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# Effect of water stress on oxidative damage and antioxidant enzyme activity in finger millet and barnyard millet

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ABSTRACT

Plants experienced mostly biotic and abiotic stresses that have a great impact on their survival rate. Drought stress is one of the most serious environmental problems that affect plant growth and productivity. In the current study, oxidative damage and antioxidant responses under water stress were compared in 2 millets crops, finger millet and barnyard millet. Effects of water stress on physiological parameters like chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll), malondialdehyde (MDA), proline content, and antioxidant enzymes including catalase (CAT), phenol and flavanoid content in both the millets through different levels of drought (15, 30, 45 days) as compared to control were measured. A significant increase in proline accumulation was detected with increasing drought stress in finger millet as compared to barnyard millet. Chlorophyll content showed significantly increased activity in finger millet rather than in barnyard millet.

A significant increase in MDA content was detected more in roots rather than leaves in case of finger millet as similar to barnyard millet. Catalase showed significantly increased activity in roots rather than leaves in case of finger millet as similar to barnyard millet. Significant phenol accumulation was detected more in leaves than roots and stems in case of finger millet as similar to barnyard millet respectively. A significant rise in flavanoid content was found more in leaves rather than roots and stems in case of finger millet as compared to barnyard millet. These results suggest that finger millet is more tolerant against drought stress than barnyard millet. In both the millets crop tolerance against drought is due to an increase in the capacity of antioxidants and the increase of proline activity. Comparing these responses against drought stress will help to identify tolerance mechanisms in the millets crop.

## 1. Introduction

Abiotic stresses are the essential environmental factors that limit the productivity of many crops and also affect the quality and quantity of crop yields. Particularly water stress directly affects the physiology of plants, especially photosynthesis. In mountains, summer (kharif) crops often encounter water stress. Water stress is a natural phenomenon in rain-fed (unirrigated) cultivated areas. Manifestation of a drought stress condition is an annual event, almost persistently. As we know, Indian agriculture is rich in millet crops, particularly in minor millets, grown extensively

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from temperate north Himalayan region to peninsular region. In terms of world agriculture production, millets are among the important drought-resistant crops. Drought stress can have a significant impact on agro-ecosystems as well as on food crops. For improving the yield under drought stress conditions it becomes a major challenge in plant breeding practices. Apart from characteristic changes in plant morphology, drought stress can be identified on the basis of their physiological and biochemical changes. Millets perform better than cereals such as wheat and rice in semi-arid environments. In semi-arid and arid environments, millets are the prevailing crops. Drought or inadequate moisture stress affects its productivity. Millets are major sources of food and feed in the developing world, including in semi-arid and arid regions of India and Africa. Millets are the crops capable to resist in extreme environmental conditions, especially in water stress, and are rich in nutritional property. Millets might provide alternative climate-smart crops as their adaptation to challenging environments is better than the current major crops of the world. Millets are grouped in small grained cereal crops belonging to the Poacea family. Millets are ancient food crops which are highly nutritious and grown under marginal environmental conditions. Millets are also known as famine crops because these are the only crops assuring yields in famine conditions. About 80% of the millets are used for food and the rest for stock feed. Finger millet (*Eleusine coracana* L.) and barnyard millet (*Echinocloa esculenta* A. Braun) are the two major millet crops growing intensively in the mountain areas of Uttarakhand. Cultivated in kharif season in rain-fed areas, these millet crops often experience water stress. The present study attempts to bring to the fore the morphological changes and physiological and biochemical indicators that emerge during drought conditions.

Climate change is expected to originate exaggerated temperatures across the planet within the range of 1°C to the maximum of 6°C by 2050. As per IPCC (2007) and different studies, temperature will increase of 1°C to 2°C can lead to a rise in production of a number of the world's major staples with progressively negative impacts. Phenological traits like early flowering and maturity square measure major parts of crop adaptation, significantly in environments wherever the season is restricted by terminal drought and (Subbarao *et al.,* 1995). Severe drought stress conjointly inhibits the chemical change of plants by inflicting changes in pigment content, by poignant pigment elements and by damaging the chemical change equipment (Iturbe-Ormaetxe *et al.,* 1998). Ommen *et al.,* 1999) reported that leaf pigment content decreases as a result of drought stress and this drought stress is especially the result of harm to chloroplasts caused by active gas species (Smirnoff, 1995). The accumulation of osmolytes might make sure the maintenance of the structural integrity of membranes. Plant area unit shows a lot of tolerance to water deficit once water is withheld beneath conditions that favor diffusion adjustment (Moinuddin and Khanna-Chopra, 2004; Talebi *et al.*, 2013). Amino acid is one in all the osmolytes that increase quicker than alternative amino acids in plants beneath water deficit stress and facilitate the plants to take care of cell state (Zhao *et al.*, 2008). Thus, amino acid accumulation is used as a criterion for drought resistance assessment of sorts (Gunes *et al.*, 2008) Among varied abiotic stresses, drought is one in all the fundamental factors for limiting crop production (Vallivodan and Nguyen, 2006). In fact, it's foretold that one third of world population are vulnerable by water shortage in year 2025.

Drought stress is taken into account to be a loss of water that ends up in stomatal closure and limitation of gas exchange. Drought stress is characterized by reduction of water content, diminished leaf water potential, stomatal closure, disturbance in metabolism and at last the death of plant (Jaleel *et al.*, 2008). It reduces plant growth by varied poignant physiological and organic chemistry processes, like chemical change, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Farooq *et al.,* 2008, Jaleel *et al.*, 2008; Razmjoo *et al.*, 2008). Water stress may be a limiting factor for agriculture production by preventing a crop from reaching the genetically determined theoretical potential yield (Begg and Turner, 1976).

Millets crop are highly adaptable and stress tolerant compared to most of the other major crops like rice and wheat. These millet crops often experience water stress and this problem seems to intensify with the on-going erratic behavior of weather and climate change. The present study attempts to bring to the fore the oxidative damage and antioxidant enzyme activity that emerge during drought stress.

## 2. Materials and Methods

The Present investigation was carried out during October to March, 2018-2019 at the glass house of the college of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar. This site is situated at $29^0$N latitude, $7929^0$E longitude and an altitude of 243.84 m above the mean sea level which lies in the foot hills of the Himalayas in a narrow strip called Tarai. The climate of Pantnagar is humid subtropical with severe cool and hot dry during winter and summer respectively. Average temperature recorded during the experimental period was $28\pm5^0$C.

### *Experimental material and details*

The seeds of both the millet crops (finger millet and barnyard millet) were collected from a mid-altitude Himalayan village of Bimola situated in district Almora in Uttarakhand. After surface sterilization, seeds were grown in plastic pots containing loamy sandy soil without any use of fertilizer. Plants were grown in glass house but not under controlled conditions. The glass house was chosen to conduct the experiment only to provide protection to plants from rainfall, fog, mist etc. Experiment was laid out in randomized complete block design (RCBD) with five replications for both the millet crops.

### *Water treatment*

In this experiment regular irrigation treatments were imposed after 7 days to maintain control conditions and water stress was imposed by reducing irrigation at 15, 30, 45 days' intervals in different pots. In each water-treatment 500 ml water was given at the fixed period of time. For analysis of Physiological parameters including Chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll), Proline, MDA, CAT, Phenol and Flavanoid were measured.

### *Determination of Chlorophyll content*

The chlorophyll content was estimated at vegetative and reproductive stage according to Hiscox and Israelstam (1979). Chlorophyll content was estimated in freshly harvested leaves at vegetative and reproductive stage by DMSO method. To estimate chlorophyll content 50 mg of finely chopped leaves were taken in test tube. Then 10 ml of dimethyl sulfoxide (DMSO) was added in each test tube. It was incubated at $65^0$C for three hours in an oven. After incubation, absorbance of DMSO containing chlorophyll was determined at 663 and 645 nm using a spectrophotometer against pure DMSO as Blank. The chlorophyll content was then calculated by using following formula:

Chlorophyll 'a' = $[(12.7\times A663-2.63\times A645)\times V)]/[\text{Weight (g)}\times 1000]$

Chlorophyll 'b' = $[(22.9\times A645-4.48\times A663)\times V]/[\text{Weight (g)}\times 1000]$

Total Chlorophyll = $[(20.2\times A645)+(8.02\times A663)\times V])/[\text{Weight (g)}\times 1000]$

### *Proline Content Determination*

The proline content was estimated from leaves, roots and stems at vegetative and reproductive stage according to standard method described by Bates *et al.*, 1973. About 0.3 g of leaf tissues from both control and stressed plants were homogenized with liquid nitrogen, and the tissue powders were suspended in 1 mL of 3% sulfosalicylic acid. Following centrifugation at 1000 × g for 5 min at 4°C, 0.1 mL of supernatant was mixed with 0.2 mL of acid ninhydrin, 0.2 mL of 96% acetic acid, and 0.1 mL of 3% sulfosalicylic acid. The mixtures were incubated at 96 °C for 1 h, mixed with 1 mL of toluene, and further centrifuged at 1000 × g for 5 min at 4 °C. Upper phases were collected, and the absorbances were read at 520 nm. The proline concentration was determined using standard curve then the proline was calculated and expressed on fresh weight (fr.wt) basis as follows:

$$\mu \text{ moles proline } g^{-1}\text{fr.wt.} = \frac{(\mu g \text{ proline /ml}\times \text{ ml toluene}\times 5)}{(115.5\times \text{ g sample})}$$

### *Estimation of MDA content*

MDA content was estimated from leaves and roots, according to Sun and Hu (2005). Approximately 0.15 g of wheat seedling leaf was homogenized in 3ml of 5% trichloroacetic acid (TCA) on the ice bath. The homogenate was centrifuged at 4000 rpm for 5 min at $25^0$C. Five ml of 0.5% thiobarbituric acid (TBA) was added to the supernatant. The mixture was kept in a boiling water bath (100 $^0$C) for 10 min, and then quickly cooled on ice. The content was re-centrifuged at 1000 rpm for 5 min. The supernatants were collected and used for the measurement of the absorbance at 450 nm, 532 nm and 600 nm.

### *Determination of Catalase activity*

The Catalase activity was estimated from leaves and roots, according to Kumar and Knowles (1993). The activity of catalase (CAT) was determined through the decline in absorbance at 240 nm for 1 min showing decomposition of $H_2O_2$. The reaction mixture contained 50 mM phosphate buffer (pH 7.0) and 15 mM $H_2O_2$. The reaction was initiated by adding 50µl enzyme extract. CAT activity (unit /min/g/fw) was defined using molecular extinction coefficient $\varepsilon = 39.4\ mM^{-1}sm^{-1}$

### *Determination of Flavanoid activity*

The total flavanoid activity was estimated from leaves, roots and stem using the method of Ordonez *et al.* (2006). To 1.5 ml of sample solution, 1.5 ml of 2% $AlCl_3$ ethanol solution was added. The mixture was incubated for 1 hr at room temperature. After that the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids. Total flavonoid contents were calculated as quercetin equivalent from the standard curve.

### *Determination of Phenol activity*

The total phenol activity was estimated from leaves, roots and stem using the method of Malick *et al.* (1980). Aliquots (0.1 to 1 ml) were pipette out from the prepared solutions into the test tubes then the volume was made up to 3 ml with distilled water. To it 0.5 ml of Foline-Ciocalteau reagent was added. After 3 min 2 ml of 20% $Na_2CO_3$ solution was added to each test tube. Then the mixture was mixed thoroughly and the tubes were placed in boiling water for exactly one min, then cooled and the absorbance was measure at 650 nm against a reagent blank. Total phenol was calculated from standard curve of catechol prepared by using different concentrations.

### *Statistical analysis*

The statistical analysis for the above mentioned parameters was carried out using three and two-way Analysis of Variance (ANOVA) technique according to Gomez and Gomez (1984) procedure for RCBD, through microcomputer 32 (with the help of STPR-15 & 2) at the Computer Center of GB Pant University of Agriculture and Technology.

## 3. Results and Discussion

**Chlorophyll content:** In two millet crops (finger millet and barnyard millet) the effect of water stress on chlorophyll content (chlorophyll 'a', chlorophyll 'b' and total chlorophyll) at two developmental stages is given in table 1 at different days of water treatment. As the level of drought increased, level of chlorophyll content gradually decreased. The chlorophyll content varied significantly ($p<0.05$) at all levels of drought (days of water treatment) during developmental stages. These data showed that water stress affected both the millet crops significantly. The maximum chlorophyll 'a' was obtained in barnyard millet (1.86 mg/g fr.wt.) at vegetative stage in case of control conditions and the minimum chlorophyll 'a' was observed in barnyard millet (1.19 mg/g fr. wt.) at reproductive stage in case of 45 days water treatment (prolonged drought). In finger millet the maximum chlorophyll 'a' (1.93 mg/g fr.wt.) was at vegetative stage in control condition and the minimum chlorophyll 'a' (1.22 mg/g fr.wt.) at reproductive stage in plants exposed to 45 days long drought conditions.

The maximum and minimum chlorophyll 'b' in barnyard millet was 0.64 and 0.23 (mg/g fr.wt. at vegetative stage in control condition and reproductive stage at 45 days of water stress, respectively. In finger miller the maximum chlorophyll 'b' was 0.70 mg/g fr. wt. at vegetative stage in control condition and the minimum chlorophyll 'b' was 0.25 mg/g fr.wt. at reproductive stage in 45 days water treated plants, respectively.

The maximum and minimum total chlorophyll obtained in barnyard millet was 2.46 and 1.40 mg/g/fr.wt. at vegetative stage in control condition and reproductive stage in case of 45 days of water treated plants. In finger millet the maximum total chlorophyll was found to be 2.59 mg/g fr.wt. at vegetative stage in control condition and the minimum total chlorophyll was 1.45 mg/g/ fr.wt. at reproductive stage in 45 days water treated plants respectively. As we compare both the millet crops, the data indicate that chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were more in finger millet than in barnyard millet. In many studies similar results were observed in wheat (Moaed Almeselmani, 2011; Farooq *et al.*, 2009), Juniperus (Arif *et al.*, 2014), finger millet (Assefa and Fetene, 2013; Khatoon and Singh, 2014), chick pea (Talebi *et al.*, 2013) etc.

**Table 1. Effect of different levels of drought stress on Chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) in finger millet and barnyard millet at developmental stages**

| Millets crop | Drought levels | Chlorophyll a (mg/g fr. Wt) | | Chlorophyll b (mg/g fr. Wt) | | Total chlorphyll (mg/g fr. Wt) | |
|---|---|---|---|---|---|---|---|
| | | Developmental stage | | Developmental stage | | Developmental stage | |
| | | Vegetative Stage | Reproductive Stage | Vegetative Stage | Reproductive Stage | Vegetative Stage | Reproductive Stage |
| Barnyard millet | Control | 1.84 | 1.63 | 0.77 | 0.62 | 2.58 | 2.22 |
| | 15 day | 1.64 | 1.44 | 0.66 | 0.55 | 2.28 | 1.97 |
| | 30 day | 1.49 | 1.37 | 0.51 | 0.43 | 1.97 | 1.77 |
| | 45 day | 1.25 | 1.18 | 0.37 | 0.31 | 1.6 | 1.46 |
| Finger millet | Control | 1.92 | 1.74 | 0.82 | 0.70 | 2.71 | 2.63 |
| | 15 day | 1.66 | 1.56 | 0.70 | 0.65 | 2.33 | 2.18 |
| | 30 day | 1.58 | 1.4 | 0.61 | 0.51 | 2.16 | 1.92 |
| | 45 day | 1.31 | 1.21 | 0.43 | 0.37 | 1.73 | 1.55 |
| | | S.Em | c.d at 5% | S.Em | c.d at 5% | S.Em | c.d at 5% |
| I factor a | | 0.020 | 0.0392 | 0.016 | 0.0315 | 0.041 | 0.021 |
| II factor b | | 0.028 | 0.0554 | 0.022 | 0.0446 | 0.058 | 0.029 |
| III factor c | | 0.020 | 0.0392 | 0.016 | 0.0315 | 0.041 | 0.021 |
| a ×b | | 0.028 | 0.0784 | 0.022 | 0.0630 | 0.083 | 0.029 |
| b × c | | 0.028 | 0.0784 | 0.022 | 0.0630 | 0.083 | 0.029 |
| a × c | | 0.020 | 0.0554 | 0.016 | 0.0456 | 0.058 | 0.021 |
| a × b ×c | | 0.039 | 0.1108 | 0.032 | 0.0891 | 0.117 | 0.041 |
| a= millets crop | | a | ** | a | ** | a | ** |
| b=drought level | | b | ** | b | ** | b | ** |
| c= developmental stage | | c | ** | c | ** | c | ** |
| | | a×b | ns | a×b | ns | a×b | * |
| | | b×c | ns | b×c | ns | b×c | ns |
| | | a×c | ns | a×c | ns | a×c | * |
| | | a×b×c | ns | a×b×c | ns | a×b×c | * |

**Highly significant, * Significant at $p<0.05$ and ns- non-significant at $p>0.05$.

**Proline content:** The effect of water stress (different levels of drought) on proline content in two millet crops (leaves, stems and roots) is presented in table 2. In the present experiment, proline content increased in the plants of both millet crops exposed to 15, 30 and 45-day long drought conditions. Highest proline content accumulation was in plants with 45 day-long drought period. The proline content was reported 24.51 µmol $g^{-1}$fr.wt in leaves, 22.14 µmol $g^{-1}$fr.wt. in stems and 21.51 µmol $g^{-1}$fr.wt in roots in case of barnyard millet, while it was 26.8 µmol $g^{-1}$fr.wt. in leaves and 28.02 µmol $g^{-1}$fr.wt. in roots at reproductive stage in case of barnyard millet. The proline content was lowest in control plants 3.01 µmol $g^{-1}$fr.wt. in leaves and 4.78 µmol $g^{-1}$fr.wt. in roots in barnyard millet and finger millet respectively, while µmol $g^{-1}$fr.wt. in leaves and 5.25 µmol $g^{-1}$fr.wt. in roots. The accumulation of proline content during water stress was significant in finger millet as compared to barnyard millet.

**Table 2. Effect of different levels of drought stress on proline content from different plant parts (leaves, stem and roots) in finger millet and barnyard millet at developmental stages**

| Millets crop | Drought levels | Proline content from leaves (µmol $g^{-1}$fr.wt.) | | Proline content from stems (µmol $g^{-1}$fr.wt.) | | Proline content from roots (µmol $g^{-1}$fr.wt.) | |
|---|---|---|---|---|---|---|---|
| | | Developmental stage | | Developmental stage | | Developmental stage | |
| | | **Vegetative stage** | **Reproductive stage** | **Vegetative stage** | **Reproductive stage** | **Vegetative stage** | **Reproductive stage** |
| Barnyard millet | Control | 4.36 | 6.73 | 4.62 | 7.05 | 6.77 | 8.67 |
| | 15 day | 7.84 | 11.94 | 8.81 | 12.51 | 10.92 | 14.95 |
| | 30 day | 12.42 | 14.30 | 13.02 | 15.13 | 15.7 | 17.28 |
| | 45 day | 17.75 | 21.51 | 18.07 | 22.14 | 20.71 | 24.51 |
| Finger millet | Control | 7.12 | 9.04 | 7.39 | 9.25 | 8.7 | 11 |
| | 15 day | 10.79 | 14.4 | 10.97 | 14.66 | 12.42 | 16.81 |
| | 30 day | 14.52 | 18.95 | 14.8 | 19.2 | 16.97 | 21.86 |
| | 45 day | 19.44 | 25.4 | 19.82 | 25.4 | 21.97 | 28.21 |

| | S.Em | Sig. |
|---|---|---|
| I factor a (Crop) | 0.090 | ** |
| II factor b (Developmental stages) | 0.090 | ** |
| III factor c (Plant parts) | 0.111 | ** |
| IV factor d (Drought levels) | 0.128 | ** |
| a ×b (Crop ×developmental stages) | 0.090 | ** |
| b × c (Developmental stages × Plant parts) | 0.111 | ns |
| a × c (Crop × plant parts) | 0.111 | ** |
| a × d (Crop × drought) | 0.128 | ** |
| b ×d (Developmental stages × drought) | 0.128 | ** |
| c ×d (Plant parts × drought) | 0.157 | * |
| a × b× c× d (crop× developmental stages × plant parts× drought) | 0.313 | ** |

**Highly significant, * Significant at $p<0.05$ and ns- non-significant at $p>0.05$.

The increased level of proline content helps plants to survive against drought stress by increasing osmotic strength of cell sap. Similar results were observed that water deficit causes sharp increase in proline content in finger millet (Bhatt *et al.*, 2011). Accumulation of proline has been shown to be positively correlated with abiotic stress tolerance (Bhatt *et al.*, 2011). Moreover, it has also been reported that exogenous supply of proline to cosmetically stressed callus of rice increase the growth of callus in vitro (Kavi Kishore *et al.*, 1989). An increase in proline content is a common response of plants to drought stress (Mostajeran and Rahimi-Eichi, 2009) and has been found in other species such as wheat (Keyvan, 2010; Akhkha *et al.*, 2011), chickpea (Mafakheri *et al.*, 2010), rice (Vajrabhaya *et al.*, 2001; Hien *et al.*, 2003), cotton (Parida *et al.*, 2008), and barley (Kabir *et al.,* 2015). Pireivatlou *et al.* (2010) observed that proline content accumulated in wheat cultivars under drought stress. The SA greatly improves the dehydration tolerance through the increment of proline content.

**Malondialdehyde content (MDA) in leaves and roots:** The effect of water stress (different levels of drought) on MDA content in two millet crops (leaves and roots) is presented in Table 3.

**Table 3. Effect of different levels of drought stress on MDA and Catalase content from different plant parts (roots and leaves) in finger millet and barnyard millet at reproductive stages.**

| Millets crop | Drought levels | MDA (unit/min/g-fw.) | | Catalase(unit /min/g) | |
|---|---|---|---|---|---|
| | | Plant parts | | Plant parts | |
| | | roots | leaves | roots | leaves |
| Barnyard millet | Control | 3.01 | 4.78 | 8.36 | 9.33 |
| | 15 day | 8.34 | 9.71 | 13.73 | 14.98 |
| | 30 day | 16.82 | 17.71 | 19.59 | 20.36 |
| | 45 day | 25.36 | 26.94 | 26.16 | 27.13 |
| Finger millet | Control | 3.43 | 5.06 | 9.42 | 10.31 |
| | 15 day | 9.10 | 10.59 | 15.08 | 16.21 |
| | 30 day | 17.41 | 18.66 | 22.60 | 23.51 |
| | 45 day | 26.80 | 27.70 | 27.04 | 28.08 |
| | | S.Em | c.d at 5% | c.d at 5% | |
| I factor a | | 0.213 | 0.426 | 0.658 | |
| II factor b | | 0.213 | 0.426 | 0.658 | |
| III factor c | | 0.301 | 0.426 | 0.931 | |
| a ×b | | 0.213 | 0.602 | 0.931 | |
| b × c | | 0.301 | 0.851 | 1.315 | |
| a × c | | 0.301 | 0.851 | 1.315 | |
| a × b ×c | | 0.426 | 1.204 | 1.86 | |
| a=crop | | a | ** | a | ** |
| b=plants part | | b | ** | b | ** |
| c=drought level | | c | ** | c | ** |
| | | a×b | ns | a×b | ns |
| | | b×c | ns | b×c | ns |
| | | a×c | ns | a×c | ns |
| | | a×b×c | ns | a×b×c | ns |
| **Highly significant, * Significant at p<0.05 and ns- non-significant at p>0.05. | | | | | |

MDA content increased from 15 days to 45 days drought conditions in the leaves of both the millet crops. Highest MDA content accumulation was in plants with 45 days water treatment. The MDA content was reported 25.36 unit/min/g$^{-1}$fw in leaves and 27.00 unit/min/g-fw. in roots in case of barnyard millet, while it was 26.8 unit/min/g$^{-1}$fw. in leaves and 28.02 unit/min/g$^{-1}$fw. in roots in case of finger millet. The MDA content was lowest in control plants 3.01(unit/min/g-fw.) in leaves and 4.78 unit/min/g-fw. in roots in barnyard millet and finger millet respectively, while 4.08 unit/min/g-fw. in leaves and 5.25 unit/min/g-fw. in roots. The accumulation of MDA during water stress was significant in finger millet as compared to barnyard millet. The higher level of drought (45 days) during developmental stage increased MDA content in leaves in both barnyard millet and finger millet.

MDA content is an indicator of membrane lipid peroxidation that may reflect the degree of damage at adverse conditions. The increase in MDA content under different water stress conditions showed that drought may induce membrane lipid peroxidation by means of ROS (Moussa and Aziz, 2008). Similar results were observed in many cases in finger millet (Kotapati *et al.*, 2014), wheat cultivars (Mohammadkhani, 2016), canola (Mirzaee *et al.*, 2013), sugarcane (Abbas *et al.*, 2014), melon (Kavas *et al.*, 2013) etc. According to Allen (1995), stress caused injury to plants which may be associated with oxidative damage at cellular level such as cell membrane damage.

### *Catalase activity in leaves and roots*

According to (Mittler 2002), catalase neutralizes hydrogen peroxide and converts it into water and inactive molecular oxygen. The effect of water stress (different levels of drought) on catalase activity in two millet crops (leaves and roots) is presented in Table 3. In the present experiment, catalase activity increased from 15 days to 45 days in both millet crop roots. Highest catalase activity was observed in plants with 45 days water treatment. The catalase activity was reported 26.16 (unit/min/g-fw.) in roots and 27.13 (unit/min/g-fw.) in leaves in case of barnyard millet, while it was 27.04 (unit/min/g-fw.) in roots and 28.08 (unit/min/g-fw.) in leaves in case of finger millet. The catalase activity was lowest in control plants 8.36 (unit/min/g-fw.) in roots and 9.33 (unit/min/g-fw.) in leaves in barnyard millet and finger millet respectively, while 9.42 (unit/min/g-fw.) in roots and 10.31 (unit/min/g-fw.) in leaves. The catalase activity during water stress was significant in finger millet as compared to barnyard millet (Table 3). Under drought conditions, catalase activity showed its protective role against stress. According to (Gupta and Gupta, 2005), the high activity of antioxidant enzymes under water stress, acts as a damage control mechanism and also provides protection from oxidative stress that may otherwise could cause lipid peroxidation which might lead to damage in cell membrane and organelles, proteins and their structure. Perhaps the increased activities of CAT result in the removal of the $O_2$- radicals and its product $H_2O_2$ induced by drought. Similar result observed in many cases in barley leaves (Salekjalal *et al.*, 2012), safflower leaves and roots (Hojati *et al.*, 2011), wheat (Shao *et al.*, 2005), canola (Hosseini *et al.*, 2015).

### *Flavanoid content from stem, root and leaves*

The effect of water stress on flavnoid content during different days of water treatment (drought state) in both barnyard millet and finger millet at maturity stage is presented in Table 4. The flavanoid content gradually increased with increasing level of drought. The higher level of drought (45 day) during maturity stage increased flavanoid content in both barnyard millet and finger millet significantly ($p<0.05$). The optimal flavanoid content acquired by barnyard millet was from stem (16.85 unit/ min/g- fw.), roots (17.89 unit/min/g-fw.) and from leaves (20.87 unit/min/g-fw.) at maturity stage in case of 45 day water treatment. In finger millet it was 17.53 from stem, 18.34 from roots and 23.34 unit/min/g-fw. From leaves at maturity stage in case of 45 day water treated plants. The minimum flavanoid content acquired by barnyard millet was 3.22 from stems, 5.28 from roots and 6.28 unit/min/g-fw. from leaves at maturity stage in case of 45 day water treatment, while the minimum flavanoid content was found in finger millet, i.e. 4.9 in stems, 8.07 in roots and 6.7 unit/min/g-fw. in leaves at maturity stage in case of control condition. The more flavanoid content was recorded from leaves of finger millet as compared to barnyard millet.

### *Phenol content from leaves, stems and roots*

The effect of water stress on phenol content from leaves, stems and roots in both barnyard millet and finger millet at maturity stage during different days of water treatment is presented in Table 4. The phenol content gradually increased with increasing levels of drought. The higher level of drought treatment (45 days) during maturity stage increased phenol content from leaves in both barnyard millet and finger millet. In the present experiment phenol content increased from 15 days to 45 days of drought period in the leaves of both the millet crops. Highest phenol content was observed in plants with 45 days of water stress.

**Table 4. Effect of different levels of drought stress on Phenol and Flavanoid content from different plant parts (leaves, stem and roots) in finger millet and barnyard millet at developmental stages.**

| Millets crop | Drought levels | Phenol(unit /min/g-fw) | | | Flavanoid (unit/min/g-fw.) | | |
|---|---|---|---|---|---|---|---|
| | | Plant parts | | | Plant parts | | |
| | | stem | root | leaves | stem | root | leaves |
| Barnyard millet | Control | 4.04 | 7.99 | 8.40 | 3.22 | 5.28 | 6.28 |
| | 15 day | 7.50 | 9.01 | 11.10 | 6.56 | 7.74 | 9.02 |
| | 30 day | 13.22 | 15.58 | 16.94 | 11.58 | 13.08 | 15.1 |
| | 45 day | 21.53 | 25.22 | 26.50 | 1685 | 17.89 | 20.87 |
| Finger millet | Control | 5.28 | 8.45 | 9.26 | 4.9 | 8.07 | 6.7 |
| | 15 day | 9.77 | 12.19 | 13.00 | 8.27 | 9.15 | 11.31 |
| | 30 day | 14.18 | 17.98 | 18.13 | 12.25 | 14.5 | 15.27 |
| | 45 day | 24.02 | 27.09 | 28.98 | 17.53 | 18.31 | 23.34 |
| | | S.Em | | c.d at 5% | S.Em | | |
| I factor a | | 0.672 | | 0.287 | 0.125 | | |
| II factor b | | 0.672 | | 0.351 | 0.153 | | |
| III factor c | | 0.950 | | 0.406 | 0.177 | | |
| a ×b | | 0.950 | | 0.497 | 0.153 | | |
| b × c | | 1.34 | | 0.497 | 0.217 | | |
| a × c | | 1.34 | | 0.406 | 0.177 | | |
| a × b ×c | | 1.90 | | 0.703 | 0.307 | | |
| a=crop | | a | | ** | a | ** | |
| b=plants part | | b | | ** | b | ** | |
| c=drought level | | c | | ** | c | ** | |
| | | a×b | | ns | a×b | ns | |
| | | b×c | | ns | b×c | ** | |
| | | a×c | | ns | a×c | ** | |
| | | a×b×c | | ns | a×b×c | ** | |

**Highly significant, * Significant at $p<0.05$ and ns- non-significant at $p>0.05$.

## 4. Conclusion

On the basis of the above-mentioned results, it could be suggested that the finger millet had higher drought stress tolerance as compared to the barnyard millet. The millet crops in the hills appear to be highly adapted to water-stress conditions which are likely to aggravate amidst the climate change scenario. Both the millets are cultivated in hills, however finger millet showed better adaptation capabilities and gave more promising results under drought stress. So, looking at the overall results, it is clear that these parameters could explain some of the mechanisms which indicate tolerance of millet crops to water stress. This investigation brings to the fore the information that could be critical for sustaining and improving the performance of crop cultivars under the spell of global warming and consequent climate change leading to acute water stress for certain period the drought-ridden areas of India.

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