

Effect of Drought Stress on *Vigna radiata*

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Abstract

Plant growth and productivity are adversely affected by nature's wrath in the form of various biotic and abiotic stress factors. Drought stress is one of the major abiotic stresses in agriculture worldwide. This adversely affects crop growth and yield. An experiment has been carried out to understand the effect of drought on biochemical contents and enzymatic activities of mung bean (*Vigna radiata* (L.) Wilczek). The plant tissues were analyzed to investigate photosynthetic pigment concentration, proline, lipid peroxidation, relative water content and antioxidant enzymes activity. We observed a great decline in the photosynthetic pigments content, but increase in carotenoids to total chlorophyll (Chl) and increase in proline and lipid peroxidation under drought stress conditions. In the var. K 851 chlorophyll a, chlorophyll b, total chlorophyll concentration and antenna size were higher than in the sensitive variety (Var). The improved performance of the var. K 851 as compared to var. RMG 62 under drought was accompanied by an increase in ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and catalase (CAT). These results suggest that var. K 851 is potentially more tolerant to drought stress and is associated with better adaptive responses.

Keywords

Antioxidant enzymes, Drought, Photosynthetic pigment, Physiological parameters, *Vigna radiata*

Introduction

Abiotic stress is a worldwide problem which affects growth and yield of crops. Among abiotic stresses, drought stress is one of the most serious problems in crops production in the arid and semi-arid regions, which causes serious damages in plants and it is recognized as a limiting factor for growth and production (Farooq *et al*, 2009; Yarnia *et al*, 2011). Generally drought stress occurs when the available water in the soil is reduced, and atmospheric conditions cause continuous loss of water by transpiration.

Mung bean (*Vigna radiata* L.) is one of the most important legumes in the tropics and subtropics regions, and it has a large role in nutrition of developing countries (Dhingra *et al*, 2003). *Vigna radiata* is grown all over India as a protein rich supplement. In India, the important states growing this crop with a total area of about 30 lakh hectares are Orissa, Maharashtra, Andhra Pradesh, Madhya Pradesh, Gujarat, Rajasthan and Bihar. Mung bean is superior to other plants for second culture because of short-term growth, nitrogen fixation capability, soil reinforcement and prevention of soil erosion. Moreover, mung bean can be used as a crop with export potential, but drought is a major limitation to legume production.

Photosynthetic pigments are important to plants mainly for harvesting light and production of reducing powers. Both chlorophyll (Chl) a and b are prone to soil drying

(Farooq *et al*, 2009). It also aids in the recycling of nutrients and may aid DNA repair (Ommen *et al*, 2003) reported that leaf Chl content decreases as a result of drought stress. Carotenoids (Car) are a large class of isoprenoid molecules, which are *de Novo* synthesized by all photosynthetic and many non-photosynthetic organisms (Andrew *et al*, 2008). Drought stresses produced changes in the ratio of Chl a and b and Car (Anjum *et al*, 2003b; Farooq *et al*, 2009).

Drought may trigger an increased formation of ROS (reactive oxygen species) which are highly reactive and bring about cellular damage. Oxidative damage generated by drought stress in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant systems. Plant ROS-scavenging mechanisms include the action of some key enzymes, such as H₂O₂ may be detoxified to H₂O by ascorbate peroxidase, catalase and other enzymes.

Lipid is an important component surrounding cells and cellular organelles. The content of MDA has been considered an indicator of oxidative damage (Moller *et al*, 2007). The rise in MDA content under stress conditions suggests that water stress could induce membrane lipid peroxidation by means of ROS (Sairam *et al*, 2000). Strong water deficit leads to a disturbance of the association between membrane lipids and proteins as well as to a

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decrease in the enzyme activity and transport capacity of the bilayer.

Proline, an α -amino acid, is one of the most common compatible osmolytes in drought stressed plants. Proline can act as a signalling molecule to modulate mitochondrial functions, influence cell proliferation or cell death, and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure, 2009).

Leaf water potential is considered to be a reliable parameter for quantifying plant water stress response. Significant differences in water potential among different varieties under drought stress were observed (Singh *et al.*, 1990). Among several methods used to characterize internal plant water status, RWC is an integrative indicator (Parsons and Howe, 1984) and was used successfully to identify drought resistant cultivars (Matin *et al.*, 1989). Therefore, the objective of the study was to investigate the effect of drought stress on physiological parameters in two *Vigna radiata* varieties.

Materials and Method

Growth and Stress Conditions

Two varieties of *Vigna radiata* were selected after screening of var. K 851, var. RMG 62, var. RMG 268, var. RMG 344, var. RMG 492 and var. SML 668 of *Vigna radiata* via pigment content (Chl a, b, total Chl, antenna size, Car), proline content and as per yield data obtained from Durgapura Research Station, Jaipur. Var. K 851 was selected as tolerant and var. RMG 62 as sensitive. Seeds of *Vigna radiata* varieties were washed thoroughly in running water, sterilized by mercuric chloride (0.1%) and washed in distilled water. Thereafter, seeds were soaked in a beaker for 24 hours. After that seedlings were transferred to pots containing autoclaved soil and kept in a growth room at 27°C, 16 h of photoperiod. Ten days old plantlets were used for experiments. Leaves of plants were excised and used for various biochemical experiments.

Photosynthetic Pigments Estimation

The photosynthetic pigments e.g. total Chl, Chl a, b and Car were extracted in 5 ml of chilled 80% acetone by grinding the leaves of drought stress treated seedlings in a chilled mortar and pestle. The homogenate was centrifuged at 3000 rpm for 10 min. at 4°C. The absorbance of the resulting supernatant was taken at 480, 645, 663 and 740 nm. Different pigments were estimated using the following formula by Arnon (1949) as given below:

$$\begin{aligned} \text{Total Chl (mg/gm)} &= \text{Chl a} + \text{Chl b} \\ \text{Chl a} &= 0.0127 * E663 - 0.00269 * E645 \end{aligned}$$

$$\text{Chl b} = 0.0229 * E645 - 0.00468 * E663$$

$$E645 = A645 - A740$$

$$E663 = A663 - A740$$

$$\text{Car} = A480 * 200$$

Proline Estimation

The level of proline in control and stress treated seedlings was estimated following the method of Bates *et al.*, (1973). The absorbance of the chromophore extracted in toluene was taken at 520 nm.

Lipid Peroxidation Estimation

Lipid peroxidation was estimated measuring the formation of malondialdehyde (MDA), a breakdown product of lipid peroxidation, with 2-thiobarbituric acid (TBA) according to De Voss *et al.*, (1989). The absorbance of resulting supernatant was taken at 532 and 600 nm.

RWC Estimation

RWC was determined by the method of Schonfeld *et al.*, (1988). 0.1 gram fresh weights (WF) of leaves were determined. Turgid weight (WT), were obtained after soaking leaves in distilled water in test tubes for 16 to 18 h at room temperature (about 20 °C), and under the low light conditions of laboratory. After soaking, leaves were quickly and carefully blotted dry with tissue paper prior to determination of turgid weight. Dry weights (WD) were obtained after oven drying the leaf samples for 48 h at 70°C. RWC was calculated from the equation-

$$\text{RWC (\%)} = [(WF - WD) / (WT - WD)] * 100.$$

Antioxidant Enzymes Estimation

For enzyme extraction, leaf samples of *Vigna radiata* were homogenized in pre-cooled mortar and pestle in 5 ml extraction mixture (1mM ascorbate in 50mM phosphate buffer of pH 7). The homogenate was centrifuged at 13,500 (rpm) for 25 min at 4°C.

Estimation of Ascorbate Peroxidase Activity

For the estimation of APX activity, 0.1 ml of enzyme extract was added to 2.8 ml reaction mixture composed of 0.5 mM ascorbic acid in 50 mM phosphate buffer (pH 7.0) and then 0.1 ml H₂O₂ was added. Changes in absorbance at 290 nm were recorded after 25 seconds for 3 min according to Asada (1984).

Catalase Activity

CAT activity was measured following Luck (1975). 0.04 ml of enzyme extract was added to 3 ml of H₂O₂ phosphate buffer. Absorbance was taken at 240 nm.

Guaiacol Peroxidase

0.1 ml of enzyme extract was added to 1.8 ml of phosphate buffer followed by 0.1 ml guaiacol and 5 μ L of H₂O₂. Absorbance was taken at 436 nm according to the method of Putter (1974).

Statistical Analysis

Physiological and biochemical data were presented as mean \pm standard deviation (SD) of three biological replicates.

Results and Discussion

Effect on Photosynthetic Pigments

Chlorophyll estimation is one of the important biochemical parameters which is used as the index of production capacity. Drought stress caused a large decline in the chlorophyll a content, the chlorophyll b content, and the total chlorophyll content in both stress and

tolerant varieties of *Vigna radiata* (Table 1). The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). In the var. RMG 62 reduction of total chlorophyll is higher than var. K 851. Less change was observed in Chl a content in var. K 851 then var. RMG 62 respectively. The Chl a:b ratio increased more in var. K 851 then RMG 62.

The values are expressed as mean \pm SD for triplicates (n=3).

Effect on Proline

Proline content of both varieties was elevated linearly with the increase of water deficit. Proline contents in water-stressed plants were higher than non-water-stressed plants. The control plants maintained lower concentrations in both the varieties of *Vigna radiata*. Higher level of proline was noticed on 5th day of stress condition in var. K 851 as compared to var. RMG 62 (Fig.1).

Table 1. Pigments content for two varieties, var. K-851 and RMG-62 after 1st, 2nd, 3rd, 4th, 5th, day of drought stress

| Varieties of <i>Vigna radiata</i> | Condition | Days | Chl a (mg/gm) | Chl b (mg/gm) | Total Chl (a+b) (mg/gm) | Chl a:b |
|-----------------------------------|-----------|------|-----------------|-----------------|-------------------------|-----------------|
| RMG-62 | Control | 1 | 1.10 \pm 0.04 | 0.38 \pm 0.05 | 1.48 \pm 0.02 | 3.28 \pm 0.02 |
| | | 2 | 1.17 \pm 0.02 | 0.40 \pm 0.06 | 1.57 \pm 0.04 | 3.44 \pm 0.00 |
| | | 3 | 1.23 \pm 0.02 | 0.49 \pm 0.04 | 1.71 \pm 0.04 | 3.56 \pm 0.02 |
| | | 4 | 1.32 \pm 0.04 | 0.53 \pm 0.04 | 1.86 \pm 0.03 | 3.62 \pm 0.03 |
| | | 5 | 1.40 \pm 0.01 | 0.59 \pm 0.04 | 1.99 \pm 0.04 | 3.68 \pm 0.01 |
| | Stress | 1 | 1.20 \pm 0.06 | 0.62 \pm 0.04 | 1.82 \pm 0.10 | 3.24 \pm 0.02 |
| | | 2 | 1.21 \pm 0.07 | 0.64 \pm 0.07 | 1.86 \pm 0.12 | 3.27 \pm 0.02 |
| | | 3 | 1.14 \pm 0.04 | 0.43 \pm 0.05 | 1.57 \pm 0.08 | 3.37 \pm 0.01 |
| | | 4 | 0.91 \pm 0.04 | 0.35 \pm 0.02 | 1.26 \pm 0.04 | 3.45 \pm 0.02 |
| | | 5 | 0.80 \pm 0.03 | 0.29 \pm 0.03 | 1.09 \pm 0.04 | 3.51 \pm 0.02 |
| K-851 | Control | 1 | 1.35 \pm 0.01 | 0.59 \pm 0.11 | 1.94 \pm 0.11 | 3.33 \pm 0.04 |
| | | 2 | 1.43 \pm 0.05 | 0.61 \pm 0.09 | 2.04 \pm 0.04 | 3.42 \pm 0.03 |
| | | 3 | 1.59 \pm 0.02 | 0.63 \pm 0.09 | 2.22 \pm 0.08 | 3.47 \pm 0.01 |
| | | 4 | 1.68 \pm 0.06 | 0.77 \pm 0.07 | 2.45 \pm 0.08 | 3.51 \pm 0.02 |
| | | 5 | 1.76 \pm 0.02 | 0.84 \pm 0.05 | 2.60 \pm 0.05 | 3.57 \pm 0.02 |
| | Stress | 1 | 1.66 \pm 0.08 | 0.92 \pm 0.04 | 2.58 \pm 0.12 | 3.28 \pm 0.02 |
| | | 2 | 1.50 \pm 0.04 | 0.86 \pm 0.01 | 2.36 \pm 0.06 | 3.35 \pm 0.02 |
| | | 3 | 1.38 \pm 0.02 | 0.69 \pm 0.06 | 2.06 \pm 0.07 | 3.46 \pm 0.03 |
| | | 4 | 1.26 \pm 0.04 | 0.65 \pm 0.03 | 1.91 \pm 0.03 | 3.50 \pm 0.02 |
| | | 5 | 1.13 \pm 0.03 | 0.61 \pm 0.02 | 1.73 \pm 0.01 | 3.56 \pm 0.02 |

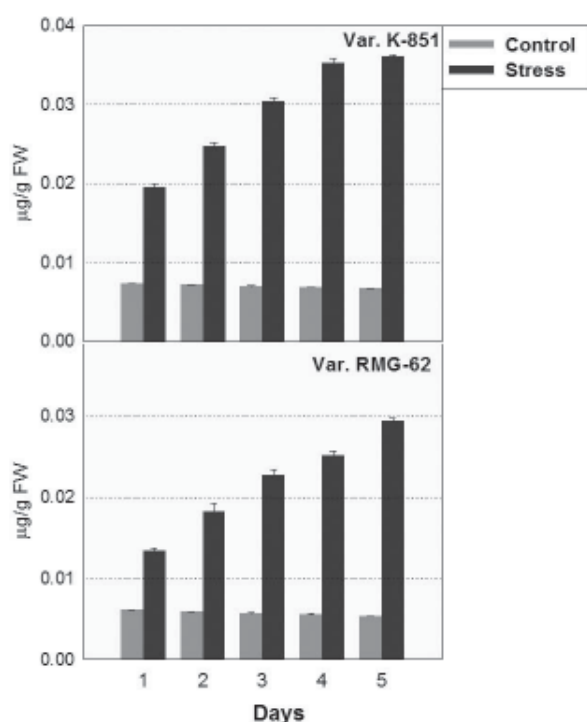


Fig.1. Changes in proline in var. RMG-62 and Var. K-851 of *Vigna radiata* exposed to drought stress

Effect on Lipid Peroxidation

Lipids are the most abundant component of membranes, and they play a role in the resistance of plant cells to environmental stresses (Yordanov *et al*, 1987). Enhanced rate of lipid peroxidation was recorded as indicated by gradually increasing malondialdehyde (MDA) contents in *Vigna radiata* leaves exposed to drought stress as compared to control (Fig.2). The content of MDA was higher in var. RMG 62 (1402.16nm/gm) then var. K 851 (1238.7 nm/gm) on 5th day of drought stress suggesting more susceptibility to damage.

Effect on Relative Water Content

RWC is the appropriate trait of plant water status pertaining to the physiological consequence of cellular water deficit. RWC content in *Vigna radiata* decreased with an increase in water stress in both the varieties. A lower level of relative water content was noticed in var. RMG 62 following stress. The measurement of RWC under low soil moisture is of importance since high RWC appears to be a common trait in drought resistant species as var. K 851 which exhibit restricted changes in RWC and can be considered to be relatively drought resistant (Fig.3).

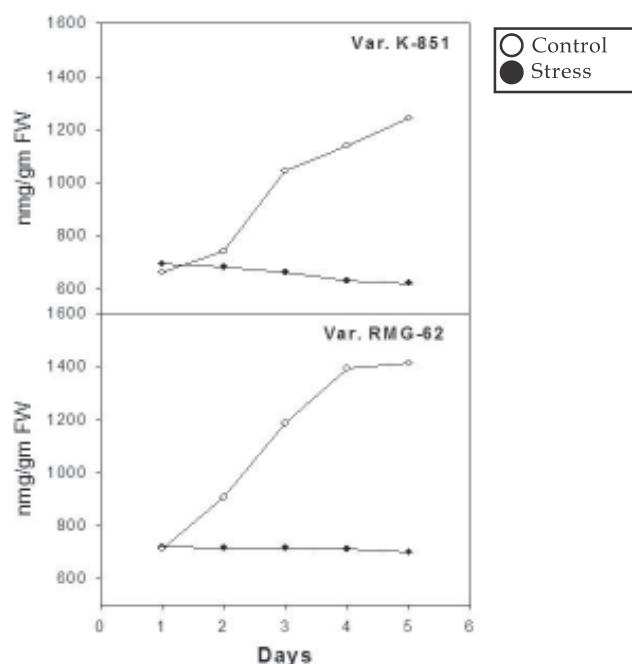


Fig.2. Changes in MDA in var. RMG-62 and var. K-851 of *Vigna radiata* exposed to drought stress

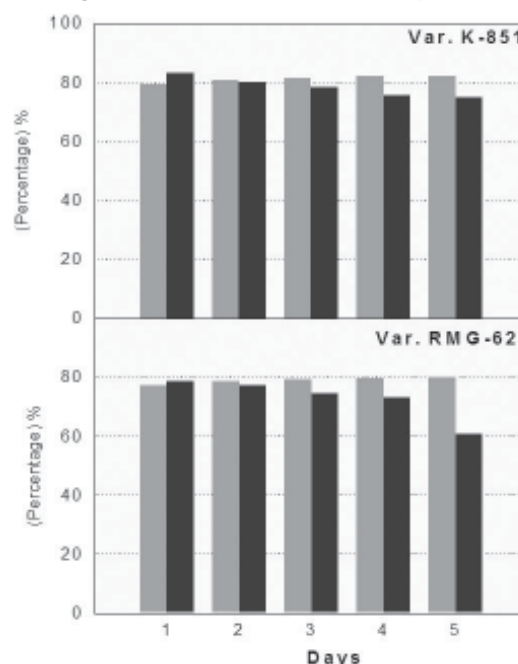


Fig.3. Changes in RWC in var. RMG-62 and var. K-851 of *Vigna radiata* exposed to drought stress

Effect on Antioxidant Enzymes

Catalase (CAT) Activity

The antioxidant enzyme catalase plays a major role in several metabolic pathways. In this study, the catalase

gradually increased in leaves of *Vigna radiata* with incipient drought stress. The var. K 851 and var. RMG 62 showed a differential response in CAT activity as compared to control on 4th day of stress with a higher increase being recorded in var. K 851 (Fig. 4). This increase in catalase activity due to the H₂O₂ is one of the crucial ROS produced in response to different environmental stresses. Maximum CAT activity was recorded after 96 hrs of exposure and thereafter, a gradual decrease was observed in CAT activity in both varieties of *Vigna radiata*.

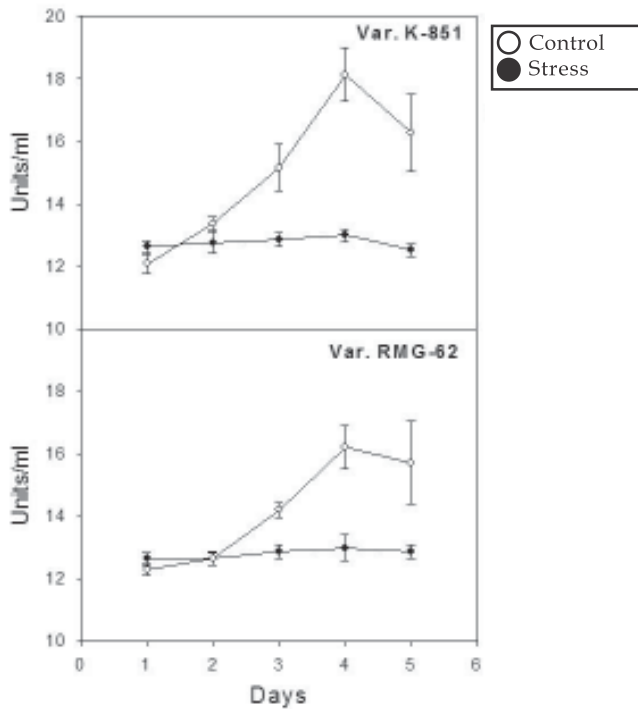


Fig. 4. Changes in CAT in var. RMG-62 and var. K-851 of *Vigna radiata* exposed to drought stress

Ascorbate Peroxidase (APX) Activity

APX can scavenge H₂O₂ that is inaccessible for catalase because of their high affinity for H₂O₂ and their presence in different subcellular locations (Breusegen 2001). The analysis of APX in two *Vigna radiata* varieties showed high activity in var. K 851 as compared to var. RMG 62 on 3rd day in stressed plants (Fig. 5). Both varieties of *Vigna radiata* differed markedly in APX activity after exposure to drought stress.

Guaiacol Peroxidase (GPX) Activity

GPX activity plays an important role in antioxidative defence system. The activities of GPX in leaves were higher as compared to control in stressed plants of both varieties. There was an initial significant increase in the activity of GPX. Furthermore, the activity of this antioxidant enzyme gradually decreased but remained over the control (Fig. 6).

As compared to the var. RMG-62, var. K-851 maintained higher GPX activity on 5th day of stress.

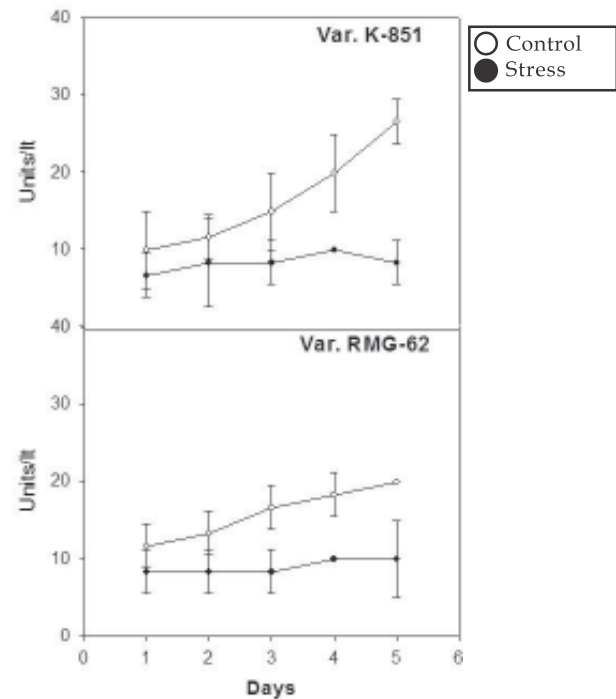


Fig.5. Changes in GPX in var. RMG-62 and var. K-851 of *Vigna radiata* exposed to drought stress

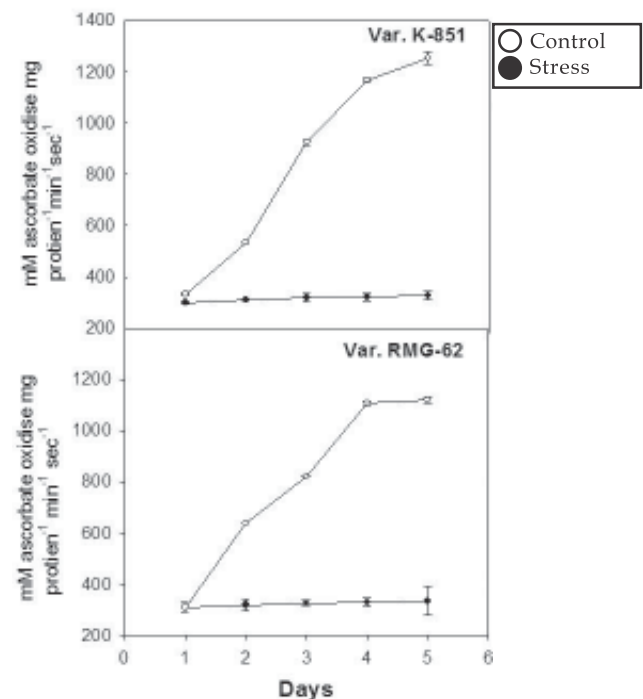


Fig.6. Changes in APX in var. RMG-62 and var. K-851 of *Vigna radiata* exposed to drought stress

Drought stress is a global phenomenon. A plant responds to a lack of water by halting growth and reducing photosynthesis and other plant processes in order to reduce water use. This may be partly due to lower turgor pressure and decrease of photosynthetic rate in the cells (Cha-Um *et al*, 2007; Regier *et al*, 2009). The characteristic feature of drought stress is low water potential due to high solute concentration. It reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel *et al*, 2008; Farooq *et al*, 2008).

Under mild or moderate drought stress stomatal closure (causes reduction of leaf internal CO₂ concentration) is the major reason for reduction of photosynthetic ratio in leaf (Chaves, *et al*, 2002; Cornic, 2004; Flexas *et al*, 2004). Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting chlorophyll components and by damaging the photosynthetic apparatus (Iturbe Ormaetxe *et al*, 1998). Ommen *et al* (1999) reported that leaf chlorophyll content decreases as a result of drought stress.

Plants can partly protect themselves against drought stress by accumulating osmolytes. Proline is one of the most common compatible osmolytes in drought stressed plants. The accumulation of proline in plant tissues is also a clear marker for environmental stress, particularly in plants under drought stress (Routley *et al*, 1999). Proline accumulation may also be part of the stress signal influencing adaptive responses (Maggio *et al*, 2002).

Drought induces the generation of ROS causing lipid peroxidation which leads to membrane injury, protein degradation, enzyme inactivation and the disruption of DNA strands (Becana *et al*, 1998). Rapid and nonspecific reaction of ROS result in severe cell biochemical changes during oxidative stress, including lipid peroxidation and damage to protein and DNA, which may lead to cell death (Yang *et al*, 2009). The ion leakage is an indicator of cell membrane stability and integrity, which is commonly considered as one of the best physiological components of drought tolerance in plants (Kocheva *et al*, 2004). MDA content is usually used to measure the extent of lipid peroxidation resulting from oxidative stress (Smirnoff 1992). The increasing of MDA content was accompanied by an increase of ion leakage in both varieties which indicated that lipid peroxidation led to membrane fluidity resulting in enhanced membrane permeability as suggested by other authors (Lima *et al*, 2002; Reddy *et al*, 2004; Zhou *et al*, 2006). Increased MDA levels indicate either increased oxidation of ascorbate or decreased efficiency of ascorbate regeneration, or a combination of both (Hebert *et al*, 2003).

In the present investigation, the activity of antioxidant enzymes showed a progressive increase in both the *Vigna radiata* varieties at drought condition. Drought leads to significant increase in CAT activity. CAT and APX are the most important enzymes involved in regulation of intracellular level of H₂O₂ (Prasad *et al*, 2000). APX convert H₂O₂ into H₂O along with the regeneration of NADP⁺ therefore, the enzymes play important roles in stress conditions (Jimenez *et al*, 1998). Omidi *et al*, (2010) recently noticed that enzyme activities of APX and CAT were highly significant difference between drought and genotypes in respect to CAT and APX. These were increase under drought stress. Increase in activity of CAT, GPX, and APX in response to drought stress, as well as higher antioxidant activity, was observed in var. K 851 as compared to var. RMG-62 of *Vigna radiata*. These results suggest that var. K 851 is potentially more tolerant to drought and is associated with better adaptive responses than var. RMG 62.

Conclusion

The purpose of the present study was to contribute to a better understanding of the physiology responses of *Vigna radiata* plants to drought stress. This study was conducted to evaluate characters of resistance under drought stress, and the results showed that chlorophyll, proline, antioxidant activity, RWC, made a difference between tolerant and sensitive varieties. Overall, from the results of this experiment, all physiological parameters responses of drought adapted (K-851) and drought sensitive (RMG-62) varieties of *Vigna radiata* to limited water supply showed similar patterns: drought stress decreased pigments content but increased the ratio of Car / Chl a+b. Decreased chlorophyll a, b, a/b concentrations, transpiration and yield associated with increased proline. Photosynthesis is limited by drought stress due to stomatal (stomatal closure) and non-stomatal (impairments of metabolic processes) factors. Prolonged severe drought stress largely decreased accumulations of osmotic solutes but significantly increased proline content and peroxidase activity in both varieties. The positive relationships were observed among activities of antioxidant enzymes, and between contents of osmotic solutes and activities of antioxidant enzymes. Differences between varieties were mainly found in water relation parameters, which indicate adaptations in physiology (stomata) or osmotic adjustments. Results of this research demonstrate that var. K-851 performs better than var. RMG-62 under drought stress, as indicated by a lower sensitivity to photoinhibition, proline accumulation and higher antioxidant enzyme activity. Results of this research suggest that antioxidant enzymes activity as CAT, APX and GPX could be a part of the drought resistance

mechanisms developed by var. K 851 of *Vigna radiata* and could be exploited in breeding programs for improved drought stress tolerance.

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