Mysore J. Agric. Sci., 56 (4) : 424-431 (2022)

Physiological Basis of Drought Adaptation in Finger Millet [*Eleusine coracana* (L.) Gaertn.]

VARSHA V. MOHAN AND Y. A. NANJA REDDY
Department of Crop Physiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : varshamohan134@gmail.com

AUTHORS CONTRIBUTION

VARSHA. V. MOHAN : Carried out the experiment and perfromed the statistical analysis;
Y. A. NANJA REDDY : Conceived the study, designed & drafted the manuscript and final apporval

Corresponding Author :

VARSHA V. MOHAN
Department of Crop Physiology, College of Agriculture, UAS, GKVK, Bengaluru

Received : August 2022
Accepted : November 2022

ABSTRACT

Finger millet (*Eleusine coracana*) is known to be one of the most nutritious millet grown mainly under rainfed situations globally. Various environmental stresses affect the productivity of the crop. In the current study, the aim was to assess the physiological (relative water content and total chlorophyll content), biochemical (proline content, malondialdehyde content, Evan's blue technique) analysis parameters and total dry matter content of tolerant (GE-845, KMR-630) and sensitive genotypes (GE-1309 and GE-5123) to study the relevance of a few physiological / biochemical traits that impart drought tolerance. The experiment was done at the early seedling stage under laboratory conditions. The results revealed that, taking into consideration the physiological and biochemical parameters, among the four genotypes KMR-630 was proved to be the most drought tolerant genotype whereas, GE-5123 was found to be the most drought sensitive genotype. The results provides an innovative information for effective breeding strategies in view of deriving drought tolerant genotypes in finger millet.

Keywords : Finger millet, Drought, Proline, Evan's blue technique, Malondialdehyde

GLOBAL concerns over malnutrition, food insecurity and loss in agricultural productivity due to uncertain climatic changes have resulted in an increased demand for climate resilient crops. With climate change, the total rainfall is projected to increase (Jalihal *et al*., 2019), but due to irregular rainfall, increased frequency of drought episodes and water is becoming a limitation for agriculture (Dash *et al*., 2009 and Anonymous, 2019). Under this scenario, finger millet has gained focus of scientific research for their extraordinary potential to grow under low moisture, high temperature and poor soils (Pradhan *et al*., 2019). Its wide adaptability to diverse environments and cultural conditions makes it a potential food crop and being cultivated as a rainfed crop (Davis *et al*., 2019), and therefore finger millet could be a better alternative for semi-arid regions of the world (Dwivedi *et al*., 2012) with its C_4 nature (Ueno *et al*., 2006) in addition to its high nutritional value (Hiremath *et al*., 2018). However, productivity decline by drought is up to the extend of 100 per cent depending on the intensity and duration of stress period (Maqsood & Ali, 2007 and Krishna *et al*., 2021).

The reduction in grain yield under drought stress was reported to be attributed to impairment in physiological responses and a reduction in yield components (Krishna *et al*., 2021). Drought stress is presently the principal risk on world's food quantity and; limiting yield by affecting, physiological and biochemical processes. The main strategies employed by plants to sustain water deficit are (i) drought escape, (ii) drought avoidance and (iii) drought tolerance. The strategy of drought avoidance relies on enhanced water uptake and reduced water loss, whereas drought tolerance is mediated by osmotic adjustment, extension of antioxidant capacity and development

of desiccation tolerance. In India, finger millet is mainly grown under rainfed conditions where drought stress of 15-30 days is a usual concern, thus finger millet genotypes with high drought tolerance is very essential (Antre *et al*., 2021).

This study deals with the assessment of physiological and biochemical changes in finger millet genotypes to drought stress during seedling stage. The aim of the study was to ascertain the physiological / biochemical traits that have relevance in imparting the drought tolerance. Thus an experiment was carried out using field performed drought tolerant (GE-845, and KMR-630) and sensitive genotypes (GE-5123, and GE-1309). This will aid in inclusion of adaptive traits and stable genotypes in effective crop improvement programmes aimed at drought adaptation.

Materials and Methods

Plant Material

Seeds of four finger millet genotypes (drought tolerant, GE-845, and KMR-630) and (drought sensitive, GE-5123, GE-130) from the previous field study were selected for the present study to identify the physiological basis of drought adaptation.

Pot Experiment

A pot experiment was conducted in the greenhouse, Department of Crop Physiology (garden), University of Agricultural Sciences, GKVK, Bengaluru during the month of February, 2022. The seeds of all the four varieties were thoroughly washed with distilled water and surface sterilized with 6 per cent sodium hypochlorite. The seeds were soaked overnight before directly sowing in the pots containing red sandy loam soil and FYM mixture in a 3:1 ratio followed by usual production practices. The plants were subjected to two field capacity (FC) levels; 100 per cent (control) and 40 per cent (water stressed). Water deficit condition was imposed on the 15th day (early vegetative stage), by withholding irrigation following the gravimetric approach (Parvathi *et al*., 2019). The soil moisture was allowed to reduce gradually and it reached 40 per cent FC after 5-days of withholding irrigation. The stress period was maintained for seven days, after which the plants were allowed to recover. The leaf samples for various physiological and biochemical studies were collected from both the control and stressed plants when they were in stress period (5th day after stress imposition).

Physiological Parameters

Relative Water Content (%)

The relative water content (RWC) of control and stressed plants were measured according to the method described by method of Barrs and Weatherley (1962). The fully expanded leaves were excised and weighed immediately to record the fresh weight (FW). Then the leaves were immersed in distilled water and incubated for 6 h to record the turgid weight (TW). Finally, the leaves were oven dried at 80^{o}C to obtain a constant dry weight (DW). RWC was calculated using the formula :

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

Total Chlorophyll Content

The total chlorophyll content from the plant leaf samples were estimated by following the method described by Arnon (1949). The chlorophyll was extracted using the solvent, Acetone / DMSO (1:1 ratio). The absorbance of the samples was taken at 645 nm and 663 nm with acetone / DMSO (1:1) as the blank. The chlorophyll content was calculated using the following formula :

$$\text{Chlorophyll a} = \frac{(12.7 \times A663) - (2.54 \times A645)}{W \times 1000} \times V$$

$$\text{Chlorophyll b} = \frac{(22.9 \times A645) - (4.68 \times A663)}{W \times 1000} \times V$$

$$\text{Total chlorophyll content (TCC)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

where, W is the weight of the leaf sample (g), V is the volume made (ml), and A663 and A645 represents the absorbance measured at 663and 645 nm, respectively.

Biochemical Parameters

Proline Content

The leaf proline content was estimated in control and water stressed plants according to the method of Bates *et al*. (1973). Proline content was measured at 520 nm using UV-visible spectrophotometer and the content was calculated in leaf tissues from a standard curve prepared using L-proline. The concentration of proline was expressed as µmol g^{-1} FW.

Malondialdehyde (MDA) Quantification and Membrane Stability Index (MSI)

The estimation of MDA in the leaf samples to determine lipid peroxidation was done by following the protocol of Heath and Packer (1968). Leaf samples (0.1g) of both control and water stressed plants were homogenized in 0.1 per cent TCA and centrifuged at 12,000 rpm at 4°C for 15 min. The supernatant was collected and used to determine the MDA concentration by measuring the absorbance at 532 and 600 nm. The calculation was done using the Lambert-Beer law with an extinction coefficient εM = 155 mM^{-1} cm^{-1} and the values were expressed as µM MDA g^{-1} FW.

Membrane stability index (MSI) was recorded using the method described by Sairam (1994). The formula for the calculation is as follows:

$$MSI = [1-(C_1/C_2)] \times 100$$

where, C_1 and C_2 are the electric conductivities recorded at 40°C and 100°C, respectively.

Evan's Blue Staining

The quantification of membrane damage in leaf samples collected from control and stress were done using Evan's blue technique. The leaf samples were immersed in 0.25 g Evans blue solution prepared in $CaCl_2$ (0.1 M, pH 5.6) and incubated for 1 h. The unbound dye on the surface of the samples was removed by rinsing thoroughly with distilled water and the stained leaf samples were observed under a compound microscope. Further, 1 per cent sodium dodecyl sulphate (SDS) was used as blank to record the absorbance at 600 nm and the amount of dye which stained the leaf tissues was calculated with the standard curve prepared using different concentration of Evan's blue dye (Vijayaraghavareddy *et al*., 2017).

Total Dry Matter (TDM) Content

The plants along with the roots were removed from the pot to obtain the total dry matter content. Afterwards, the plants were placed in brown paper covers and kept in an oven at 70^0C temperature for 72 h to obtain the constant dry weight (DW).

Results and Discussion

The present study was aimed to understand the physiological and biochemical basis of drought tolerance. A comparative analysis of the genotypes (tolerant, GE-1309, KMR-630 and sensitive, GE-5123, GE-845), under control and water deficit condition revealed the tolerance level of each genotype for drought.

Physiological Parameters

Relative Water Content (%) and Total Chlorophyll Content

All the four genotypes showed significant decrease in RWC under water deficit condition compared to the control (Fig. 1a, Table 1) which was in accordance with the study conducted by Naik *et al* (2020) in finger millet. Among the genotypes, relatively higher

Table 1

Effect of drought stress on physiological parameters in different finger millet genotypes

Geno types	RWC (%)		Total chlorophyll content(mg g^{-1} FW)	
	Control	Stress	Control	Stress
GE-1309	90.33	75.68	1.52	1.91
GE-5123	92.03	65.61	1.34	2.36
GE-845	91.30	84.49	1.54	1.95
KMR-630	91.65	88.65	1.51	1.23
SEM±	1.87		0.15	
CD at 5%	5.65		0.47	

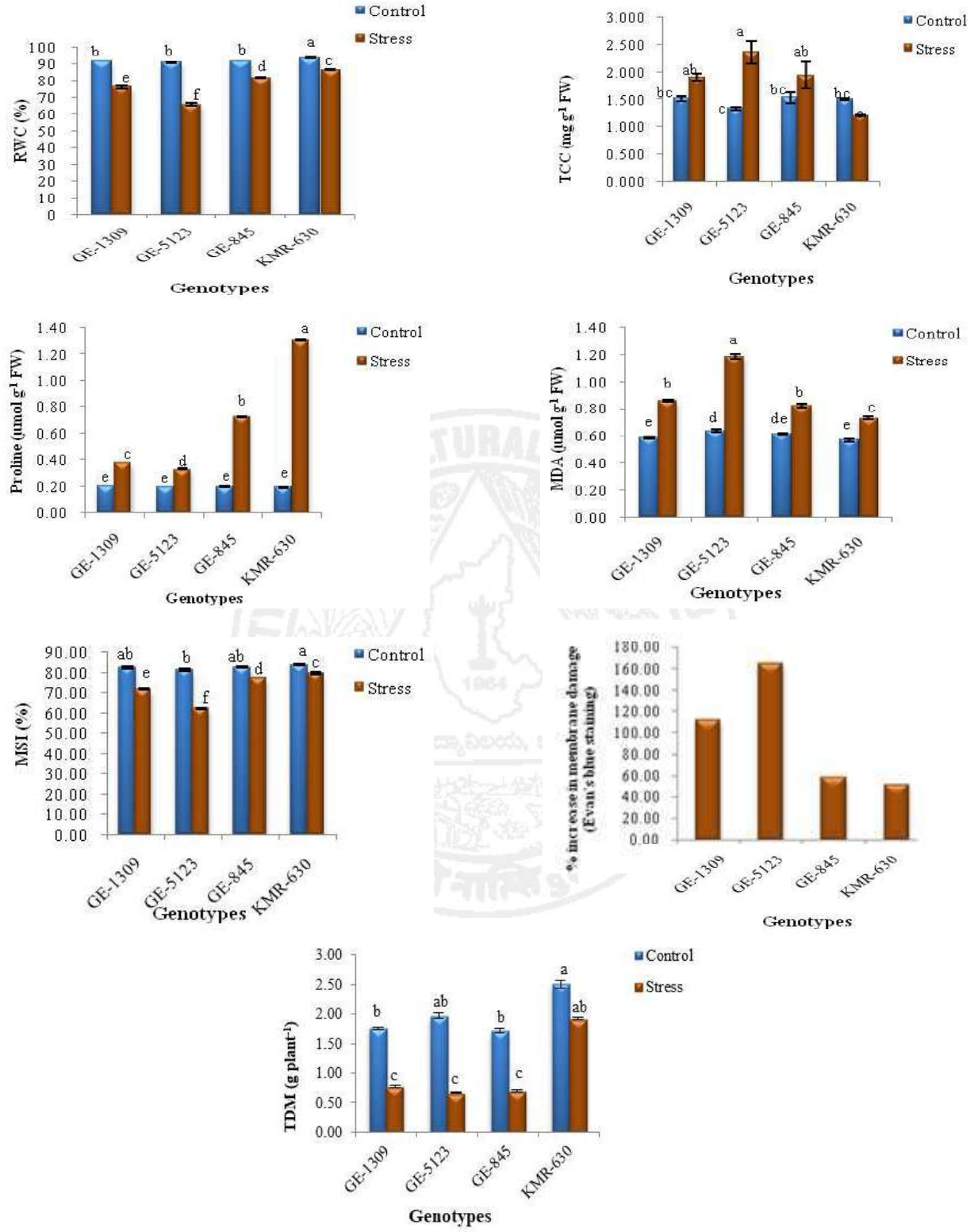

Fig. 1 : Physiological, biochemical and total dry matter content of the under drought stress in comparison to control

Legend : (a) Relative water content [RWC (%)], (b) Total chlorophyll content [TCC (mg g^{-1} FW)], (c) Proline content (µmol g^{-1} FW), (d) MDA (µmol g^{-1} FW), (e) MSI (%), (f) Percentage increase in membrane damage (Evan's blue staining technique) and (g) Total dry matter [TDM (g $plant^{-1}$)].

(86.45%) and least (66.05%) RWC was observed in KMR-630 and GE-5123, respectively under water stress condition. Higher RWC under water stress condition indicates relatively higher ability of the genotype to withstand the tissue dehydration (Vijayalakshmi *et al*., 2012).

Under water deficit condition, total chlorophyll content tends to increase in genotypes *viz*., GE-1309, GE-5123, GE-845 except KMR-630 which showed lesser chlorophyll content compared to the control (Fig. 1b, Table 1). The total chlorophyll content in plants is the presence of photosynthetic pigment and light harvesting system available, and the reduction in chlorophyll content under water deficit condition could be an adaptive strategy of the plants to reduce photo-oxidative damage and to maintain photosynthetic function (Chen *et al*., 2016).

Biochemical Parameters

Proline Content

The proline content of all genotypes increased when subjected to water deficit condition. Among all the genotypes, KMR-630 and GE-845 showed significantly higher whereas, GE-5123 and GE-1309 showed least proline content under water stress condition compared to the control condition (Fig.1c, Table 2). The accumulation of proline in plants is considered to be a protective mechanism against water deficit condition as proline is an osmo-protectant (Kotapati *et al*., 2014).

Malondialdehyde (MDA) Quantification and Membrane Stability Index (MSI)

In the present study, lower MDA content (29.2%) and higher MSI (76.6%) was recorded in the genotype KMR-630 whereas higher MDA content (85.9%) and lower MSI (62.4%) was recorded in the genotype GE-5123 under stress condition (Fig. 1d-e, Table 2). The measurement of MDA and MSI indicates the extent of lipid membrane peroxidation and electrolyte leakage in the plant tissues under water deficit condition. Even under water the stress condition the genotypes showing less MDA content and more MSI refers to the ability of the plants to tolerate the drought stress without damaging the membrane (Slama *et al*., 2011).

Evan's Blue Technique

The Evan's blue staining of the leaf samples showed a clear difference among the genotypes as well as between the control and water deficit condition of the genotypes (Fig. 2 a-d). The intensity of the stain was observed more in water stressed plants than the control as there is more chance of membrane damage under stress condition (Lekshmy *et al*., 2021). Highest (163%) and lowest (51%) percentage of stain was observed in GE-5123 and KMR-630, respectively whereas GE-1309 and GE-845 showed 111 and 58 per cent of the stain (Fig. 1f). The quantification of membrane damage through staining with Evan's blue supported the result obtained from MDA content and MSI measurement in the genotypes.

TABLE 2

Effect of drought stress on biochemical parameters in different finger millet genotypes.

Geno types	Proline (µmol g^{-1} FW)		MDA (µmol g^{-1} FW)		MSI (%)	
	Control	Stress	Control	Stress	Control	Stress
GE-1309	0.20	0.39	0.58	0.86	82.66	71.76
GE-5123	0.20	0.33	0.64	1.19	81.30	62.42
GE-845	0.20	0.73	0.62	0.82	82.68	77.35
KMR-630	0.19	1.31	0.57	0.73	83.94	79.57
SEM±	0.00		0.01		0.46	
CD at 5%	0.01		0.04		1.40	

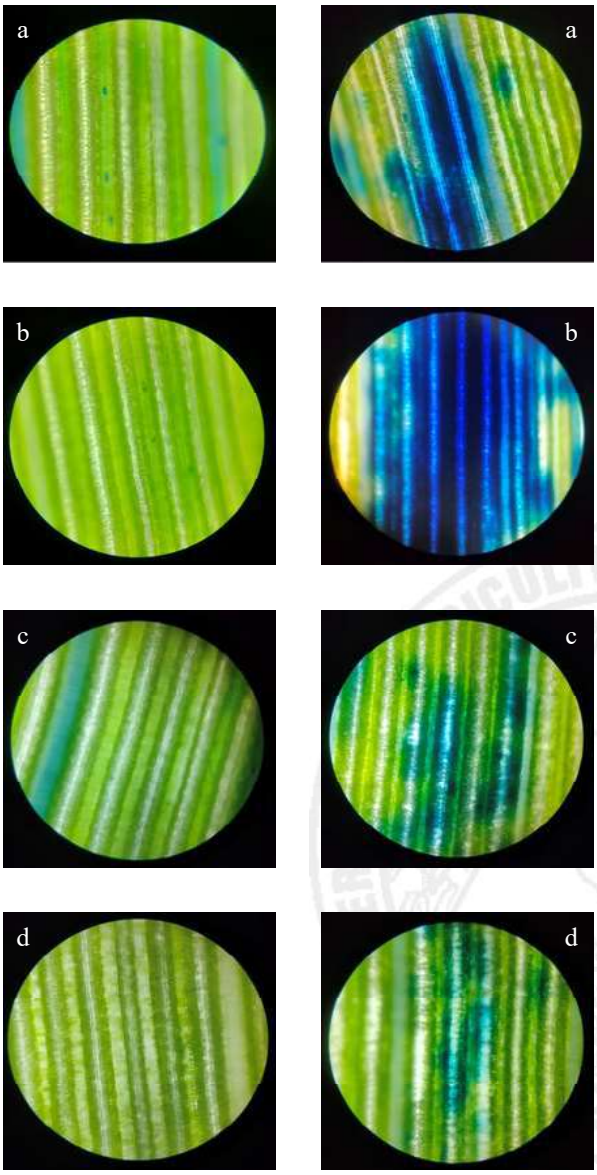

Fig. 2 : Microscopic images of Evan's blue staining of finger millet genotypes

Legend : (a) GE-1309, (b) GE-5123, (c) GE-845 and (d) KMR-630

Total Dry Matter (TDM) Content

The water deficit condition significantly affected the total dry matter production of the genotypes. There was a reduction in TDM content in all the genotypes under stress and among that KMR-630 showed the highest while GE-5123 showed the least TDM content (Fig. 1g, Table 3). The production of TDM refers to the accumulation of photosynthates in the plant. In the study, the genotypes producing more TDM under water deficit condition may be considered as drought tolerant as it indicates the ability of the genotype to accumulate more CO_2 and produce more photosynthates even under the stress condition (Kato *et al*., 2004).

TABLE 3

Effect of drought stress on total dry matter content in different finger millet genotypes

Geno types	Total dry matter (g plant^{-1})	
	Control	Stress
GE - 1309	1.76	0.78
GE - 5123	1.97	0.67
GE - 845	1.72	0.70
KMR - 630	2.51	1.93
SEM ±	0.42	
CD at 5%	1.28	

The present study described that at the early seedling stage in finger millet, the drought stress causes physiological and biochemical changes. Each genotype showed different tolerance level as a result of various physiological, biochemical and total dry matter content analysis. In the study, taking the physiological, biochemical and total dry matter content at early seedling level in consideration, it is concluded that the genotype KMR-630 and GE-845 have a drought tolerant mechanism while, the genotypes GE-1309 and GE-5123 are susceptible to drought. Through this study, we put forward that the basic selection for drought tolerant and sensitive genotypes for molecular analysis and omics studies can be done using these physiological and biochemical approaches.

REFERENCES

ANONYMOUS, 2019, http://uas banagalore.edu.in/index.php/research-en/agromateorology-en.

ANTRE, S. H., RAVIKUMAR, R. L., SUBRAMANYA, A. S. S. AND MEGHANA, K. J., 2021, Selection of stable drought tolerant genotypes in finger millet [*Eleusine coracana* (L.) Gaertn] using combination of drought tolerance indices. *Mysore J. Agric. Sci*., **55** (4) : 340 - 348.

ARNON, D. I., 1949, Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*., **24** : 1 - 15.

BARRS, H. D. AND WEATHERLEY, P. E., 1962, A re-examination of the relative turgidity technique for estimating water deficit in leaves. *J. Biol. Sci*., **15** : 413 - 428.

BATES, L. S., WALDREN, R. P. AND TEARE, I. D., 1973, Rapid determination of free proline for water-stress studies. *Plant Soil*, **39** : 205 - 207.

CHEN, D., WANG, S., CAO, B., CAO, D., LENG, G., LI, H., YIN, L., SHAN, L. AND DENG, X., 2016, Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Front. Plant Sci*., **6** : 1241.

DAVIS, K. F., CHHTRE, A., RAO, N. D., SINGH, D. AND DEFRIES, R., 2019, Sensitivity of grain yields to historical climate variability in India. *Environmental Research Letters*, **14** : 1 - 10.

DASH, S. K., KULKARNI, M. A., MOHANTY, U. C. AND PRASAD, K., 2009, Changes in the characteristics of rain events in India. *Journal of Geophysical Research Atmospheres*, **114**, D10. https://doi.org/ 10.1029/ 2008JD010572.

DWIVEDI, S., UPADHYAYA, H., SENTHILVEL, S., HASH, C., FUKUNAGA, K., DIAO, X., SANTRA, D., BALTENSPERGER, D. AND PRASAD, M., 2012, Millets : Genetic and genomic resources. In: Plant Breeding Reviews. Hoboken, New Jersey: Wiley-Blackwell, pp. : 247 - 375. ISBN 9781118100509

HEATH, R. L. AND PACKER, L., 1968, Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*., **125** : 189 - 198.

HIREMATH, N., GEETHA, K., VIKRAM, S. R., NANJA REDDY, Y. A., JOSHI, N. AND SHIVALEELA, H. B., 2018, Mineral's content in finger millet [*Eleusine coracana* (L.) Gaertn.]: A future grain for nutritional security. *International Journal of Current Microbiology and Applied Sciences* (Special Issue), **7** : 3448 - 3455.

JALIHAL, C., SRINIVASAN, J. AND CHAKRABORTY, A., 2019, Modulation of Indian monsoon by water vapour and cloud feedback over the past 22,000 years. *Nature Communications*, **10** : 1 - 8.

KATO, M., KOBAYASHI, K., OGISO, E. AND YOKOO, M., 2004, Photosynthesis and dry-matter production during ripening stage in a female-sterile line of rice. *Plant Prod. Sci*., **7** (2) : 184 - 188.

KOTAPATI, K. V., PALAKA, B. K., ANITHAMMA, K., PAMURU, R. R. AND AMPASALA, D. R., 2014, Response of antioxidative enzymes and lipoxygenase to drought stress in finger millet leaves (*Eleusine coracana* (L.) Gaertn). *Int. J. Plant Animal Env. Sci*., **4** (3) : 644 - 653.

KRISHNA, S. S., NANJA REDDY, Y. A. AND RAVI KUMAR, R. L., 2021, Assessment of traits for grain yield under drought in finger millet, *Plant Physiology Reports*, **26** (1) : 84 - 94.

LEKSHMY, V. S., VIJAYARAGHAVAREDDY, P., NAGASHREE, A. N., RAMU, V. S., RAMEGOWDA, V., MAKARLA, U. AND SREEMAN, S., 2021, Induction of acquired tolerance through gradual progression of drought is the key for maintenance of spikelet fertility and yield in rice under semi-irrigated aerobic conditions. *Front. Plant Sci*., **11** : 2275.

MAQSOOD, M. AND ALI, A. S. N., 2007, Effects of environmental stress on growth, radiation use efficiency and yield of finger millet (*Eleusine coracana*). *Pakistan Journal of Botany,* **39** (2) : 463 - 474.

NAIK, L. M., MONDAM, M., GUJJULA, V., JINKA, S., PINJARI, O. B., REDDY, Y. A. N. AND PATAN, S. S. V. K., 2020, Morpho-physiological and biochemical changes in finger millet [*Eleusine coracana* (L.) Gaertn.] under drought stress. *Physiol. Mol. Biol*., **26** (11) : 2151 - 2171.

NANJA REDDY, Y. A., JAYARAME GOWDA, ASHOK, E. G. AND KRISHNE GOWDA, K. T., 2020, Effect of moderate drought stress on photosynthetic rate and grain yield in finger Millet genotypes. *International Journal of Current Microbiology and Applied Sciences*, **9** (5) : 2951 - 2959.

NETRAVATI, H., GEETHA, K., VIKRAM, S. R. AND NITHYASHREE, K., 2018, Antioxidant property of finger millet (*Eleusine coracana* L.). *J. Pharmaco. Phytochem*., **1** : 793 - 795.

PARVATHI, M. S., NATARAJA, K. N., REDDY, Y. A., NAIKA, M. B. AND GOWDA, M.V., 2019. Transcriptome analysis of finger millet (*Eleusine coracana* (L.) Gaertn.) reveals unique drought responsive genes. *J. Genet*., **98** (2) : 1 - 12.

PRADHAN, A., PANDA, A. K. AND BHAVANI, R. V., 2019, Finger millet in tribal farming systems contributes to increased availability of nutritious food at household level: Insights from India. *Agric Res*., **8** (4) : 540 - 547.

RAMYA, V. AND REDDY, Y. A. N., 2018, Leaf temperature : A screening trait for drought tolerance in finger millet. *Mysore J. Agric. Sci*., **52** (3) : 529 - 535.

SAIRAM, R. K., 1994, Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Indian. J. Exp. Biol*, **32** : 584 - 593.

SLAMA, I., TAYACHI, S., JDEY, A., ROUACHED, A. AND ABDELLY, C., 2011, Differential response to water deficit stress in alfalfa (*Medicago sativa*) cultivars : Growth, water relations, osmolyte accumulation and lipid peroxidation. *Afr. J. Biotechnol*., **10** (72) : 16250 - 16259.

UENO, O., KAWANO, Y., WAKAYAMA, M. AND TAKEDA, T., 2006, Leaf vascular systems in C_3 and C_4 grasses : A two-dimensional analysis. *Annals of Botany,* **97** (4) : 611 -621.

VIJAYARAGHAVAREDDY, P., VANITHA, P., VEMANNA, R., SHESHSHAYEE, M. S. AND MAKARLA, U., 2017. Quantification of membrane damage/cell death using Evan's blue staining technique. *Bio-Protocol*, **7** : e2519.

VIJAYALAKSHMI, T., VARALAXMI, Y., JAINENDER, S., YADAV, S. K., VANAJA, M., JYOTHILAKSHMI, N. AND MAHESWARI, M., 2012, Physiological and biochemical basis of water-deficit stress tolerance in pearl millet hybrid and parents. *Am. J. Plant Sci*., **3** (12) : 1730 - 1740.