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Differential responses of growth, antioxidant enzymes and osmolytes in the leaves of two groundnut (*Arachis hypogaea* L.) cultivars subjected to water stress.

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The present study was carried out to study the variation of antioxidative potentials in terms of activities of antioxidative enzymes along with the accumulation of osmolytes and plant growth in the leaves of two groundnut (*Arachis hypogaea* L.) cultivars namely K-134 and JL-24 under three levels of water supply conditions. Growth retardation in terms of root and shoot length, and in relation to the severity of drought stress was more pronounced in susceptible cv. JL-24 than in in tolerant cv. K-134.The leaves of cv. JL-24 showed greater reduction in dry weight and relative water content (RWC) when compared to cv. K-134 with increasing stress intensity. The activities of antioxidative enzymes which include superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), glutathione reductase (GR) and osmolytes such as proline and glycinebetaine were significantly high in the water-stressed leaves of both cultivars and the magnitude of increase was dependent on the severity of stress. These results highlight the ability of groundnut cultivars to up-regulate the enzymatic antioxidant system and osmolytes to withstand oxidative during water deficit conditions. However, the extent of up-regulation varied between the cultivars K-134 and JL-24, leading to the higher amounts of antioxidants and osmolytes in cv.K-134, supporting its drought tolerance. Furthermore, the drought tolerant nature of cultivar K-134 was well supported by lower rates of membrane lipid peroxidation, electrolytic leakage and chlorophyll breakdown.

Key words: Groundnut, Antioxidants, Water stress, Drought tolerance, Osmolytes

Groundnut (*Arachis hypogaea* L.), is an important oil seed, food and feed crop grown worldwide in an area of 23 million ha with an annual production of about 35 million tons (Akcay *et al.,* 2010). It is produced mainly for its high quality edible oil and proteins. In the semi-arid tropics, where about 70% of the peanuts are grown, drought is a major constraint to peanut production. The crop frequently experiences drought stress, which can vary in severity and duration. The adverse effect of drought on pod yield (14–88% decrease) and seed grade (100-seed mass) of groundnut is well documented (Clavel *et al.,* 2006). Despite its agronomic and economic importance, very little is known about its adaptive responses to drought (Clavel *et al.,* 2005). Therefore, an understanding of the physiological and biochemical mechanisms conferring drought tolerance of this species is very important in terms of developing selection and breeding strategies. The effects of various environmental stresses in plants are known to be mediated, partly, by an enhanced generation of activated oxygen species (AOS) (Foyer and Noctor 2005). This hypothesis is very plausible because chloroplasts, mitochondria and peroxisomes of plant cells are important intracellular (sites of generators) generations of activated oxygen species. These include superoxide anion (radicals) ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), which cause oxidative damage to membrane lipids, proteins, pigments and nucleic acids which ultimately results in the cell death (Foyer *et al.,* 1994). Plants have developed several antioxidation strategies by an array of enzymatic and non-enzymatic antioxidants (Apel and Hirt, 2004), that can protect cells from oxidative damage and scavenge harmful AOS created in excess of what is often needed for several metabolic reactions. The non-enzymatic antioxidants include the major cellular redox buffers, ascorbate (AsA) and glutathione (GSH), as well as tocopherol, flavonoids, alkaloids, and carotenoids which remove, neutralize and scavenge the AOS. Important AOS scavenging enzymes include superoxide dismutase (SOD, EC 1.15.1.11), peroxidase (POX, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) (Vaseva *et al.,* 2012). They take part in the elimination of superoxide radical and hydrogen peroxide (H_2O_2), which are created either directly or indirectly by the Mehler reaction and photorespiration in plants, preventing the highly toxic hydroxyl radical from forming through Haber-Weiss or Fenton processes (Mittler 2002). SODs are found in the cytosol, peroxisomes, mitochondria, and chloroplasts. Unlike to CAT enzymes, which are only found in peroxisomes, POX activities are localized in the cytosol, cell walls, and vacuoles. Glutathione reductase (GR, 1.6.4.2), is a potential enzyme of ascorbate-glutathione cycle and plays essential role in defense system against AOS by sustaining the reduced status of GSH. It is found in chloroplasts as well as in mitochondria and cytoplasm. It was demonstrated that elevated levels of reactive oxygen species, such as hydroxyl radicals, under drought are capable to induce oxidative stress, causing lipid peroxidation and consequently membrane injury (Mittler, 2002). Malondialdehyde (MDA) content, a product of lipid peroxidation is induced by large accumulation of AOS under water stress (Zhang *et al.*, 2007) and is regarded as biomarker for evaluation of the damages in plasmalemma and organelle membranes caused by oxidative stress (Mittler 2002, Vaseva*et al*, 2012). Therefore, activities of antioxidant enzymes and MDA content determine the toxic degree to crops (Turkan *et al.*, 2005; Zhang *et al.,* 2007). Cell membrane stability (CMS) measured in leaf fragments has been extensively used for assessing the stress intensity in plants (Vaseva *et al*, 2012) and in some cases higher membrane stability could be correlated with better performance (Sudhakar *et al.,* 2001; Meloni, *et al.,* 2003). The accumulation of low-molecular weight metabolites that act as osmoprotectants, such as proline and glycine betaine is a part of plant adaptation towards water deficiency (Ramanjulu and Sudhakar, 2000; Hoekstra *et al.*, 2001; Hasheminasab*et al.,* 2012). Proline is known to form long-lived adducts with free radicals, thus mitigating their damaging potential (Floyd and Zs-Nagy 1984) and stabilizes membranes and maintains protein conformation at low leaf water potentials (Reddy *et al.,* 2004). Proline was also found to be effective hydroxyl radical scavenger in vitro (Smirnoff and Cumbes, 1989). There are many reports in the literature that underline the intimate relationship between enhanced or constitutive antioxidant enzyme activities and increased resistance to water stress (Zhang *et al.,* 2007; Akcay *et al.,* 2010; Vaseva *et al.*, 2012; Kavas *et al.,* 2013).

Identification of the physiological and biochemical components of antioxidative defense system, which has a potential to confer drought tolerance, is essential for the characterization of tolerant cultivars and improve drought stress tolerance in crops. In this direction, to gain the better knowledge of understanding adaptive mechanisms underlying differential tolerance to drought, we made an attempt to study changes in growth parameters, RWC, chlorphyll stability index, cell membrane stability, osmolytes such as proline, glycinebetaine and activities of antioxidant enzymes represented by SOD, CAT, POX and GR in two high yielding cultivars of groundnut with contrasting drought tolerance.

MATERIALS AND METHODS

Plant material and water stress treatments

Seeds of groundnut (*Arachis hypogaea* L.) cultivars namely (K-134 and JL-24) were procured from Andhra Pradesh Agricultural Research Station Kadiri, Anantapur district. Seeds were surface sterilized with 0.1 % (w/v) sodium hypo chlorite solution for 5 min, thoroughly rinsed with distilled water and then germinated in plastic pots containing 2 kg of soil and sand (2:1) mixture and allowed to grow for thirty days. The pots were maintained in the departmental botanical garden under natural photoperiod of 10-12 h and temperature 28 4°C. Thirty-day-old plants were then divided into four-sets and arranged in randomized complete black design. One set of pots received water daily to field capacity and served as control (100 %). Water stress was induced by adding of water daily to 75, 50 and 25 % soil moisture levels respectively. Leaf samples were collected on day-12 after stress induction for analysis of various parameters.

Growth parameters

The length of the root and shoot was measured after inducing water stress. The plants were washed with deionized water and blotted dry with filter paper. Root and leaves were separated and dry weights were recorded. For the determination of dry mass, the roots and leaves were separately dried at 80°C in a hot air oven until a constant mass was formed.

Relative water content (RWC)

Fully expanded leaves were excised and fresh weight (FW) was immediately recorded from control and stressed plants. Then the leaves were immersed in distilled water and after 4h they were blotted dry and the turgid weight (TW) was taken. The leaves were kept at 80°C in a hot air oven for 48h and dry weights (DW) were recorded. The relative water content (RWC) was calculated from the formula (2), according to Turner, (1981). RWC was calculated according to Turner, (1981) using the following formula RWC (%) = [(FW – DW)/TW – DW)] X 100.

Proline content determination

Free proline content was extracted from leaves of both cultivars in 3% aqueous sulphosalicylic acid and estimated using ninhydrin reagent (Bates *et al.,* 1973). Fresh leaves were homogenized using mortar and pestle in 3% aqueous sulfosalicylic acid and filtered through four layered muslin cloth. 2 cm^3 of the filtrate was then added to acidic ninhydrin and glacial acetic acid 2 cm^3 each and incubated in a boiling water bath for 1 h. The tubes were then transferred to an ice bath to terminate the reaction and 4 cm^3 of toluene was added and vortexed for 15 s. Free Pro content in the mixture was measured by reading the absorbance at 520 nm against toluene.

Glycine betaine

Quaternary ammonium compounds (QACs) were extracted and measured as glycinebetaine (GB) equivalents according to Grieve and Grattan (1983) using KI-I2 reagent.

Enzyme extraction and assays

Superoxide dismutase

Leaf material was homogenized in 50 mM phosphate buffer (pH 7.0) containing 1% polylvinylpyrrolidine. The homogenate was filtered and centrifuged in a refrigerated centrifuge at 15000 × g for 15 min and the supernatant obtained was used as source of enzyme. All steps in the preparation of enzyme extract were carried out at 4°C. An aliquot of 0.1 ml enzyme extract was used for the determination of the protein content. The ability of superoxide dismutase to prevent the photochemical reduction of nitro blue tetrazolium was used to measure the enzyme's activity (Beauchamp and Fridovich, 1971). The reaction mixture (3 ml) consisting of 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM nitrobluetetrazolium, 0.1 mM EDTA, 2 µM riboflavin and 0.1 ml of enzyme extract. Riboflavin was added lastly and test tubes were shaken and placed 30 cm below a light souce (40 W fluorescent lamps). The reaction was started by switching on the lamps. The reaction was allowed for 30 min and then stopped by switching off the lights. The tubes were covered with black cloth. The reaction mixture which was not exposed to light did not develop colour and served as control. The absorbance was measured at 560 nm in a spectrophotometer. Log A_{560} was plotted as a function of the volume of enzyme extract used in the reaction mixture. From the resultant graph, the volume of the enzyme extract corresponding to 50% inhibition of the reaction was read and considered as one enzyme unit, and expressed as units per gram fresh weight per minute).

Peroxidase and Catalase

The leaf material was placed in a pre-cooled mortar and ground with cold 0.05 M Tris-HCl buffer pH 7.0. The extract was passed through cheese cloth and centrifuged at 1000 rpm to remove cellular debris. The supernatant solution was centrifuged again at 10000 rpm for 20 min. The supernatant was used as crude enzyme source for the assay of catalase and peroxidase. The procedures were carried out in a cold room. Kar and Mishra (1976) method was used to measure the peroxidase activity. The reaction mixture containing 0.1 M Tris-buffer (pH 7.0), 0.01 M pyrogallol and 0.005 M H_2O_2. The reaction was started by adding enzyme solution and the mixture was incubated at 25°C for 5 min. The reaction was stopped by adding 1.0 ml 2.5 N H_2SO_4. The amount of pyrogallin formed was estimated by measuring the absorbance at 425 nm.

Catalase activity was assayed and estimated as per the method of Barber, (1980). The reaction mixture consisted of enzyme extract, 0.005 M H_2O_2 and 0.05 M Tris-buffer (pH 7.0). After incubating it for 1 min at 25°C, the reaction was stopped by adding 1.0 ml of 2.5 N H_2SO_4. 0.01 N KMnO4 was used to titrate the residual H_2O_2. A blank was kept with the reaction mixture at zero time. Catalase activity was expressed as mg H_2O_2 oxidized per gram fresh weight per min.

Glutathione reductase

The plant material was powdered in liquid nitrogen using a mortar and pestle and extracted in 2.0 ml of 100 mM potassium phosphate buffer (pH 7.0). The homogenate was centrifuged for 10 min at 11000 g in a microcentrifuge. The supernatant was passed through a Sephadex G-25 column, active fractions were collected and used as enzyme source for the assay of glutathione reductase. Glutathione reductase activity was assayed as per the method of Foster and Hess, (1980).

The reaction mixture consists of enzyme extract, 100 mM potassium phosphate buffer, pH 7.0 containing 1.0 mM EDTA, 150 µM NADPH and 500 µM oxidised glutathione. The enzyme activity was measured at 340 nm. Activity was calculated using the extinction coefficient for NADPH of 6.22 mM^{-1} cm^{-1} and expressed as µ mol NADPH oxidized mg^{-1} protein min^{-1}.

Lipid peroxidation

Lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituricacid (TBA) reaction as described by Peever and Higgins, (1989). One gram of tissue (FW) was homogenized in 5 ml of 0.1% (w/v) TCA. The homogenate was centrifuged at 10,000 × g for 5 min and 4 ml of 20% TCA containing 0.5% (w/v) TBA was added to 1 ml of the supernatant. The mixture was quickly cooled on ice after being heated at 95°C for 30 minutes. The contents were centrifuged at 10,000 × g for 15 min and the absorbance was measured at 532 nm and 600 nm. The MDA concentration was calculated by its molar extinction coefficient (155 mM^{-1} cm^{-1}) after subtracting the non-specific absorbance (600 nm).

Chlorophyll stability index (CSI)

Chlorophyll stability index was determined by adopting the method of Koloyereas, (1958). The chlorophylls was estimated after heating the excised leaf discs of stressed plants incubated in distilled water for 30 minutes at 56 ± 1^0 C. Excised leaf discs incubated in distilled water without heating served as control. CSI was expressed as the difference between these two values.

Cell membrane stability (CMS)

Cell membrane stability was determined in two groundnut cultivars according to Leopold *et al.,* (1981). Leaf discs of 1 cm diameter were prepared using cork borer from control and stressed plants and leaf discs were incubated in 10 mL of water for 2 h. The solution was filtered and OD was examined at 273 nm (Initial OD). Subsequently leaf discs were boiled in the same solution for 30 minutes, cooled, filtered and OD was examined at 273 nm (Final OD). Percent leakage was computed using the formula, Per cent leakage = [(Initial OD/ Final OD) X 100].

Statistical Analysis

The data obtained in all parameters were subjected to analysis of variance (ANOVA) and the mean values were compared by Duncan's Multiple Range (DMR) test at 0.05% level as described by Duncan (1955).

RESULTS

Different response of root and shoot growth were observed due to water stress in both the cultivars (table1). There were no significant changes in root length of both cultivars during mild and moderate stress treatments. However, at severe stress treatments the root length was reduced to 15% and 9% in cultivars JL-24 and K-134, respectively when compared to control. The differences in the shoot growth between the stressed cultivars and control plants were not significant at mild stress levels, whereas at moderate stress level, shoot growth was reduced by 8% in cv. K-134 compared with a decrease of 13% in the cv. JL-24. Further, this decrease was more pronounced in cv. JL-24 (25%) than in cv. K-134 (12%) at severe stress level.

Root and leaf dry mass was decreased in water stressed plants of both cultivars when compared to controls (table1). However, cv. K-134 roots showed a slight increase (insignificant) in root dry mass upon exposure to mild stress when compared to control. Growth retardation was more pronounced in cv. JL-24 when compared to cv. K-134 in both root and leaf. Exposure to mild, moderate and severe water stress resulted in significant decline in RWC in leaves of both cultivars as compared to controls (table1). The magnitude of decrease was found to be stress intensity dependent. Nevertheless, a lesser degree of decline in RWC was observed in cv. K-134 compared to cv. JL-24 at all stress levels.

The free proline content was significantly increased in the stressed plants of both cultivars at all stress regimes over controls (table1). There was a linear increase in proline accumulation with increasing severity of stress. However, a difference in the accumulation of free proline content was observed between the two cultivars, a more pronounced increase was observed in the cultivar K-134 compared to JL-24. Proline content was increased by about 2.5-fold and 3.7-fold in the leaves of JL-24 and K-134, respectively, on the 12th day at severe stress treatments (table1). The glycinebetaine content significantly increased in the water stressed leaves of both cultivars throughout the experimental period (table1). The pool size of accumulated glycinebetaine increased with the stress intensity. However, the magnitude of increase was greater in the leaves of cultivar K-134, than in cultivar JL-24 (table1). The amount of glycinebetaine was increased by 11-fold in the stressed leaves of K-134 cultivar and 8-fold in JL-24 cultivar when compared to corresponding controls during severe stress conditions. From the results, it is also clear that at every stage of water stress the levels of glycinebetaine were higher in the cultivar K-134 compared to JL-24.

The activities of SOD, CAT, POX and GR are given in figures (table 2). Constitutive and induced antioxidant enzymes studied were higher in cv. K-134. A significant increase in the SOD activity was registered in both cultivars at all stress levels. Further the rise in SOD activity was dependent on stress intensity and cultivar specific (table 2). The increase in SOD activity was relatively greater in cv.K-134 compared to cv. JL-24. Thus, in cultivar K-134, severe stress treatment brought about 3 fold higher SOD activity over the respective control. While in cultivar JL-24, at severe stress approximately 2.2 fold increase in SOD activity was observed as compared to the control (table 2). The CAT and POX activities were significantly elevated in the leaves of both cultivars at all stress levels (table 2).

Table 1. Effect of water stress on root length, shoot length, dry weight (DW) of roots and leaves, leaf relative water content (RWC), leaf proline and glycine betaine contents of two groundnut cultivars. Means from 5 experiments ± SD. The mean values in a row followed by a different letter for each plant species are significantly different ($P \leq 0.05$) according to Duncan's multiple range (DMR) test.

Cultivars	JL-24				K-134			
Parameters	**Control**	**Mild**	**Moderate**	**Severe**	**Control**	**Mild**	**Moderate**	**Severe**
Root length (cm plant^{-1})	28.12a ± 0.28	29.09a ± 0.24	26.80a ± 0.35	24.58b ± 0.59	29.94a ± 0.34	31.15a ± 0.22	30.11a ± 0.48	27.47a ± 0.51
Shoot length (cm plant^{-1})	15.72a ± 0.21	14.52a ± 0.48	13.68b ± 0.39	11.72c ± 0.59	16.32a ± 0.19	15.82a ± 0.56	15.02a ± 0.30	14.32b ± 0.63
DW of roots (g plant^{-1})	0.2145a ± 0.003	0.2041a ± 0.004	0.1691b ± 0.002	0.1299c ± 0.006	0.2594a ± 0.004	0.2549a ± 0.003	0.2258b ± .006	0.1822c ± 0.007
DW of leaves (g plant^{-1})	1.078a ± 0.036	0.9432b ± 0.058	0.7755c ± 0.064	0.5400d ± 0.048	0.6872a ± 0.029	0.6214b ± 0.047	0.5326c ± 0.042	1.078a ± 0.036
RWC	91.58a ± 2.08	83.52b ± 3.21	62.34c ± 3.56	37.68d ± 2.54	90.12a ±1.09	81.62b ± 1.72	65.01c ± 2.94	48.53d ± 3.88
Proline contents (µg/gm^{-1} fresh wt.)	30.37a ±5.42	40.91b ±7.65	55.38c ±6.40	77.04d ±5.98	36.75a ±5.61	54.78b ±6.82	86.53c ±7.43	138.40d ±6.91
Glycinebetaine (µ mol g^{-1} DW)	1.42a ±1.06	3.16b ±1.10	6.67c ±0.98	11.42d ±1.12	1.66a ±0.99	4.52b ±1.12	10.11c ±1.01	18.27d ±1.28

Table 2. Effect of water stress on superoxide dismutase, peroxidase, catalase, glutathione reductase and malonialdehyde content in the leaves of two groundnut cultivars. Means from 5 experiments ± SD. The mean values in a row followed by a different letter for each plant species are significantly different ($P \leq 0.05$) according to Duncan's multiple range (DMR) test.

Cultivars	JL-24				K-134			
Parameters	**Control**	**Mild**	**Moderate**	**Severe**	**Control**	**Mild**	**Moderate**	**Severe**
Superoxide dismutase (units g^{-1} FW min^{-1})	6.90a ± 0.61	8.63b ± 0.82	11.98c ± 0.35	15.44d ± 0.90	8.10a ± 0.58	10.41b ± 0.75	16.46c ± 0.89	27.46 ± 0.98
Peroxidase (units g^{-1} FW min^{-1})	2.50a ± 0.18	3.29b ± 0.20	4.22c ± 0.25	5.26d ± 0.28	3.01a ± 0.21	4.61b ± 0.26	6.40c ± 0.28	8.23d ± 0.23
Catalase (mg H_2O_2 oxidised g^{-1} FW min^{-1})	2.31a ± 0.34	3.20b ± 0.42	4.28c ±0.38	5.52d ± 0.36	2.64a ± 0.28	4.53b ± 0.33	6.57c ± 0.40	8.69d ± 0.39
Glutathione reductase (µ mol NADPH oxidized mg^{-1} protein min^{-1})	29.08a ± 4.01	49.47b ± 4.38	76.93c ± 4.96	108.42d ± 5.92	34.97a ±4.04	63.85b ± 5.63	102.65c ± 6.31	150.40d ± 6.72
Malonialdehyde (µ mol g^{-1} FW)	10.04a ± 0.84	14.98b ± 0.86	20.84c ± 1.02	27.29d ± 1.32	9.01a ±0.91	12.43b ± 1.10	16.08c ± 1.21	19.83d ± 1.34

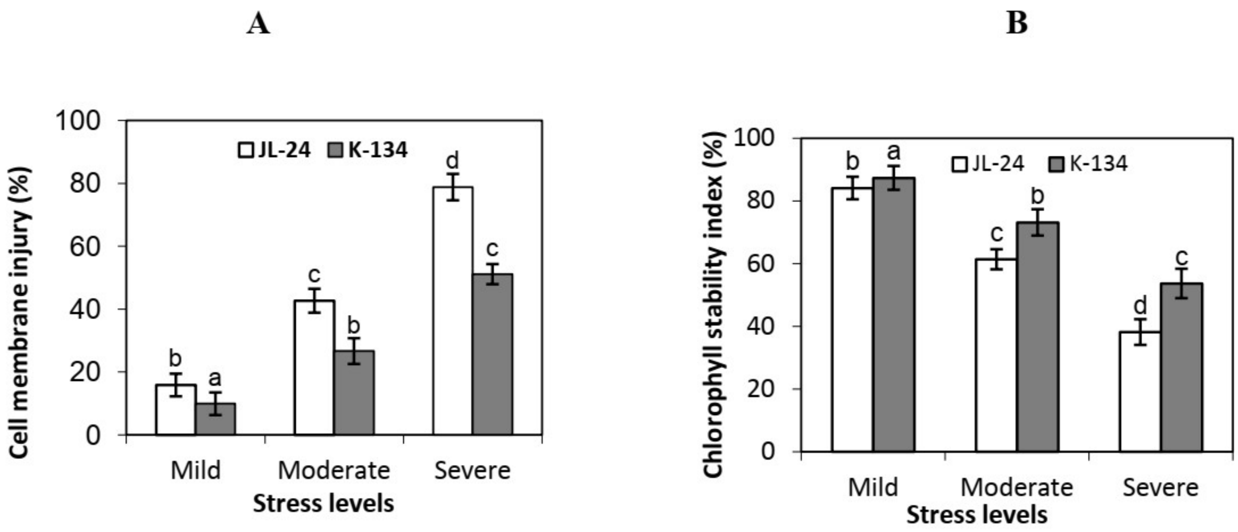

Figure 1. . Effect of water stress on (a) cell membrane stability and (b) chlorophyll stability index in two groundnut cultivars. Means from 5 experiments ± SD. The mean values in a row followed by a different letter for each plant species are significantly different (P ≤ 0.05) according to Duncan's multiple range (DMR) test.

However, the percent increase in the enzyme activities were high in K-134 than in JL-24 cultivar at all stress levels. The activities of H_2O_2 scavenging enzymes (CAT and POX) in leaves of water-stressed plants at the end of experimental period, was 3.3- and 2.7- fold in cv. K-134, and 2.3- and 2.1 fold in cv. JL-24, respectively (table 2). The enzyme GR was significantly increased in the leaves of all stressed plants. The rate of increase in enzyme activity was found to be dependent on severity in both cultivars. The percent increase was about 182, 293 and 430% in cv. K-134 and 170, 264 and 372% in cv. JL-24 under mild, moderate and severe stress respectively (table 2). The oxidative damage of lipids was evaluated by measuring the changes in malonedialdehyde (MDA) content. The MDA content was gradually increased with increase in stress intensity from mild to severe stress in both cultivars (table 2). However, MDA content was significantly higher in cultivar JL-24 (2.7 fold) than in cultivar K-134 (2 fold) under severe stress. Water stress caused a decline in chlorophyll stability index (CSI) and cell membrane stability (CMS) in both the cultivars (figure 1). The magnitude of decline was dependent on the severity of stress. However, the percent of decrease was low in K-134 than in JL-24 cultivar at all stress levels. The per cent membrane injury was 51% in cultivar K-134 and 78% in cultivar JL-24 under severe water stress. The loss of chlorophyll in cultivar K-134 is about 47% whereas in cultivar JL-24 it is 62% at the end of experiment.

DISCUSSION

Drought stress has adverse influence on water relations, biochemical and physiological processes, growth and yield of groundnut (Reddy *et al.,* 2003). It is known that plants may escape drought stress by cutting short their growth duration, and avoid the stress with the maintenance of high tissue water potential either by reducing water loss or improved water uptake, or both (Farooq *et al.,* 2009). In this study, groundnut may postpone dehydration by increasing root length up to mild stress levels and the ability of groundnut to maintain a viable root system during water stress may contribute to the crop's drought resistance (Sanders *et al.,* 1993). Although severe stress has caused inhibition in root length in both cultivars, cv. K-134 exhibited relatively lesser inhibition. This reduced growth under water stress may be ascribed to the reduced turgor, sufficiently enough to stop cell elongation or to dry soil conditions The decreased shoot growth during water stress has been reported by many workers in groundnut (Srinivasan *et al.,* 1987; Akcay *et al.*, 2010) and in other plants (Turkan *et al.,* 2005; Sankar *et al.,* 2007a; Singh *et al.*, 2021). The present study also revealed a reduction in shoot length during stress conditions in both cultivars and the reduction in plant growth could be attributed to decline in rapid cell division, elongation and enlargement due to low turgor pressure in the plant under water stress. (Sankar *et al.,* 2007a). In our investigation, root growth under water stress is less inhibited than shoot growth. This distinct response of the groundnut's root and shoot is an adaptation by plants to prevent severe water stress, while utilising moisture present at shallow layers of drying soil (Suma *et al.,* 2006). In assessing drought resistance of peanut genotypes, biomass can be used to indicate their potential productivities under drought stress (Purushotham *et al.*, 1998; Pimratch *et al.,* 2008). Dry weight (root and leaf) was decreased in water stressed plants of both cultivars compared to controls. These results agree with earlier reports in groundnut (Clavel *et al.,* 2005; Latha and Reddy, 2007). Dry mass accumulation in roots and shoots was relatively less in JL-24 than in K-134. Wright and Rao, (1992) noted that groundnut cultivars with vigorous early growth, a relatively large biomass accumulation and capacity for remobilizing stored assimilates to reproductive sinks may be better adapted to drought stress.

To identify legumes with contrasting differences in drought tolerance, RWC is considered as an alternative measure of plant water status that reflects the metabolic activity in tissues (Sinclair and Ludlow, 1986). The decline in RWC was reported by several investigators under stress conditions in groundnut (Madhusudan *et al.,* 2002; Quilambo 2004;, Clavel *et al.,* 2005; Udhayabharathi *et al.*, 2022) and other plants (Turkan *et al.,* 2005; Farooq and Azam, 2006; Ansari *et al.*, 2018). Similarly in the present study, there was gradual reduction in leaf RWC in groundnut cultivars with the increase of stress severity, but decrease in RWC was least in cv. K-134. This genotypic variation in RWC may be attributed to difference in the ability of varieties to absorb more water from soil and /or the ability to control water loss through stomata (Fazeli *et al.,* 2006). The patterns of RWC and biomass accumulation in leaves ran in parallel and showed decrease in relation to the degree of stress in both cultivars. It is suggested that the relative water content could help the tolerant cultivar to perform physio-chemical processes more efficiently leading to the maintenance better dry matter than the sensitive cultivar

Plants are known to withstand the prevailing drought stress by synthesizing and accumulating compatible solutes like sugars, polyols, betaines and proline which play a pivotal role in stress tolerance (Ramanjulu and Bartels, 2002). Many studies have proved a positive correlation between stress tolerance and the synthesis of osmolytes like proline and glycine betaine (Giridharakumar *et al.*, 2003; Asraf and Foolad, 2007). In the present study, we observed a substantial increase in proline and glycinebetaine levels in both groundnut cultivars under water stress. Similar reports were made for many plant species (Reddy *et al.,* 2004, Sankar *et al.,* 2007a, Mannivannan *et al.,* 2008; Chaitanya *et al.,* 2009). However, the percent of increase in proline and glycine betaine was more in cv. K-134 than cv. JL-24. Genotypic differences in proline and glycinebetaine accumulation during drought stress have been reported (Reddy *et al.,* 2004; Sankar *et al.,* 2007a; Abd ElAziz *et al.*, 2022) and a positive correlation between magnitude of proline accumulation and drought tolerance has been suggested as an index for determining drought tolerance potential of groundnut cultivars (Jharna *et al.,* 2001; Madhusudan *et al.,* 2002). On the basis of higher proline and glycine betaine accumulation and in consideration of manifold increase of the same over the control, the cultivar K-134 might be considered as drought tolerant. These facts showed that these compatible solutes not only function as osmolytes, but also protects the macromolecules (protein folding or membrane stability) from damaging effect of stress (Kusvuran *et al.*, 2013).The difference in osmolytes accumulation observed between the two cultivars during water stress is clearly reflected in their RWC and dry mass pattern.

The measurement of specific antioxidant enzyme activities during water stress treatments has been generally accepted as an approach to assess the involvement of the scavenging system during drought stress. Adaptation to drought may depend on the capacity to maintain a high level of antioxidants and lipid anti-peroxidation under water deficit (Fotelli *et al.,* 2002). Hence, the activities of antioxidant enzymes as well as MDA content determine the degree of damage to water-stressed crops. (Mencon *et al.,* 1995) The changes in activities of antioxidant enzymes and MDA content can be marked differently under water stress depending on the drought resistance of crop varieties, methods used and the degree of water stress (Arora *et al.,* 2002; Ge *et al.,* 2006). Many test results are employed to prove higher activities and variation of antioxidant enzymes as well as lower MDA content for a drought tolerant variety than a drought sensitive one under drought stress (Kavas *et al.,* 2013). Thus, a drought tolerant variety can possess a stronger ability to eliminate ROS and lipid peroxidation (Mencon *et al.,* 1995). As a confirmation, in the present work, we also observed higher constitutive and induced activities of SOD, CAT, POX and GR along with lower MDA content in cv. K-134 than in cv. JL-24 under water stress. SOD is the key enzyme in the active oxygen scavenger system and considered to be the first line of defense against ROS (Hamilton & Heckathorn, 2001). In the present study, water stress caused a significant increase in SOD activity during stress conditions in both cultivars, suggesting that SOD may function as a ROS scavenger, by converting $O_2^{\bullet-}$ to H_2O_2 (Alscher *et al.,* 2002). It is note worth that the contribution of SOD for the total $O_2^{\bullet-}$ scavenging activity was about 301.72% in the leaves for K-134, while for the JL-24 it was 223.76% in the leaves respectively at the end of experimentation (table 2). Thus, the percent increase was more in K-134 than in JL-24 cultivar, reflecting the ability of cultivar K-134 to scavenge $O_2^{\bullet-}$ radicals more effectively than JL-24. This implied that the tolerance of cv. K-134 may be at least partially associated with increased SOD activity. Similar genotypic variations in the activity of SOD differing in drought tolerance have been reported earlier (Turkan *et al.,* 2005; Dutta and Beera, 2007; Khanna-Chopra and Selote, 2007; Abd ElAziz *et al.*, 2022). Even though a high SOD activity protects the plant against the superoxide radical, it cannot be considered solely responsible for membrane protection against peroxidation because it converts $O_2^{\bullet-}$ to H_2O_2, which is also a ROS. This ROS should be then scavenged by other enzymes, such as CAT and POD, which destroy the H_2O_2 produced by SOD and other reactions (Foyer *et al.,* 1994). Increased activity of CAT and POD was observed under water stress in groundnut (Sankar *et al.,* 2007b) and in other plants (Gupta *et al.,* 2005; El-Tayeb 2006; Dutta and Beera, 2007; Khanna-Chopra and Selote, 2007; Jaleel *et al.,* 2008, Ansari *et al.*, 2018) Similarly in the present study, a significant elevation in the activity of CAT and POD was recorded in both cultivars at different regimes of water stress and the degree of elevation was relatively higher in cv. K-134 than cv. JL-24. This showed that cv. K-134 was more efficient scavenger of H_2O_2, which may result in better protection against H_2O_2. Similar results has been observed when comparisons were made between genotypes in wheat (Gupta *et al.,* 2005), in broad bean (El-Tayeb, 2006) in muskmelon (Ansari *et al.*, 2018) and in teosinte (Abd ElAziz *et al.*, 2022) differing in drought tolerance. Increased CAT and POD activity is likely to be adaptive trait, possibly helping to overcome the damage to the tissue metabolism by reducing toxic levels of hydrogen peroxide produced during cell metabolism as reported by Rasheed *et al.,* (1991). POD is thought to be involved in various plant processes, including lignification (Hendriks *et al.,* 1991), oxidation of phenolics (Largrimini, 1991), regulation of cell elongation (Fry, 1986) and detoxification of toxic compounds such as H_2O_2, which are produced as a result of oxidative stress (Chaparzadeh *et al.,* 2004). It is noteworthy that drought-induced SOD activity in the leaves was accompanied by an increase in CAT and POD activities in both cultivars. Thus, our results suggest that CAT and POD activities coordinated with SOD activity play a central protective role in the $O_2^{\bullet-}$ and H_2O_2 scavenging process (Liang *et al.,* 2003; Badawi *et al.,* 2004; Ansari *et al.*, 2018; Thippeswamy *et al.*, 2021) and the active involvement of these enzymes is related, at least in part, to drought-induced oxidative stress tolerance in groundnut plants. When the activity values of the H_2O_2 scavenging enzymes were compared (table 2), it was observed that CAT had a much higher H_2O_2 scavenging activity than POX in leaves of both drought -stressed cultivars of groundnut plants. Therefore, it could be hypothesized that CAT is the most important H_2O_2 scavenging enzyme in leaves of groundnut. These results are in agreement with those of Sudhakar *et al.,* 2001 and Azevedo Neto *et al.,* 2005. Glutathione reductase (GR) plays a key role in oxidative stress by converting the oxidized glutathione, GSSG to GSH maintaining a high GSH/GSSG ratio (Fadzilla *et al.,* 1997). Increased GR activity in leaves of stress plants has been reported to be closely related with drought tolerance capacity of plants (Turkan *et al.,* 2005; Khanna-Chopra and Selote, 2007). As a good confirmation with these findings, in our study, GR activity was increased in K-134 cultivar compared to JL-24 cultivar. The increase in GR in water stressed K-134 cultivar might have resulted in a higher pool of GSH, which could be used in ascorbate generation. Increases in GR activity make NADP available, which can take electrons from ferrodoxin and minimize the chance of superoxide production in addition to scavenging H_2O_2 (Arora *et al.* 2002). In the present study, when compared to JL-24 cultivar, K-134 cultivar would might have more efficiently eliminated H_2O_2 in the leaves by ascorbate-glutathione cycle in which POX acts as a strong catalyst together with GR. Both groundnut cultivars possessed better correlation amongst RWC, osmolyte accumulation and antioxidant enzymes with increasing stress intensity.

Malondialdehyde is a product of lipid peroxidation and is regarded as a biomarker for evaluation of the damages in plasmalemma and organelle membranes caused by oxidative stress. (Lin and Kao, 2000). A number of ROS appear to have initiated the lipid peroxidation in plant cells. Plants' sensitivity to salt stress and their tolerance to oxidative stress can both be assessed using the rate of lipid peroxidation measured in terms of MDA (Jain *et al.,* 2001). Parallel to these observations, in the present study it was observed a significant increase in MDA content in leaves of JL-24 cultivar and a less increase in K-134 cultivar. The lower level of lipid peroxidation in K-134 cultivar suggests that this is better protected from oxidative damage under water stress. Most often cellular membranes represent the first target of various abiotic stresses and this parameter characterizes their integrity (Bajji *et al.*, 2002). According to Bandurska (2000), the maintenance of the physical–chemical integrity of membranes under drought stress can be considered as one of the best physiological indicators of protoplasmic tolerance in plants. Cell membrane stability has often been used to assess drought and salinity tolerance in different crops (Sudhakar *et al.,* 2001; Farooq and Azam, 2006; Khanna-Chopra and Selote, 2007; Chowdhury *et al.*, 2017; Ansari *et al.*, 2018; Maishanu and Rabe, 2019).

In the present study, cell membrane stability was negatively correlated with the accumulation of MDA in groundnut cultivars and a smaller per cent injury was observed in cultivar K-134 compared to JL-24, which supports tolerance nature of cultivar K-134. The CMS values were correlated with drought tolerance in groundnut, and the drought tolerant cultivar showed low per cent membrane injury than in sensitive cultivar (Venkateswarlu and Ramesh, 1993; Gopalakrishna *et al.,* 2001; Babitha *et al.,* 2006; Udhayabharathi *et al.*, 2022). The less injury index in K-134 during water stress reflects maintenance of equilibrium in favour of synthetic processes. Relatively higher CMS and antioxidant activity, coupled with lower MDA content have been reported in drought tolerant genotypes of wheat (Renu and Devarshi, 2007, Hashmeinsab 2012), bean (Zlatev *et al.,* 2006), Mulberry (Ramachandra *et al.,* 2004) and safflower (Thippeswamy *et al.*, 2021). Chlorophyll pigments are thermosensitive in nature and their degradation occurs when it is subjected to high temperature. The recorded observations in respect to chloropyll stabiity clearly supported the tolerance nature of cv. K-134 compared with cv. JL-24. The chlorophylls are closely associated with drought tolerance and these parameters are used as biochemical markers for the identification of drought tolerant genotype in groundnut (Vyas *et al.*, 2001) and other plants (Arjun, 2019; Vishnu *et al.*, 2022). A higher CSI helps to plants with stand stress through better availability of chlorophyll which leads to increased photosynthetic rate, more dry matter production and higher productivity. It appears that relative tolerance of groundnut cultivars, as reflected by its lower lipid peroxidation and high membrane stability with the levels of its antioxidant enzymatic activity, characterizing cultivar K-134 as the less susceptible to membrane damage provoked by drought.

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