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Research Article

BIOCHEMICAL RESPONSES TO DROUGHT STRESS IN *CICER ARIETINUM* L.

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ABSTRACT
Chickpea (*Cicer arietinum* L.) is an important legume crop as a protein source across the world. It is mostly grown on arid and marginal lands where it faces drought stress at different growth stages. Present study was carried out to understand the drought- induced changes in various antioxidant (guaiacol peroxidase, superoxide dismutase, catalase) and other key enzymes (α- amylase, invertase, acid phosphomonoesterase) of two varieties (C-235 and BG-391) of *C. arietinum*. The biochemical study clearly indicates that *C. arietinum* variety C-235 has high drought tolerance as compared with BG-391. *C. arietinum* variety C-235 responds well against drought stress by modifying the activity of various key enzymes to minimize adverse effects of water deficit condition.

Keywords: *Cicer arietinum L., Drought Stress, Guaiacol Peroxidase, Superoxide Dismutase, Catalase, α-Amylase, Invertase, Acid Phosphomonoesterase*

INTRODUCTION
Drought stress is one of the major abiotic stresses that affects plant growth and metabolism adversely around the world. The plant responses to water stress include changes in stomatal conductance, growth, osmolyte accumulation, and expression of specific genes. Plants respond to drought at morphological, biochemical, physiological and molecular levels (Cochard *et al.,* 2002). Under water deficit condition, plants modify the expression of various genes and accumulate metabolites to prevent from drought-induced osmotic damage. Drought also inhibits photosynthesis, resulting from limited stomatal conductance (Flexas *et al.,* 2004).

Drought causes disruption of ionic equilibrium, inhibition of enzymatic activity, osmotic imbalance, membrane disorganization, inhibition of cell division and expansion, reduction in photosynthesis and overproduction of toxic reactive oxygen species (ROS). Plants have developed an antioxidant system to minimize the toxic effects of ROS, like antioxidant enzymes superoxide dismutase, peroxidase, catalase, and antioxidant compounds ascorbate, glutathione (Apel and Hirt, 2004; Kaur *et al.,* 2013). Plants also respond to drought through the over-expression or down-expression of other genes to reduce the adverse effects of water deficit condition.

Cicer arietinum L. (Chickpea) is the second most important grain legume (pulse) globally (Pang *et al.,* 2017). In subtropical areas, it is cultivated after the rainy season on stored soil moisture; while in Canada and Mediterranean climatic areas, it is cultivated in the rainy season (Leport *et al.,* 1999). Whether grown on stored soil moisture or current rainfall, chickpea is severely exposed to water deficit conditions during the reproductive phase, a situation referred to as 'terminal drought' (Siddique *et al.,* 1999). The crop, in particular, is affected drought stress because of late sowings.

Terminal drought is normally accompanied by increasing temperature towards maturity, which finally reduce pod production (Wery *et al.,* 1994). With terminal drought, seed yields can be reduced by 58–95% compared to irrigated plants (Leport *et al.,* 2006). In the present study, efforts were carried out to identify the effects of drought on various antioxidant (guaiacol peroxidase, superoxide dismutase, catalase) and other key enzymes (α- amylase, invertase, acid monophosphoesterase) in two varieties (BG-391 and C-235) of *C. arietinum*.

MATERIALS AND METHODS

Plant Materials and Growing Conditions: *C. arietinum* varieties C-235 and BG-391 were analyzed concerning their ability to endure drought stress. Seeds were obtained from Agriculture Research Station (Jaipur, Rajasthan, India) and were sown *in vivo* in germination trays containing containing compost soil and farmyard manure in a ratio of 3:1) at Research Nursery, School of Life Sciences, Jaipur National University, Jaipur, India (Figure 1A). Prior to sowing, surface sterilization of seeds was done with 10% (v/v) chlorox followed successive washings with distilled water. Seedlings were watered twice a day.

Drought Treatment: The germinated plants were equally well watered for one month prior to exposure to water stress treatment. The plants were divided into two sets (each of four plants), out of which one set was subjected to water stress by withholding of water supply till wilting symptoms appeared, while the second set was watered regularly and served as a control. Biochemical analyses of enzymes were carried out in the leaves of *C. arietinum*. The samples were collected regularly at an interval of three days till day 21.

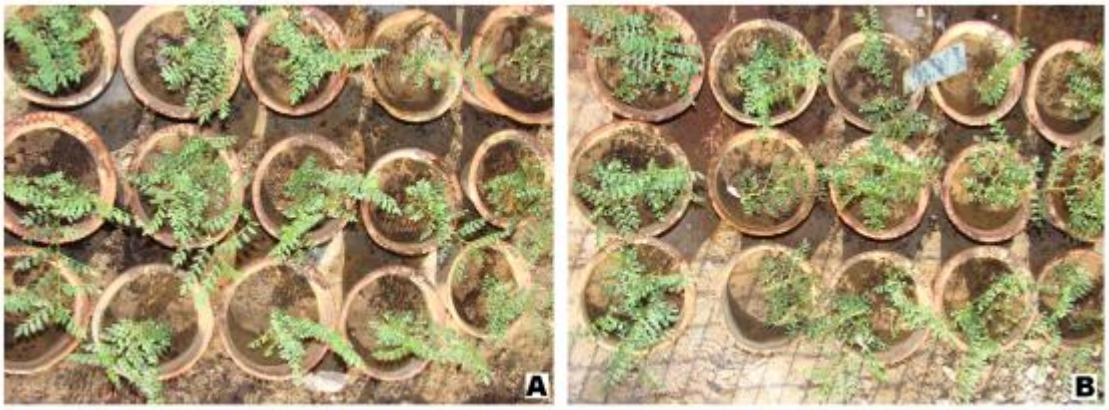

Figure 1: *Cicer aricetinum* L. variety C-235 (A) and BG-391 (B)

Sample Preparation: Leaf samples of *C. arietinum* were macerated in cold pestle mortar by adding 50 mM potassium-phosphate buffer (pH 7.4) to fine slurry and then centrifuged at 10,000 g for 20 minutes at 40 C. The supernatant thus collected was used for biochemical assay. Results are the averages of three replicates.

Biochemical Assay: Guaiacol H_2O_2 method was used for assaying the activity of guaiacol peroxidase (GPX; Racusen and Foote, 1965). The activity of superoxide dismutase (SOD) was assayed following the method of Kono (1978). Catalase (CAT) activity was determined by the method of Teranishi *et al.* (1974). Bernfeld's method was used for assaying the activity of α- amylase. A modified method of Harris and Jeffcoat (1974) was used to determine the activity of acid invertase. The activity of acid phosphomonoesterase was assayed by using p-nitrophenyl phosphate as substrate (Zink and Veliky, 1979).

RESULTS AND DISCUSSION

In *C. arietinum* cv. C-235, GPX activity increased continuously with an increasing drought stress period (Figure 2 A). On the other hand, enzyme activity enhanced continuously till day 15 and then declined sharply in BG-391 plants subjected to severe drought stress (Figure 2 B). The control plants of both the genotypes exhibited marginal variation in GPX activity. Similar results were also noted in bean leaves (Alekseeva and Ramazanova, 1973) and rice (Jha and Singh, 1997) under water stress. Increased peroxidase activity appears to be caused by over expression of peroxidase coding genes to reduce the ROS concentration. SOD is one of the key components of the cell protection system against oxidative stress. In the present study, SOD activity was increased continuously in the leaves of both the genotypes of chickpea. Higher activity of SOD enzyme in C-235 and BG-391 was recorded on day 18 and 15

respectively (Figure 2 C, D). SOD activity increased by six folds in BG-391 plants subjected to moderate drought condition. On 18 day of water deprivation, a sharp decline in enzyme activity was observed in BG-391. The induction of SOD indicates the biochemical adaptation in C-235 and BG-391 in response to drought.

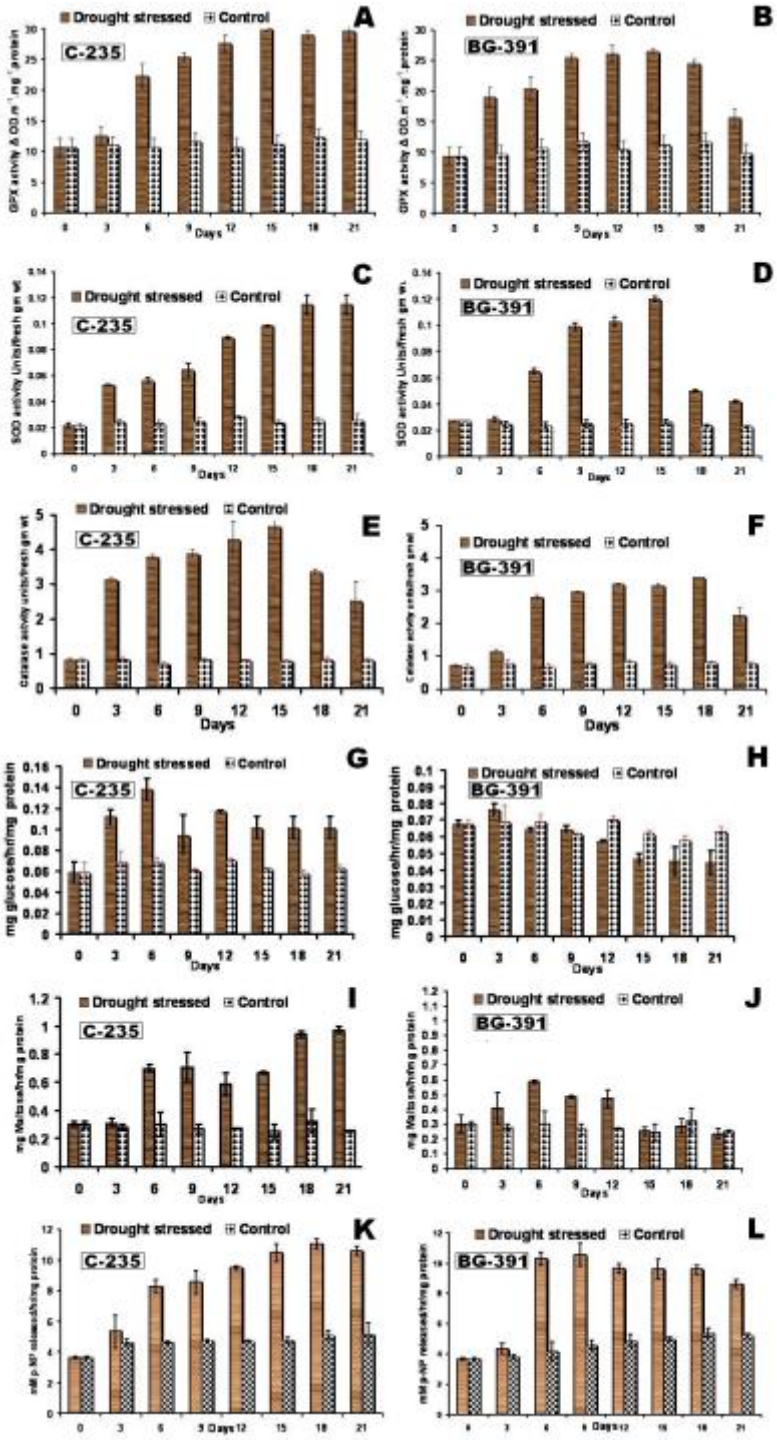

Figure 2: Drought induced changes in the activities of guaiacol peroxidase (A,B); superoxide dismutase (C,D), catalase (E,F), invertase (G,H); α-amylase (I,J); acid phosphomonoesterase (K,L) in C-235 and BG-391 varieties of *C. arietinum*

CAT activity is effective on reducing the adverse influences of environmental stresses. Drought condition significantly enhanced the activity of catalase enzyme in C-235 as compared with BG-391 (Figure 2 E, F). Higher activity of catalase enzyme was recorded on day 15. On day 18 of water deprivation, a sharp reduction in catalase activity was observed in C-235 plants. The catalase activity increased continuously till day 18 and declined thereafter in drought stressed BG-391 plants. The enzyme activity significantly increased on day 6 of water deprivation. Under moderate stress condition, plants exhibited a marginal increase in the activity of catalase enzyme. A remarkable reduction in enzyme activity was recorded on day 21 of water deprivation. The diminish activity of CAT during the later phase of drought stress might be caused on one hand by the inactivation of CAT and the disturbance of metabolism under severe drought stress and on the other hand by the occurrence of light-deficient inactivation.

Plant invertases are essential for growth processes since they make sugars available for cell elongation. In *C. arietinum* cv. C-235, invertase activity initially increased till day 6 and then declined with an increasing water starvation (Figure 2 G). The enzyme activity was slightly decreased under moderate and severe drought condition. In *C. arietinum* cv. BG-391, invertase activity was initially increased on day 3 and then declined slightly with an increasing water starvation (Figure 2 H). The higher activity of invertase activity in C-235 shows its involvement in water stress tolerance mechanism. The variation in invertase activity in the plants growing under normal irrigated condition was insignificant.

A wide variation in α-amylase activity was observed in both the genotypes of chickpea. Drought stress significantly increased the activity of α-amylase enzyme in C-235 (Figure 2 I). α-amylase activity was initially increased till day 6 and then reduced slightly in the leaves of *C. arietinum* cv. BG-391 plants subjected to the drought stress (Figure 2 J). The control plants exhibited marginal enhancement in α-amylase activity on day 18. Drought survival is enhanced by rapid hydrolysis of starch due to the over expression of α-amylase genes. The higher amylase activity observed in both varieties of chickpea might be associated with the *de novo* synthesis of α-amylase to maintain survival under adverse drought condition.

Acid phosphomonoesterase is known to maintain a certain level of inorganic phosphate in plant cells under stress (Chiung-Yueh and Ching-Hvei, 1998). Drought-induced activity of acid phosphomonoesterase was observed in both the genotypes of *C. arietinum* L. Acid phosphomonoesterase activity was increased continuously with an increasing water starvation in C-235 (Figure 2 K). Maximum enzyme activity was recorded on 18^{th} day of drought stress. In BG-391 the enzyme activity was significantly increased on day 6 and then slightly declined with increasing water deprivation period (Figure 2 L). Maximum enzyme activity in BG-391 was observed on day 9 Induction of acid phosphomonoesterase activity may be due to fact that under conditions of water stress, growth is restricted and delivery of phosphate is impaired, resulting in the activation of the cellular acid phosphomonoesterases that release soluble phosphate from its insoluble compounds inside or outside the cells (Sharma *et al.,* 2004). Olmos and Hellin (1997) observed that acid phosphomonoesterase under salt and water stress maintained a certain level of inorganic phosphate which could be co-transported with H^+ along a gradient of proton motive force. Similar changes in acid phosphomonoesterase activity under water stress were also reported in wheat (Barrett-Lennard *et al.,* 1982; Szabo-Nagy *et al.,* 1992), cotton species (Vieira-da-Silva, 1969), alfalfa (Ehsanpour and Amini, 2003) and rice (Shih and Kao, 1998). Barrett-Lannared *et al.,* (1982) demonstrated that salt and water stress increased acid phosphatase activity in *Pisum sativum*. On the other hand, Vyas *et al.,* (1985) reported that acid phosphatase activity progressively declined with increasing water stress in leaves of *Sesamum indicum*. The biochemical study clearly indicates that the *C. arietinum* variety C-235 has high drought tolerance as compared with BG-391.

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