As per UGC guidelines an electronic bar code is provided to secure your paper

International Journal for Modern Trends in Science and Technology
Volume 12, Issue 01, pages 43-54.
ISSN: 2455-3778 online
Available online at: http://www.ijmtst.com/vol12issue01.html
DOI: https://doi.org/10.5281/zenodo.21588709

Morpho-physiological and biochemical changes in barnyard millet *(Echinochola frumentacea)* under drought stress

J. Ajani | Shaik Parveen Banu

Department of Biochemistry, Yogi Vemana University, Vemanapuram, Kadapa – 516005, Andhra Pradesh, India
ajanireshma@gmail.com , *parveenbio1982@gmail.com*

To Cite this Article
J. Ajani & Shaik Parveen Banu (2026). Morpho-physiological and biochemical changes in barnyard millet *(Echinochola frumentacea)* under drought stress. International Journal for Modern Trends in Science and Technology, 12(01), 43-54. https://doi.org/10.5281/zenodo.21588709

Article Info
Received: 02 January 2026; Revised: 22 January 2026; Accepted: 26 January 2026.

KEYWORDS

ABSTRACT

Drought stress is one of the major abiotic threats arousing effects in almost all plants growth and yield. Several reports have shown that in vitro screening technique using PEG is one of the dependable approaches for the selection of desirable genotypes to study in detail on water scarcity on plant germination indices. The effect of drought stress in barnyard millets (Echinochloa frumentacea) varieties BYM NDL -8, VL-172, BYM NDL-1, VL-207, VMBC-350, was studied under in vitro environment in the presence of PEG 6000 with different osmotic potential 5% w/v, 10% w/v, 15% w/v, 20% w/v. Biochemical and enzymatic parameters were assessed in 7 days old seedlings imposed to 20% concentration of PEG. Parameter of oxidative stress malondianldehyde (MDA) and activities of stress responsive enzyme, catalase (CAT) and proline were determined. Maximum response to drought stress was alleviated at 20% w/v PEG as compared to that of control. However variety BYM NDL1 & VMBC 350 responded better among the other varieties. The relative drought tolerant ability associated with variety BYM NDL1 & VMBC 350 due to high antioxidant enzyme activity, high proline with low MDA content.

INTRODUCTION

Worldwide agriculture productivity has been threatened due to various abiotic stresses such as drought, salinity, heavy metals and high temperature. Drought is an important limiting factor which adversely influences the plants grow and development causing crop failure and decreasing average yields by more than 50% More than 10% of arable land has become desertified and greater than 50% of the average yields of major crops have been reduced. Under these conditions, water deficits in the plant tissues develop, along with changes in biochemical and physiological process in the plant. This affects seed germination and causes unsynchronized seedling emergence affecting the plants establishment and causing negative effects on the yield – The plants adapts to the water stress by altering is cellular mechanism and evoking its defence mechanisms.

Millets are a group of annual grasses found mainly in the arid and semi-arid areas. They are cultivated on 71.9 million acres in India, accounting for nearly 25 percent 0f the acreage under cereal crops. Barnyard millet is an important minor millet crop well adapted to low and moderate rainfall areas (500-700 mm) due to its early maturity character. The grains of barnyard millet are low in phytic acid and rich in iron and calcium (Sampath et al., 1986) [16]. In general small millets have a lots of health benefits. Among the small millets, barnyard millet place a most important role and have high micronutrient content, particularly calcium and iron, high dietary fiber, higher amount of essential amino acids and low glycemic index thus play an important role in the food and nutritional security of the poor. According to Resources Council Science and Technology Agency, Japan, (1982) the nutritional aspect, its grains are highly nutritious for example rich in protein, lipid, vitamins B1 and B2, and nicotinic acid compared with other cereals, such as rice and wheat grains. Barnyard millet have lots of health benefits but the awareness and health benefits of barnyard millets are very less in India compare to the developed countries thereby indicating a greater scope for exploitation of this millet under Indian condition. In our country, malnutrition is a major issue and commonly spread in Indian children's. Barnyard millets have high production potential under optimum conditions and millets have diverse adaptation mechanisms to grow and survive under relatively marginal environments and also present day's climate change is one of the alarming issues which have capability to alter the whole reproductive processes of the plant system and drastically affects seventy percent yield of the crop. To increase the productivity and stabilize the production in the changing climate, Development of abiotic stress tolerant genotypes is essential. Generally plants tolerant to particular abiotic stress establish a metabolic homeostasis and Carry on to their growth without suffering stress induced injuries. On the other hand sensitive plants unable to establish metabolic homeostasis that results in reduction in growth, ultimately leading to death (jogaiah et al, 2013). Noteworthy developments were made in understanding the abiotic stress at molecular, biochemical, physiological, and agronomic scales. Especially the response mechanisms and potential targets for improving crop response to drought (Tester and Landgridge 2010; chen Q et al , 2013), salt (zhang and shi , 2013), flooding (Bailery-serres et al,2012), low Temperature (Theocharis et al ,2012) and high temperature (hasanuzzaman et al, 2013), several reports have shown that in vitro screening technique using PEG is one of the dependable approaches for the selection of desirable genotypes to study in detail on water scarcity on plant germination indices (kocheva et al , 2003 ; sakthivelu et al, 2008). To achieve this we intend to understand the effect of different PEG-6000 concentrations on germination ,root length and shoot length of selective barnyard millet genotypes. It has been reported that barnyard millet to be tolerant to drought and water logging (Zegada-Lizarazu and Iijima 2005). Drought stress altered the balance between antioxidant defences and the amount Reactive Oxygen Species (ROS) resulting in oxidative stress. Excessive ROS generated in plants tend to interact with different macromolecules resulting in oxidation of protein, membrane lipids and nucleic acids and causes cellular damage, eventually affecting the growth and productivity of plants. Plants having some mechanism to cope out with drought condition to continue their normal growth conditions like accumulation of compatible solutes like amino acids, carbohydrates in plant cells that are known to play a role as security to protect cellular components. Among osmolyte, proline is well known in plants which increasing in moderate or severe stress condition, proline as a nitrogen storage reservoir or soluble which help in cytoplasmic osmotic potential decrease in acts of plant stress tolerance assists (Ghodsi et al. 1998).

MORHOLOGICAL PARAMETERS

Growth parameters

It has been established that drought stress is a very important limiting factor at the initial Phase of plant growth and establishment; it affects both elongation and expansion growth (Anjum et al., 2003). The Reduction in growth is common phenomenon in plants under water stress conditions (Jaleel et al., 2008c). Growth is accomplished through cell division, cell enlargement and Differentiation, and involves genetic, physiological, ecological and morphological events and their complex interactions. The quality and quantity of plant growth depend on these events, which are affected by water deficit. Cell growth is one of the most drought Sensitive physiological processes due to the reduction in turgor pressure. The decrease in cell enlargement is highly sensitive process, since cell growth is quantitatively Cell turgor dependent and the cell turgor decreases with any dehydration in cell water potential. It has been proposed that environmental and developmental controls of growth Rate act by regulating cyclin-dependent kinase (CDK) activity and cell division. CDKs are a family of protein kinases, those emerging as key players in regulation of cell Division and are likely to be regulated at both transcriptional and post-translational levels in response to stress. Cell expansion is a co-ordinately regulated process at the whole plant level and is influenced by external stimuli including water availability. The rate of cell expansion is mainly determined by two parameters, cell wall extensibility and cellular osmotic potential. The enlargement of plant cells involves control of wall synthesis and expansion, solute and water transport, membrane synthesis, Golgi secretion, ion transport and other processes.

Root and Shoot growth behavior

Roots play a primordial role in sensing soil water deficits. Water stress is known to influence various root attributes such as root size, morphology, depth, length, density and hydraulic conductance. The extent and pattern of root development are closely related to the ability of the plant to absorb water and hence are of great significance in drought tolerance. The growth reduction is presumably regulated by hormonal signals coming from the roots, and it's reduction is due to osmotic effect. An increase in root growth with decreasing soil moisture content has been reported in Arachis hypogaea, in Vicia faba, in Eucalyptus microtheca, in Triticum aestivum, in Abelmoschus esculentus and in Catharanthus roseus (Jaleel et al., 2008c). Other investigators have confirmed that root growth is restricted/marked reduction under severe moisture stress. Deep and dense root system enables the plant to tolerate drought by maintaining water uptake and higher water potentials. Allen et al., (1976) concluded from measured soil water attraction that during water stress, groundnut roots in lower depths continue to grow deeper even though vegetative growth appears to stop. showed that peanut had greater root length density deeper in the soil than other legumes when grown under drought stress. Production of ramified root system under drought is important to above ground dry mass and the plant species or varieties of a species show great differences in the production of roots (Jaleel et al., 2009). reported that drought tolerant wheat genotypes had significantly more roots in the crown region with the nodal and seminal roots concentrated closer to the soil surface while the susceptible genotypes had fewer roots in the crown region with most of the roots concentrated away from the soil surface. In general, extensive root systems are effective in postponing dehydration especially in deep soils.

Recorded deeper penetration of roots under moisture stress and concluded that the depth of root penetration can provide a useful selection criterion for wheat breeding under drought tolerance. Root penetration through compacted soil layers or hard pans has been recognized as an important breeding objective for drought improvement in rice.Roots is the primary sites of perception and transduction of drought signal. The information seems to be transferred to the whole plant via growth regulators (ABA or ethylene) and induces stomatal closure. A decrease in height of the plant under water stress condition was reported in Arachishypogaea in Eucalyptus micro theca in Sorghum bicolour in Triticumaestivum and Aegilopsbiuncialis in Glycine max (Zhang et al. 2004) in Abelmoschus esculentus in Helianthus and in Petroseliumcrispum. Noticed a decrease in relative growth rate of eight cultivars of maize under water stress conditions. Similarly, a decrease in relative growth rate of two cultivars of sugarcane (Singh and Singh, 1994) under moisture stress was also reported. The differential response has been correlated with drought tolerance based on lesser inhibition in relative growth rate.

Reported that roots subjected to drying soils may produce a non-hydraulic (hormonal) signal which carries an important effect on shoot growth and development, irrespective of the plant water status water deficits reduce the stands of plants. In general, shoot growth is reduced more than root growth because more severe water deficits develop in the shoots and probably persist longer. Thus, root-shoot ratios are generally increased by water stress. Increased root to shoot ratio was reported in Solanum melongena, In Arachis hypogaea, in Helianthus annuus and in Sophora davidil. Increase in root to shoot ratio may be an adaptive response to drought and to conserve water which may be an adaptation for drought survival. An increase in rout to shoot ratio under drought conditions was related to ABA content of roots and shoots In contrast, reported an increase in shoot to root ratio in rain fed low land rice cultivars under mild water stress. Similarly, increased shoot to root ratio was reported in soybean from the above reports it is clear that water stress affects the shoot growth and root growth of plants differentially.

BIOCHEMICAL CHANGES

Proline metabolism

Plants respond to a variety of stresses by accumulating certain specific metabolites, such as sugars, amino acids, amide, betaines etc. and the most conspicuous being proline. Its free accumulation is a widespread response of higher plants, algae, animals and bacteria to low water potential. The accumulation of free proline as a consequence of water stress has been documented by several investigators in groundnut and other plants. Genotypic variations in proline accumulation during water/salt stress have been noticed in a variety of crop species. Established a direct role to proline in transgenic tobacco plants genetically engineered to accumulate higher levels of proline and such plants are able to tolerate higher salinity. In addition to acting as an osmoprotectant proline also serve sink for energy to regulate redox potentials as storage of nitrogen source for rapid growth after stress (Stewart and Lee, 1974), as a hydroxy radical scavenger. A solute that protects macromolecules against denaturation an agent of detoxification of NH3 produced during water stress and as a means of reducing the acidity in the cell. Several investigators have been reported that the exogenous application of proline stimulates growth of cell and plants and to improve metabolisms under stress conditions.

The survival value of proline accumulation during water stress was contested by Lin and Kao, (2001), who suggested that high proline levels in water stressed leaves, was essentially a symptom of injury and hence might not represent a true index of drought tolerance. It is evident from the literature cited above, the increased levels of proline contributes significantly to the turgor maintenance of cells and proline accumulation has been considered as stress indicator in several plant species under water stress conditions. The accumulation of proline in stressed tissues has been attributed either to enhanced proline synthesis or inhibition of proline oxidation or impaired protein synthesis.

The pathway for the biosynthesis and degradation of proline was well established. Key enzymes involved in the proline metabolism are pyroline-5-carboxylate reductase (P-5-CR), proline oxidase and proline dehydrogenase. Proline biosynthesis can take place from glutamate as well as ornithine. Proline synthesis can takes place from the glutamate as well as ornithine by action of two important enzymes viz., pyroline carboxylic acid synthetase (P-5-CS) and pyroline-5-carboxylic acid reductase (P-5-CR). It has been demonstrated that plants expressing high levels of P-5-CS genes produced several fold more proline than the control plants concluded that P-5-CR activity might be involved in the metabolic regulation of cellular redox potential by affecting the level of reduction of NADPH. Studies on the sub cellular location of P-5-CR using Western blot assay revealed that the enzyme is localized in cytosol and plastid fractions in different parts of the plant. The location of this enzyme in plastids suggests that it may assist in counteracting photo-inhibitory damage of Rubisco enzyme under adverse conditions.

Proline catabolism is repressed under osmotic stress but once the stress is withdrawn, proline is oxidized to pyroline-5-carboxylate (P. 5-C) by proline oxidase; the first enzyme in the proline degradation pathway, P-5-C is then converted back to glutamate by the enzyme proline dehydrogenase. Thus, both proline oxidase and proline dehydrogenase form two important enzymes in the degradation of proline to glutamate in higher organisms. It has been reported that proline dehydrogenase also functions as P-5-C reductase (a proline synthesizing enzyme) and catalyzes reactions with some reactants and co-enzymes, but operating in an opposite direction. The role of proline catabolism has been highlighted in the field of nitrogen fixation. Plants such as soybean (Glycine max) synthesize high levels of proline in the host cells of nodules, and bacteroids are capable of high proline- oxidizing activity, suggesting that free proline entering the bacteroids from host cells might be an important energy source for nitrogen fixation. An increased activity of pyroline-5-carboxylate reductase under water stressed conditions was reported a significant decline in proline oxidase activity and proline dehydrogenase activity in ragi leaves under water stressed conditions. Similar reports were made in horse gram and in sweet pepper.

Malondialdehyde (MDA) content and Antioxidant enzyme assays

Drought stress involves in the production of ROS. These reactive oxygen species cause cellular damage interceded by exchanges with different cellular components, including lipids. Production of lipid peroxides is an integrated component of injury due to the stress imposed on the plants. The demolition of the lipid membranes resulted in the formation of lipid hydro peroxides, which can amplify the cellular toxicity. MDA content is measured as an indicator of oxidative damage caused by stress. Higher concentrations of PEG-induced water-deficit stress caused significant damage to membranes than control.

Plants employ an array of antioxidative and detoxifying enzymes like catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidise (APX), guaiacol peroxidise (GPX), etc., to protect from drought stress-induced oxidative damage by limiting the level of reactive oxygen species. Several reports emphasize the enhanced antioxidant enzyme activities during environmental stresses.

MATERIALS AND METHODS

Representative grain samples of five different barnyard millet varieties used in the study were collected from Regional Agricultural Research Station (RARI), Nandyal, Acharya N.G. Ranga Agricultural University (ANGRAU), Andhra Pradesh India. The names of the varieties are BYM NDL-8, VL-172, BYM NDL-1, VL-207, VMBC-350.

SEED GERMINATION EXPERIMENT

Healthy, uniform seeds of all varieties were surface sterilized with 1% sodium hypochlorite for 3 min, 70% ethanol and distilled water and surface dried using what man paper. Twenty seeds for each cultivar in each treatment were allowed to germinate on a filter paper in 9cm diameter Petri dishes. Each filter paper was moistened with solutions of 0(distilled water) as control, or 5%, 10%,15%,20%of PEG (MW 6000)concentration corresponding to final osmotic potential of -0.30,-0.51,-0.58 and -0.80 Mpa, respectively7. Polyethylene glycol 6000(Himedia, Mumbai) is of high molecular weight and is inert and non –ionic .PEG 6000 was used as it is small enough to influence the osmotic potential but cannot be fully observed by the plant and is not phytotoxic [8]. 10ml of appropriate solutions was applied to each petridish. The petriplates were arranged in completely randomized design (CRD) with two replicates for each treatment. Germination room temperature was maintained at 25°C in the dark with 8 h photoperiod and a relative humidity of 70%. Petri plates were periodically checked and respective solutions were applied to compensate evaporation. Seeds were considered germinated when radical had extended for at least 2 mm. Seedling shoot and root length of randomly selected seedlings from each replication were measured at the time of harvest (4 and 9 days after treatment application) by using a scale. Shoot and root dry weights were recorded after oven drying at 70°C for 24 hours in a hot air oven .After final count , final germination percent (FGP) were calculated by the following formula [9,10].

SEEDLING GROWTH EXPRIMENT

Seedling growth experiment was carried out in plastic pots (egg trays) filled with a mixture of the sand and peat (1:1). The pots were placed into growth cabinet with 2 replicates and 10 seeds were planted in each replication. Seeds were sown 3cm in depth and irrigate with 20% PEG – 6000 solutions as to generate osmotic potential. Pots were incubated under 25oC and 70 – 80 % relative humidity for 20 days. A seed or seedling was considered as emerged when the emerging radical reached to soil surface. The seedlings were taken off from the pots, rinsed with water to remove the debris, and used for proline estimation, enzyme analysis and estimation of MDA.

Proline

Free proline was determined by the method of Bates **et** al. (1973) . Leaf, 0.2 g, was homogenized in sulfosalicylic acid (3% **w**/v in distilled water) in the ratio of 1:10 and the homogenate was centrifuged at 10,000 rpm for **20** min. at room temperature. Supernatant 2 mL was mixed with 2 mL of glacial **acetic acid** and 2 mL of acid ninhydrin reagent. The content was incubated for one hour at 100 °C in a water bath. The reaction was stopped **by** keeping the tubes in ice bath. 4 mL of toluene was added to each tube and the chromophore was extracted **by** vigorous stirring **on a** vortex mixer. The absorbance of chromophore containing toluene layer was measured at **520** nm with toluene blank UV-VIS Spectrophotometer Concentration of proline in the sample was calculated in µg of free proline/ g fresh weight. The concentration of proline was expressed as µmol/g FW.

Malondialdehyde Content

Malondialdehyde content was assessed by the standard method Heath and Packer (1968) [38], 0.2 g of leaf material was homogenized in 0.25% (**w**/v) 2-thiobarbituric acid (TBA) prepared in 10% (w/v) TCA, in **the** ratio of 1:10. The homogenate was incubated at 95 °C for 30 min. in a water bath **and** centrifuged **at** 10,000 rpm for 30 min. The clear supernatant was collected and allowed to cool at room temperature. The absorbance was measured at 532 nm and 600 nm respectively. Absorbance at 600 nm was subtracted from **the** absorbance at 532 nm for **non**-specific absorbance. The concentration of MDA was calculated by using an extinction coefficient of $155 mM^{-1} cm^{-1}$.

Catalase Activity (CAT)

Specific catalase (CAT, EC 1.11.1.6) activity was measured according to Beers **and** Sizer (1952) [10], with minor modifications. **The** assay mixture consisted **of** 50 µL of the enzyme, 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, and 20 mM H_2O_2 in a total volume of 1.5 mL. The decrease **of** H_2O_2 was monitored by reading the absorbance at 240 nm at the moment of H_2O_2 addition and 1 min. later. The difference in absorbance (AA240) was divided by the H_2O_2 molar extinction coefficient ($36 M^{-1} cm^{-1}$) and the enzyme activity expressed as µmol of H_2O_2 min1 mg^{-1} protein.

RESULTS AND DISCUSSION

Germination percentage (%):

The PEG inhibited the germination of the susceptible lines and caused them in record low germination percentage. Almaghrabi, (2012) reported that environmentally confined seedlings in Laboratory experiments would appear to be suitable for screening large population to improve drought tolerance prior to yield testing. Usually the drought tolerant genotype will have the highest germination rate and better survival. The higher germination rates of the tolerant germplasm may be due to their capability to absorb water even under PEG induced water stress.

Final germination percentage drought stress induced by PEG had significant effects on the seeds germination. At 20% PEG concentration, seed germination in all five barnyard millet varieties was at its lowest ranging between 11% (VL-207) to 14% (VL-172) (Table-1). Germination percentages were inversely related to level of PEG concentration. The percentage of germination significantly decreased in all varieties due to increasing PEG concentrations. The final germination percentage range between 60 %(VL-207) to 75 %(BYM NDL -8) at 15% PEG concentration. At 10% PEG concentration, the range was between 70 %(VL-207) to 90 %(BYM NDL -8). Then, it ranged from 70% (VL-207) to 95% (BYM NDL-8). At 5%PEG concentration. All the five varieties exhibited a similar trend in reduction of germination percentage, however low PEG concentration (10, 15, and 20%) did not have any significant effect on the FGP of the barnyard millet seeds. Drought results in poor crop stand due to decreased seed germination. Presumably, the osmotic effect due to drought stress was the main inhibitory factor that reduced germination.

Table: 1 Effect of drought stress on final germination percentage (%) of barnyard millet seed varieties after 7 days growth.

	Seed Germinations				
	0%	5%PEG	10%PEG	15%PEG	20%PEG
BYM NDL-8	100 ± 0.2	95 ± 0.2	90 ± 0.2	75 ± 0.3	12 ± 0.1
VL-172	95 ± 0.2	80 ± 0.2	75 ± 0.2	65 ± 0.3	14 ± 0.1
BYM NDL-1	100 ± 0.2	95± 0.2	90 ± 0.2	76 ± 0.3	15 ± 0.1
VL-207	95 ± 0.2	70 ± 0.3	70 ± 0.3	60 ± 0.3	11 ± 0.1
VMBC-350	90 ± 0.2	75 ± 0.3	85 ± 0.2	60 ± 0.3	12 ± 0.1

Values represents mean + Standard Error

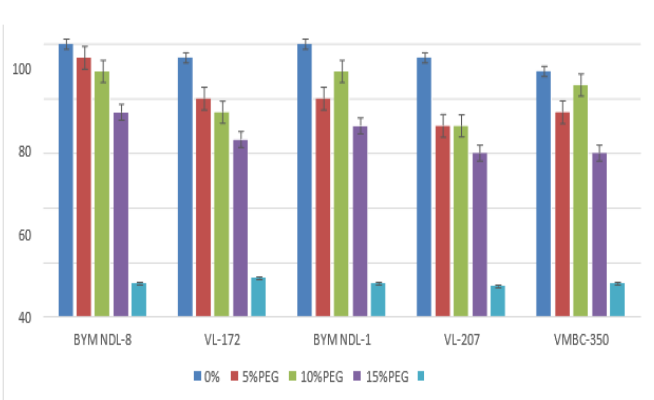

Figure 1 Effect of PEG-6000 induced osmotic stress on germination percentage of five genotypes of Barnyard millet.

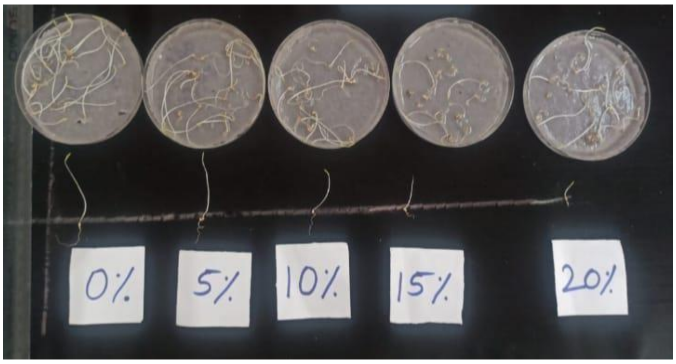

Figure 2 Screening of Barnyard millet at different concentration of PEG 600

Phenotypical assessments analysis

Root length

To find out the Drought withstand varieties root length measurement is the significant parameter in at seedling stage of plant. The root length was measured with common measuring scale in cm. Water stress due to drought is one of the most significant abiotic factors that limit the seed germination, seedling growth, plants growth and yield. Several methods have been developed to screen drought tolerant germplasm in plant species. Based on the literature available, PEG is considered as a superior chemical to induce water stress. Polyethylene glycol (PEG) molecules are inert, non-ionic, virtually impermeable chains and have been used frequently to induce water stress in crop plants. One of the important speculations is that a positive correlation between drought tolerance of the genotypes in the field and in laboratory experiments was noted.

The response of barnyard millet varieties against different PEG concentration generated informative results. Three different types of phenomenon observed among the root length of 07 days old seedlings i.e. Strong enhanced, enhanced, and declined by compared to control. In control the mean root length of BYM NDL-8, VL-172, BYM NDL-1, VL-207, VMBC-350, were 3.93cm,4.01cm,3.8cm,3.77cm,and 3.42cm respectively. Significant difference was observed in root length among the five varieties (Table 2). Among all the barnyard millet varieties examined at control to 10% PEG induced stress there was no significant difference was observed. Whereas between all varieties at 15%, and 20% PEG a clear differentiate phenomenon was recorded. Among all varieties VL-207 (2.27) was showed high mean root length and the variety BYM NDL -1(1.7) showing least root length but the remaining three varieties have very moderate mean root lengths against PEG induced drought stress.

Table: 2 Effect of PEG 6000 induced osmotic stress on root length of five genotypes of Barnyard millet.

	Radical Length				
	0%	5%	10%	15%	20%
BYM NDL-8	3.93	3.01	3.04	2.09	2.17
VL-172	4.01	3.12	2.70	2.20	2.02
BYM NDL-1	4.01	3.91	3.31	2.30	2.70
VL-207	3.77	3.33	3.30	2.27	2.10
VMBC-350	3.42	2.70	2.10	2.12	1.12

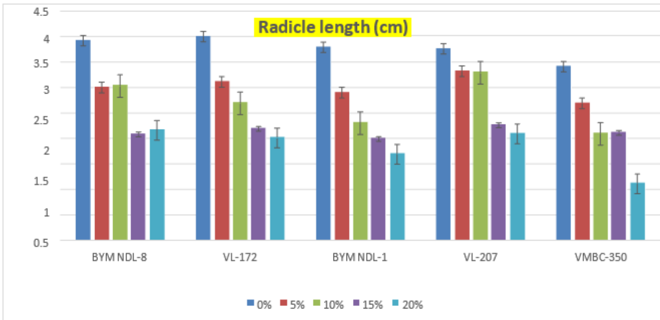

Figure 3 Effect of PEG induced drought stress on radical length (CM) of different barnyard millet varieties

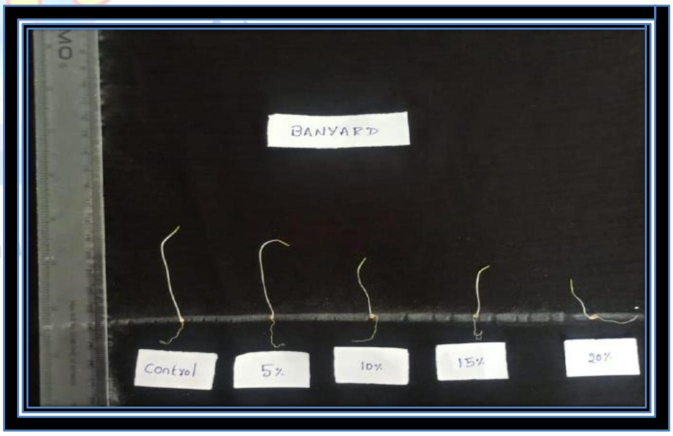

Figure 4 Barnyard millet showing the effect of water stress during early seedling growth.

Shoot length

To find out the Drought withstand varieties shoot length measurement is the clear parameter in at seedling stage of plant. The shoot length was measured with common measuring scale in cm. and mean shoot length was tabulated with standard error. The response of barnyard millet varieties against different PEG concentration generated confirmative decline from control to 20% PEG induced results. A strong negative correlation coefficient was noted in between PEG concentration and shoot length (i.e. shoot length was decreased with the increasing concentration of PEG). Five different types of phenomenon observed among the root length of 07 days old seedlings i.e. strong enhanced, enhanced, weakning and declined by compared to control. In control the mean shoot length of BYM NDL-08, VL-172, BYM NDL-1, VL-207, VMBC-350 were: 5.56, 5.79, 6.82, 6.14 and 5.82 respectively. Significant difference was observed in shoot length among the five varieties (Table 3.). Among all the barnyard millet varieties examined at control to 10% PEG induced stress there was no significant difference was observed. Whereas between all varieties at 15%, and 20% PEG a clear differentiate phenomenon was recorded. Among all varieties BYM NDL-8 (4.58) was showed high mean root length and the variety VMBC-350(2.1) showing least shoot length but the remaining three varieties have very moderate mean shoot lengths against PEG induced drought stress.

Table 3 Effect of PEG 6000 induced drought stress on plumule length (cm) of different Barnyard millets varieties.

	Plumule length (cm)				
	0%	5%	10%	15%	20%
BYM NDL-8	5.56	5.22	4.63	4.58	4.01
VL-172	5.79	5.2	4.29	3.62	3.0
BYM NDL-1	6.82	5.61	4.99	3.29	2.8
VL-207	6.14	4.77	4.49	2.48	2.4
VMBC-350	5.82	5.44	5.18	2.99	2.1

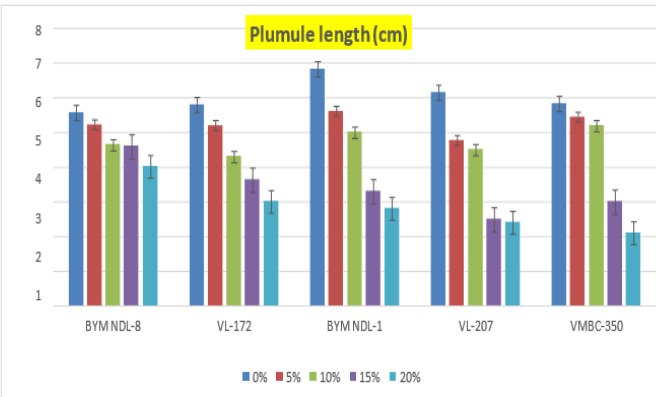

Figure 5 Effect of PEG Induced drought stress on plumule length (cm) of different barnyard millet varieties.

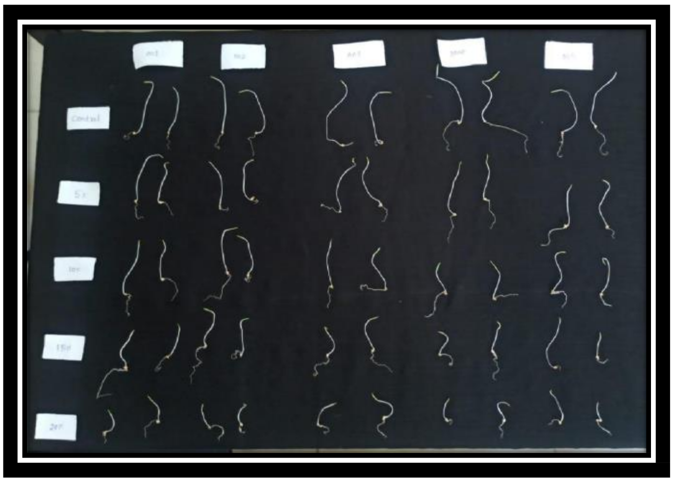

Figure 6 Effect of PEG 6000 on plumule and radical length at different concentra tions (0% w/v,5%w/v,10%w/v,15%w/v,20%w/v)of PEG.

PLUMIUM AND RADICAL FRESH WEIGHT

Plumium and radical fresh weight showed an inverse relationship with PEG concentrations. Both Parameter showed a significant decrease with increasing PEG concentration .However, plumium fresh weight was relatively less sensitive to PEG then radical fresh weight specially at higher PEG concentrations. At PEG levels of 10% and 15%,maximum plumium fresh weight was recorded VMBC-350. At 20% PEG, maximum plumium fresh weight was recorded.VL-207 The radical fresh weight decreased with increasing PEG levels as represented in Table-5 .At 10%, 15%, and 20% PEG concentration, maximum radical fresh weight was recorded VMBC-350 At 10%,15% and,20% PEG concentration , minimum radical fresh weight was recorded in BYM NDL-8

Table: 4 Effect of PEG on Fresh weight of plumule (g/seeds) of Barnyard millet varieties.

FRESH WEIGHT OF PLUMULE					
VARITIES	0%	5%	10%	15%	20%
BYM NDL-8	0.1555	0.1305	0.1155	0.09	0.0555
VL-172	0.1395	0.1235	0.118	0.075	0.0402
BYM NDL-1	0.12	0.1	0.09	0.05	0.04
VL-207	0.1355	0.1255	0.0825	0.072	0.0695
VMBC-350	0.17	0.149	0.1275	0.09	0.076

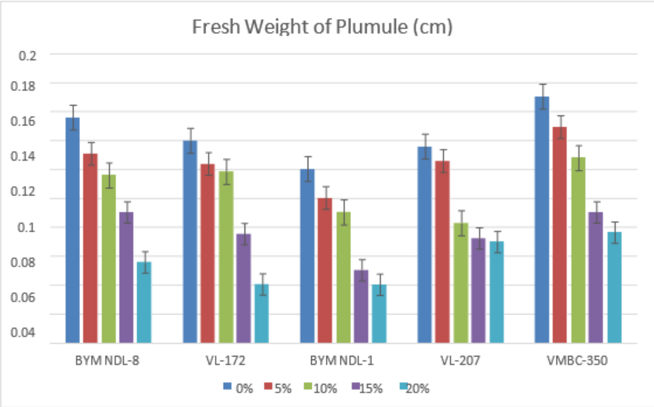

Fig: 7 Changes in the fresh Weight(Fw) In barnyard millet genotype during control & drought stress on 7th day bar represents mean value ± standard error of 3 biological replicates ,

Table: 5. Effect of PEG on fresh weight of radical g/seeds of barnyard millet varieties.

FRESH WEIGHT OF RADICAL					
VARITIES	0%	5%	10%	15%	20%
BYM NDL -8	0.051	0.048	0.03	0.02	0.0065
VL-172	0.055	0.0315	0.0235	0.009	0.004
BYMNDL -1	0.065	0.047	0.031	0.022	0.012
VL-207	0.04	0.045	0.031	0.025	0.0115
VMBC-350	0.05	0.032	0.0235	0.0222	0.0115

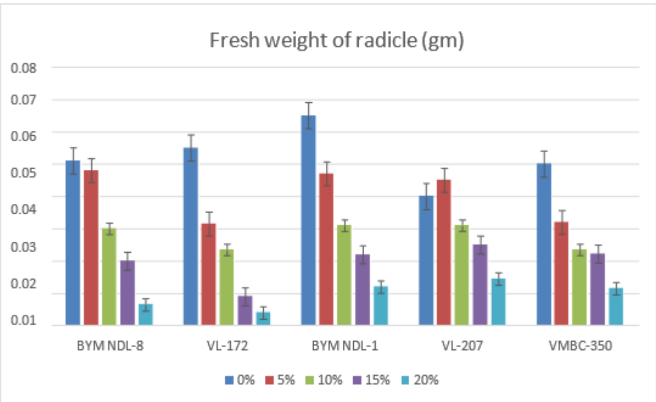

Figure 6 Changes in Fresh weight (Fresh weight) in five Barnyard millet genotypes during control & drought stress on 7th day. Bar represent mean value +, - standard error of 3 Biological replicates.

PLUMIUM AND RADICAL DRY WEIGHT

Drought stresses significantly reduce the accumulation of dry weight in all the five genotypes. Plumium and radical dry weight showed an inverse relationship with PEG concentrations. Both Parameters showed a significant decrease with increasing PEG concentration .However, plumium dry weight was relatively less sensitive to PEG then radical dry weight specially at higher PEG concentrations. At PEG levels of 10% and 15%, maximum plumium dry weight was recorded VL-172 at 20%PEG, maximum plumium dry weight was recorded BYM NDL_8. The radical dry weight decreased with increasing PEG levels as represented in Table-5 .At 10%, 15%, and 20% PEG concentration, maximum radical dry weight was recorded VL-172 .At 10%,15% and,20% PEG concentration , minimum radical dry weight was recorded in BYM NDL-1.

Table: 5.Effect of PEG on plumule dry weight g/seeds of barnyard millet varieties.

DRY WEIGHT OF PLUMULE					
VARITIES	0%	5%	10%	15%	20%
BYM NDL -8	0.125	0.095	0.09	0.09	0.075
VL-172	0.107	0.1	0 .085	0.07	0.05
BYM NDL -1	0.095	0.075	0.07	0.05	0.03
VL-207	0.12	0.1	0.09	0.065	0.04
VMBC-350	0.11	0.105	0.085	0.07	0.05

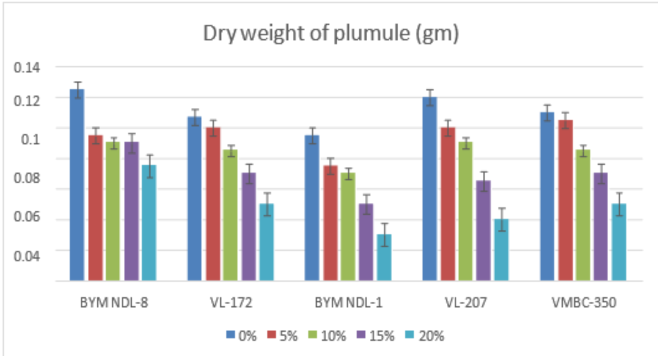

Figure 7 . Changes in dry weight of plumule in 5 barnyard millet genotype during cc control and drought stress on 7th day bar represent mean value + standard error biological replicates.

DRY WEIGHT OF RADICAL					
VARITIES	**0%**	**5%**	**10%**	**15%**	**20%**
BYM NDL -8	0.055	0.055	0.035	0.03	0.02
VL-172	0.044	0.04	0.035	0.0332	0.015
BYM NDL -1	0.051	0.045	0.036	0.0281	0.02
VL-207	0.0415	0.033	0.0311	0.023	0.02
VMBC-350	0.0399	0.0312	0.025	0.025	0.013

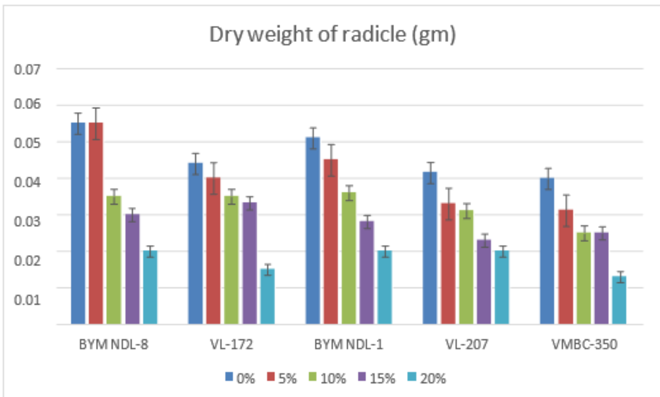

Figure 8 Changes in dry weight of radical in 5 barnyard millet genotype during control and drought stress on 7th Day bar represent mean value + standard error of 3 biological replicates

Proline analysis

Proline can act as a signalling molecule to modulate mitochondrial functions, influence proliferation or cell death and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savoure, 2009). Accumulation of proline under stress in many plant species has been correlated with stress tolerance, and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants. It influences protein solvation and preserves the quaternary structure of complex proteins, maintains membrane integrity under dehydration stress and reduces oxidation of lipid membranes or photo inhibition (Demiral and Turkan, 2004).

In generally the proline content was followed increase trend to drought stress, similarly in the present work also showed same as the previous research which was clearly mentioned in Fig 1. The varieties BYM NDL-1 and VMBC-350 are have high proline content at higher stress condition. Furthermore, it also contributes to stabilizing sub-cellular structures, scavenging free radicals, and buffering cellular redox potential under stress conditions (Ashraf and Foolad, 2007). Plants accumulate different types of organic and inorganic solutes in the cytosol to lower osmotic potential thereby maintaining cell turgor (Rhodes and Samaras, 1994). Under drought, the maintenance of leaf turgor may also be achieved by the way of osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycinebetaine, and other solutes in cytoplasm improving water uptake from drying soil.

MALONDIALDEHYDE CONTENT

The levels of MDA were higher in all the stressed plants compared to control. During unstressed condition maximum content of MDA was found in variety VL -172 among the other varieties. Minimum content of MDA was found in variety BYM NDL – 8. While as the seedlings were subjected to drought stress the maximum MDA content increased (0.26953) was found in variety VL 172 compared to control. However minimum (0.17317) increased was found in variety BYM NDL – 8 compared to control.

CATALASE ACTIVITY (CAT)

CAT was increased in all the five varieties of barnyard millet as shown in (figure 9a , b, c, d). Experiment finding indicated that highest activity of CAT was observed in variety BYM NDL 1 (0.0185) and lowest CAT activity was observed in variety VL 172 (0.015) compared to control in 20% PEG.

Table 1 Response of Proline content of millets barnyard at different drought conditions.

Barnyard Millet								
	Proline			MDA			CAT	
	ntrol	ated		Control	Treated		ntrol	eated
BYM NDL-8	0.775	1.957	BYM NDL-8	0.1417 6	0.1731 7	BYM NDL - 8	0.0052	0.0148
VL-172	0.564	2.168	VL-172	0.2272 7	0.2695 3	VL-172	0.0054	0.015
BYM NDL-1	0.8021	3.699	BYM NDL-1	0.1796 7	0.2590 7	BYM NDL-1	0.006	0.0185
VL-207	0.379	2.248	VL-207	0.1653 4	0.1916 6	VL-207	0.005	0.0108
VMBC-350	0.1151	2.886	MBC-350	0.1454 1	0.2456 2	VMBC -350	0.0058 7	0.0154

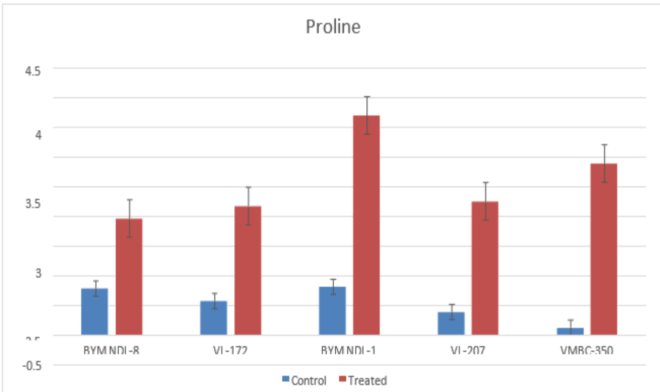

Figure 9 Effect of drought stress by PEG 6000 (20% Of V/W) on proline in 5 varieties of barnyard millets

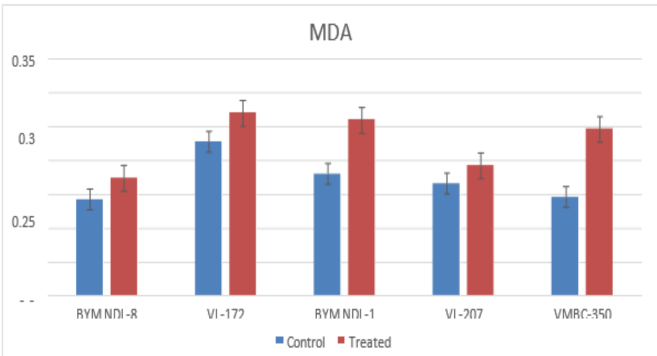

Figure 11 Effect of PEG 6000 induced osmotic stress on lipid per oxidation(in term of MDA content) in seedling of genotypes of barnyard millet

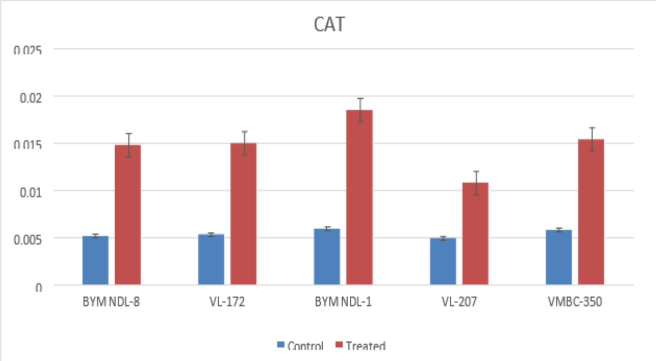

Figure 12 Effect of PEG 6000 induced osmotic stress on anti oxidant enzyme activity(CAT) in seedling of 5 genotypes of barnyard millets

CONCLUSION

Several reports have shown that in vitro screening technique using PEG is one of the dependable approaches for the selection of desirable genotypes to study in detail on water scarcity on plant germination indices. Identification of Barnyard millets genotypes that can withstand inadequate water condition is vital to increase the crop production and this can be accomplished only by exploring the drought tolerant germplasm of Barnyard millets. Current study was planned to find out appropriate criteria for simple and quick screening of barnyard millets genotypes that have higher tolerance to drought. The germplasm were exposed to PEG stress presented a significant alteration in germination index, plant growth, relative water content and proline content. From the above studies the germplasm BYM NDL-1 & VMBC-350 shows high proline and and high germination index, plant growth, fresh biomass and shoot & root ratio. The biochemical parameter proline content was increased in BYM NDL-1s and VMBC-350 varieties. Hence BYM NDL-1& VMBC-350 varieties showing resistance against PEG induced drought stress.

Our results indicated that drought stress increased oxidative level inside the plant and it resulted in accumulation of MDA content. However, variety BYM ND L-1 & VMBC-350 among the other three varieties respond better by having good correlation mechanism to minimize these effects by increasing others factors like higher accumulation of proline. To dismutase various antioxidant enzymes CAT activity was found to increase in variety BYM NDL-1& VMBC-350.

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

REFERENCES

[1] Anjum NA, Umar S, Ahmad A. Oxidative Stress in Plants: Causes, Consequences and Tolerance. 1st ed. IKInternational Publishing House Pvt Ltd, New Delhi 2003

[2] Bailey-Serres, J., Lee, S.C. and Brinton, E. 2012. Waterproofing Crops: Effective Flooding Survival Strategies. Plant Physiol., 160 (4): 1698-1709.

[3] Ehleringer, J.R. and Cooper. T.A. 1992. On the role of orientation ireducinphotoinhibitory damage in photosynthetic-twig desert shrub. Plant Cell Environ.. 15: 301-306.

[4] Gupta A, Mahajan V, Kumar M, Gupta HS. Biodiversity in the barnyard millet (Echinochloa frumentacea Link, Poaceae) germplasm in India. Genetic resources and crop evolution. 2009 Sep;56(6):883-9.

[5] Hasanuzzaman, M., Nahar, K. Mahabub, Md., Alam, R., Roychowdhury and Fujita, M. 2013,Physiological, Biochemical, and Molecular Mechanisms of Heat Stress Tolerance in Plants. t Int. J. Mol. Sci., 14: 9643-9684.

[6] Jaleel, C.A., Manivannan, P., Sankar, B., Kishorekumar, A., Gopi, R., Somasundaram R, 364. and Panneerselvam,, R. 2007. Induction of drought stress tolerance by ketoconazole inCatharanthusroseus is mediated by enhancedantioxidant potentials and secondary metabolite accumulation. Colloids Surf. B: Biointerfaces, 60: 201-20

[7] Jogaiah, S., Govind, S.R. and Tran, L.S.P. 2013. Systems biology-based approaches toward understanding droughttolerance in food crops. Crit. Rev. Biotechnol., 33: 23- 29.

[8] Kochava,k.and georgiev,g.2003.Evaluation of the reaction of two contrasting barley (Hordem vullgare L.) cultivaries in response to osmotic stress with PEG 6000. Bulg.J.plant physiol.,290-294.

[9] Larcher, W, 2000. Temperature stress and survival ability of Mediterranean clerophyllous Plants. Plant Biosyst, 134: 279-295.

[10] Sampath TV, Razvi SM, Singh DN, Bandale KV. Small millets in Indian Agriculture. In Small Millets in Global Agriculture. Ed. Seetaram, A .Riley, KU. And Hariyana, G.Oxford and IBH Publishing Co. Pvt. New Delhi, 1986, 34.

[11] Saleh ASM, Zhang Q, Chen J, Shen Q. Millet grains: nutritional quality, processing, and potential health benefits. Compr Rev Food Sci Food Saf 2013; 12:281-295.

[12] Sakthivelu, G., Devi, M.K.A., Giridhar, p., Rajasekaram, T., Ravishankar, G.A. Nedev, T.and Kosturkova, G. 2008. Drought –induced alterations in growth, osmotic potential and in vitro regeneration of soybean cultivars, Genet. APPl. Plant Physio. 34: 103 -112.

[13] Sood S, Khulbe RK, Gupta AK, Agrawal PK, Upadhyaya HD, Bhatt JC. Barnyard millet–a potential food and feed crop of future. Plant Breeding. 2015 Apr;134(2):135-47.

[14] SteWart, J.I., R.D. Misra, W.O. Pruitt and R.M. Hagan .1975. Irrigation corn and grain sorghum with a deficient water supply . Trans ASAE. 18: 270 -280.

[15] Sing, B.R. and D.P. Singh. 1995. Agronmic and Physiological responses of sorghum, maize and pearl millet to irrigation. Field Crops Res., 42; 57 -67.

[16]

[17] Tester, M. And Langride ,p.2010.Breeding technologies to increase crop production in a changing world, science 27:818-822.

[18] Theocharis ,a., clement ,C. and Barka, E.A 2012.Physiological and molecular changes in lants grown at low temperature .planta, 235:1091-105.

[19] Xiping, D., Lun, S. and Shinobu. I. 2002. Assement of the water conservations practices and Wheat adaptations to the semi –arid and eroded enivironements -12 th ISCO conference, Beijing

[20] Zegada-Lizarazu W, Iijima M. Deep root water uptake ability and water use efficiency of pearl millet in comparison to other millet species. Plant Prod Sci 2005;8:454-460

[21] Zhang J.L. and Shi, H. 2013. Physiological and molecular mechanism of plant salt tolerance. Photosunth. Res., 115:1-22.