructive oxidative processes such as lipid peroxidation, fatty acid de-esterification, chlorophyll bleaching and protein oxidation (Terziand Kadigolu, 2006; Ansari et al., 2019). Increase in ROS is known to cause alteration of the main biomolecule classes, leading to structural and functional changes in lipids, proteins, chlorophylls, and nucleic acids (Moran et al., 2010; Sood et al., 2011). To avoid ROS-induced injury, detoxifying antioxidants (ascorbic acid [AsA] and reduced glutathione) and scavenging enzymes such as ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px),

monodehydroascorbate reductase (MDHAR). dehydroascorbate reductase (DHAR) and glutathione reductase (GR) are produced in different cellular compartments of plant (Mittler et al., 2004). Catalase, present in the peroxisomes of plant cells, is important for regulation of intracellular H<sub>2</sub>O<sub>2</sub> (Shigeoka et al., 2002). In Vigna mungo upregulation of CAT activity was recorded with continuous increase till 13<sup>th</sup> day of water stress (Fig. 6). These observations clearly indicate that CAT is required to combat stress-induced ROS. Earlier studies by Jiang and Zhang (2002) also support these findings, where water stress-induced ABA accumulation triggers the increased production of ROS, which, in turn, lead to the upregulation of the antioxidant defence system.



# Fig. 6: Effect of water stress on the activity of catalase (CAT) activity ( $\mu$ mol of H<sub>2</sub>O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> protein) in Urad bean (*Vigna mungo*) at different time intervals (5, 7, 9, 11 and 13 days).

Most of the abiotic (*i.e.* water, temperature, light, ozone, ultraviolet radiations, salts, pesticides, pollutants, heavy metals etc.) and biotic (pathogens, insects, microorganisms etc.) stresses are known to generate ROS in plants (Fig. 7). ROS play a dual role: (i) these have a negative impact on growth,

development, as well as yield of the plants (ii) on the other hand, ROS accumulation has a positive effect on cells by stimulating signal transduction pathways that induce acclimation mechanisms, which, in turn, counteract the negative effects of stress, including ROS accumulation (Kochhar and Gujral, 2020).

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# Fig 7: Abiotic stresses such as water deficit and salinity lead to production of reactive oxygen species (ROS), which can impact plants negatively as well as positively.

Next set of experiments was aimed to evaluate the effect of water stress on the amount of photosynthetic pigments *viz.* chlorophyll *a*, chlorophyll *b* and carotenoids (Fig. 8 and 9). The amount of chlorophyll *a* showed more reduction on  $13^{\text{th}}$  day of stress as compared to chlorophyll *b*. However, in control

seedlings, level of both chl a and b tends to increase with the day interval and approaches maximum on 13<sup>th</sup> day (Fig. 8).Our results are compatible with the findings of Liu et al., (2011) where they studied the effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species.



Fig. 8: Effect of water stress on content of chlorophyll *a* and *b* (mg/l) in seedlings of Urad bean (*Vigna mungo*) at different time intervals (5, 7, 9, 11 and 13 days).

In the last set of experiments, total amount of carotenoids was determined in both control and waterstressed seedlings. It was observed that level of carotenoids also showed the similar trend and decreased drastically by 13<sup>th</sup> day. However, in control seedlings also, a slight decrease in carotenoids content was observed after 11<sup>th</sup> day (Fig.9). Carotenoids show multifarious roles in drought tolerance including light harvesting and protection from oxidative damage caused by drought. Therefore, decrease in content of carotenoid may lead to complete damage of the plant metabolic machinery.

Water stress, along with other physiological changes, has ability to reduce the amounts of chlorophylls and carotenoids (Havaux, 1998; Kiani et al., 2008), coupled with the production of ROS in the thylakoids (Niyogi, 1999; Reddy et al., 2004). Moreover, arrestation of photosynthesis directly causes disturbance of metabolism and finally the death of plant (Jaleel et al., 2008). Low water potential created due to water deficit has direct effects on the structural integrity of the photosynthetic apparatus. Both the photosynthetic electron transport and photophosphorylation mechanisms decline in chloroplasts, resulting in structural impairment of thylakoid membranes and ATP synthase  $(F_0-F_1)$ complex. Another impact of exposure of plants to water deficit conditions is continued photoinhibition because absorbed light energy cannot be processed due to inhibition of photosynthetic electron transport. Hence, plants have evolved both enzymatic and nonenzymatic defence systems for scavenging and detoxifying ROS, resulting in antioxidant defence capacity that is a useful criterion for the screening of resistant genotypes (Faize et al., 2011). Besides the non-enzymatic antioxidants (e.g. ascorbic acid and glutathione), carotenoids are pigments with a protective role for dissipating the excess of energy necessary to avoid ROS generation (Sircelj et al., 2007).





Our results are similar to the findings of Koutoua et al., (2016) who reported that water stress reduced height, stem diameter, leaf area, number and length of root, and the specific weight of the tomato plants. Water content of the plant, relative water content of leaves, photosynthetic pigments such as chlorophyll (*a*, *b*, total) and carotenoid were also reduced with water stress, accompanied by accumulation of proline content and an increase in catalase activity.

## Conclusion

In the present study, water stress not only affected the morphological parameters viz. shoot growth, root growth, and leaf area but also the physiological and biochemical parameters like RLWC, CAT activity, contents of photosynthetic pigments in Urad bean. Plants have developed various mechanisms to cope with water deprivation. Understanding the morphological, physiological and biochemical responses to drought is essential for a holistic perception of plant resistance mechanisms to waterlimited conditions and also to design screening techniques for drought tolerance that may be employed in crop breeding. Molecular control mechanisms for abiotic stress tolerance are based on the activation and regulation of specific stress-related genes. These genes are involved in the whole sequence of stress responses, such as signalling, transcriptional control, protection of membranes and proteins, and free-radical and toxic-compound scavenging. Hence, coupled increasing population with shrinking resources, has fuelled research into elucidating mechanisms by which plants respond to stress and manipulating these mechanisms to enhance plant productivity in suboptimal environments.

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