BIOCHEMICAL EVALUATION OF CMS (CYTOPLASMIC MALE STERILE), RESTORERS AND HYBRIDS OF PEARL MILLET [*Pennisetum glaucum*] AGAINST DROUGHT TOLERANCE

by

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MAHARASHTRA, INDIA

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A Thesis submitted to the

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RAHURI-413 722, DIST-AHMEDNAGAR,
MAHARSHTRA, INDIA

In partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE [AGRICULTURE]

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2022

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
thereof has not been submitted
by me or other person to any
other university or institute
for a Degree or
Diploma.

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CERTIFICATE

This is to certify that the thesis entitled, **"BIOCHEMICAL EVALUATION OF CMS (CYTOPLASMIC MALE STERILITY), RESTORERS AND HYBIRDS OF PEARL MILLET AGAINST DROUGHT TOLERANCE,"** submitted to the faculty of agriculture, Mahatma Phule Krishi Vidypeeth, Rahuri, Dist. Ahemednagar (M.S) for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **BIOCHEMISTRY**, embodies the result of a bonafide research carried out by **MR.LANDAGE HARIHAR BABAN** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been acknowledged.

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LIST OF ABBRIVATIONS AND SYMBOLS

%	*: Per cent*
g	: Gram (s) or gravity
h	: Hour
L	: Litre
M	: Molar
U	: Unit
V	: Volt
µg	: Microgram
µl	: Microlitre
µmol	: Micromole
^0C	: Degree centigrade (Celsius)
DW	: Dry weight
et al.	: et alli (and others)
etc.	: Etcetera
Fig.	: Figure
FW	: Fresh weight
DW	: dry weight
GB	: Glycine betaine
TW	: Turgid weight
H_2O_2	: Hydrogen peroxide
Mpa	: Mega pascals
M ha	: Million hectors
M tons (Mt)	: Metric tons
MDA	: Malondialdehyde
Min	: Minute
mM	: Millimolar
NaCl	: Sodium chloride
NADH	: Nicotinamide adenine dinucleotide phosphate
$NADP^+$	: Nicotinamide adenine dinucleotide phosphate (oxidized)
NADPH	: Nicotinamide adenine dinucleotide (reduced)
NBT	: Nitroblue tetrazolium
Nm	: Nanometer

nmol	: Nanomole
O.D.	: Optical density
O_2	: Oxygen
O_2^-	: Superoxide radical
OH^-	: Hydroxyl radical
RLWC	: Relative leaf water content
ROS	: Reactive oxygen species
S.E.	: Standard error
Sec.	: Second (s)
TCA	: Trichloroacetic acid
QAC'S	: Ouaternary ammonium compound
BADH	: Betaine aldehyde dehydrogenase
TBARS	: Thiobarbutiric acid reactive substances
SOD	: Superoxide dismutase
TBA	: Thiobarbutaric acid
CSI	: Chlorophyll stability index
GB	: Glycine betaine
GA_3	: Gibberellic acid
TW	: Turgid weight
UV	: Ultraviolet
v/v	: Volume by volume
w/v	: Weight by volume
ε	: Molar extinction coefficient

ABSTRACT

BIOCHEMICAL EVALUATION OF CMS (CYTOPLASMIC MALE STERILITY), RESTORERS AND HYBRIDS OF PEARL MILLET [*PENNISETUM GLAUCUM*] AGAINST DROUGHT TOLERANCE

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A candidate for the degree
of
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The present research work entitled "Biochemical evaluation CMS (cytoplasmic male sterile), restorers and hybrids of pearl millet against drought tolerance," by using biochemical and physiological parameters was carried out with a view to evaluate the effect of water stress by withholding irrigation water on CMS, restorers, maintainer and hybrids of pearl millet. The level of water stress evaluated in terms of RWC. Thirty-one genotype of pearl millet, 11 restorer line,7 male sterile line, 11 maintainer line and variety Dhanshakti, Hybrid Phule Adishakti were subjected to water stress for evaluation of relative leaf water content, photosynthetic rate, total chlorophyll, chlorophyll stability index, proline, glycine betaine content, activity of antioxidative enzymes such as superoxide dismutase and lipid peroxidation.

The level of proline, glycine betaine was increased in CMS, restorer lines, maintainer lines and hybrid variety of pearl millet when exposed to water stress.

Activities of antioxidative enzyme *viz* superoxide dismutase and lipid peroxidation were found to be increase under stress condition. However, the RLWC, photosynthetic rate, chlorophyll and CSI were found to be decreased during water stress. The lowest per cent decrease in RLWC 4.04 per cent was recorded in Dhanshakti variety,4.62 per cent in maintainer DHLBI-20/883, followed by restorer DHLBI-1708 and male sterile DHLB-24A. This line seen to be promising for water stress. Photosynthetic rate 5.08 per cent was recorded in Dhanshakti variety, 8.67 per cent in maintainer DHLBI-20/883, followed by restorers 9.02 per cent in DHLBI-1708, male sterile DHLBI-24A 4.26 per cent. Total chlorophyll decreased 7.5 per cent was recorded in Dhanshakti variety, 16.10 per cent in maintainer DHLBI- K-20/883, followed by restorer DHLBI-1708 11.46 per cent and male sterile DHLBI-24A 21.42 per cent.

Chlorophyll stability index 9.16 per cent was recorded in Dhanshakti variety, 10.42 per cent maintainer DHLBI-K-20/883, followed by restorer DHLBI-1708 16.27 per cent in DHLBI-1708 and male sterile DHLBI-24A 11.58 per cent.

The maximum per cent increase of superoxide dismutase activity was observed in restorer line DHLBI-1708 (36.44 %), followed by male sterile DHLBI-24A (31.87 %), in maintainer DHLBI-K-20/883 (32.72 %) and Dhanshakti (38.92 %). The maximum per cent increase of lipid peroxidation was recorded in restorers line DHLBI-1708 (13.90 %) in DHLBI-1708, followed by male sterile DHLBI-24A (14.69 %), maintainer DHLBI-K-20/883 (17.19 %) and Dhanshakti (17.68 %).The maximum per cent increase of proline was recorded in restorers DHLBI-1708 (90.13 %), followed by male sterile DHLBI-24A (135.35 %), maintainer DHLBI-K-20/883 (97.52 %) and Dhanshakti (140.00 %) and The maximum per cent increase of glycine betaine was recorded in restorers DHLBI-1708 (28.05 %), followed by male sterile DHLBI-24A (19.06 %), maintainer DHLBI-K-20/883 (19.10 %) and Dhanshakti. (30.35 %).

Form the above results it can be concluded that restorer DHLBI-1708, male sterile DHLB-24A, maintainer DHLBI-K-20/883, were found to be superior for water stress tolerance over others. Therefore, it can be concluded that above lines may be used to stress and then the breeding programme for drought tolerance.

1. INTRODUCTION

Pearl millet (*Pennisetum glaucum* L.) locally called bajra is one of the important cereal crops grown for human consumption in the tropical and subtropical areas of world (Hulse *et al.,* 1980). It exhibits superior performance to other cereals under water stress, low soil fertility and low input farming condition. It can withstand unpredictable fluctuation in ecological conditions and has a phenomenal capacity to respond to the improve agriculture inputs. Therefore, in the areas of rainfed agriculture, pearl millet is an important cereal. Most of the world's pearl millet production is concentration in the region of Asia and Africa (Salunkhe *et al.,* 1985).

In India, total area under this crop during 2020 was 6.93 million ha and the production was 8.61 million tons and productivity of 1243 $kgha^{-1}$. Pearl millet rank's forth and it is next to rice, wheat and maize (Directorate of millet development 2020). In world, India is the largest single producer of the pearl millet, both in area and production. The West and Central Africa region has large areas under millet (15.7 M ha), of which 90 per cent is pearl millet. In Maharashtra state contributes nearly 6.7 per cent and 9.2 per cent of India's pearl millet production and area respectively. In Maharashtra total area under this crop was 0.68 million ha and the production 0.61 Mt. The pearl millet cultivation is concentrated mainly in Western and Marathwada region. During the last decade the, pearl millet production decrease form 1.48 million tons in 1996-1998 to 0.75 million tons in 2011-2015. Among the cereals, pearl millet ranks second and is next to sorghum in the respect of area in the state.

Pearl millet is superior to other cereals in its protein content with an excellent balance of amino acids and relatively high vitamin-A content (Rai *et al.,* 2008). It is also considered a "high-energy" cereal as it contains more oil than maize. The protein content in pearl millet ranges from 9 to 21 per cent. Protein of pearl millet consist of albumins and globulins (22-28 per cent) of total nitrogen. The amino acid balance is also better than sorghum. The pearl millet is 40 per cent richer in amino acid methionine and lysine. Lysine content is 21 percent greater than corn and 36 percent greater than sorghum (Leader 2004). Pearl millet is also richer in fat content (5-7 g/100g) than rice, maize, wheat, and sorghum (Gopalan *et al.,*2003). About 70 per cent of dry grain is predominantly carbohydrates, consisting of 56 to 65 per cent starch, of which 20 to 22 per cent is amylose. Nutrition plays a significantly role in controlling communicable disease, is an important risk factor, for emerging chronic diseases at a later date. Malnutrition occurs mainly in children consequent to the inadequate supply of the recommended dietary allowance (RDA). The RDA is the amount of nutrients required to prevent disease. A study has shown that more than 70 per cent of pre-school children consume less than 50 per cent RDA of iron vitamin A and riboflavin (Bamji *et al.*, 2009). Despite its nutritional qualities certain anti-nutritional factors (phytate, tannins and polyphenols) are also present in pearl millet (Ranasalv and Visvanathan 2014). Presence of these factor leads to chelation of dietary minerals in the gastrointestinal tract, thereby reducing their

bioaccessibility and bioavailability (Nour *et al.,* 2014).

Food productivity is decreasing due to biotic and abiotic stresses, therefore minimizing these losses is a major area of concern to ensure food security under changing climate . Abiotic stresses such as drought extreme temperature, cold, heavy metals or high salinity. World drought being the most important environmental stress severely impairs plant growth and development, limits plant production and the performance of crop plant, more than any other enviromental factore (Shao *et al* ., 2009).

Plants experience, drought stress either when the water supply of root become difficult or when the transpirstion rate become very high. Acclimation of plant to water deficit is the result of different event, which leads to adptive changes in plant growth and physio-biochemical processes such as changes in plant structure , growth rates tissue osmotic potential and antioxidants defenses (Duan *et al*., 2007). It has becomes impressive to the response and adaption of plant to water deficit and take action to improve the drought resistance ability of crop plant and ensure the higher crop yields against unfavorable environmental stresses. Plants exhibit a several of adaptations to survive under stress situations, including reduced leaf area, stomatal closure to reduce transpiration water loss. Plant cells similarly collect solutes in order to avoid water loss and restore cell turgor. At low leaf water potentials, solute accumulation for osmotic adjustment is a common approach to stabilise membranes and preserve protein structure (Reddy *et al*., 2004).

It incluces oxidative stress because of inhibition of photosynthetic activity due to imbalanced between the light capture and its utilization. Oxidative stress affects physiological processes both at whole plant and cellular levels (Morgan, 1992).The antioxidant defence machinery protects plants against oxidative stress dameges (Gill and Tuteja, 2010).The antioxidant defence system of the plant comprises a variety of antioxidant molecules and enzyme (Arora *et al.*, 2002). Superoxide dismutase is class of enzyme that catalyses the dismutation of superoxide into oxygen and hydrogen peroxide (Sawade *et al.,* 1972). It is the most effective intracellular enzyamatic antioxidant which is ubiquitous in all aerobic oraganisms and in all subcellular compartments prone to reactive oxygen species mediated oxidative stress. It is well established that various enviromental stresses often lead to the increased generation of ROS species, where, superoxide dismutase has been proposed to be important in plant stress tolerance and provide the first line of defence against the toxic effect of elevated ROS species . Superoxide dismutase remove O_2 by catalysing its dismutase, one O_2 being reduced to H_2O_2 and other oxidized to O_2. Catalases are tetrameric heme containing enzyme with the potential to directly dismute H_2O_2 into H_2O and O_2 (Alscher *et al.,* 2002). The oxidative damage generated by drought stress in the plant tissue is alleviated by a concerted action of both enzyamatic and non-enzyamatic antioxidant systems. These include β-carotenes,ascorbate,α-tocopherol,reduced gluthione and enzymes including superoxide dismutase, peroxidase, ascorbate peroxidase, catalase, polyphenol oxidase

and glutathione reductase (Jaleel *et al.,* 2009).

Pearl millet is best suited for arid and semi-arid regions of the country due to its high temperature tolerance and improved ability to tolerate drought and flourish even in low soil fertility circumstances (Khairwal *et al.,* 2007). However, due to low and irregular rainfall, which is the single most important constraint to its production, productivity in the arid zone is higher. Since dwarf hybrids with significant yield potential have been produced. Pearl millet is a possible alternative grain crop for areas of the Great Plains with sandy soil, limited rainfall, and a short growing season. (Kajjari and Patil, 1956). (Burton, 1951) noticed a wide range of seed set in pearl millet self-fertile inbred lines. Complete sterility, partial sterility, female sterility, tip sterility, gappiness in panicles, and headless plants were all described by (Krishnaswamy *et al.,* 1962).

The Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, discovered the A1 cytoplasmic genic male-sterility (CGMS) system and produce it. Tift 23A is the first commercially successful male-sterile line (A-line) in pearl millet hybrid cultivar development (Burton, 1958 and 1965). Two new causes of cytoplasmic male sterility in pearl millet were discovered in Ludhiana, India, in 1961 and 1962, (Burton and Athwal, 1969). L 66A is a cytoplasmic male sterile line generated from the first of these, which was discovered in a self-progeny of an African variety. L 67A was the name given to another cytoplasmic male sterile line formed from the second source, which occurred in the self- progeny of an outcross with a mix of yellow and slate grey seeds. At ICRISAT, a ‘CMS' source was discovered in the Large Seeded Gene Pool, designated as LSGP-66 (Rai, *et.al.,* 1995). Six iso-cytoplasmic and eleven alloplasmic male sterile lines were synthesised from a collection of 22 novel male sterile lines of various bases (Hepziba, 1996). A new source of pearl millet cytoplasm A5 for male sterility, ICMA 5, has been discovered (Rai and Patil, 1998).

Looking into importance of crop in the national economy it is imperative to develop cultivars competent to survive under drought condition to sustain the productivity.Hence it is necessary to generate data on the levels of different biochemical constituents, osmolytes, antioxidants and antioxidative enzymes under water stress in pearl millet genotype having differential behaviour during water stress.The increase or decrease of certain molecules in the leaves is the phsiological adaptation of the plant with stress condition.Certain cultivar may high efficency in such adaptation. However, studies on biochemical factors and antioxidant enzyme during drought stress in a drought tolerant crop like pearl millet still remain elusive.

Therefore, the present study was framed to compare the biochemical and antioxidative responses in the leaf tissue of pearl millet under drought stress.Pearl millet is thus an ideal candidate for mining genes for abiotic stress tolerance, since it is relatively tolerant to drought salinity and heavy metals. Hence information on definite association of one or more biomolecules in such adaptation will be quiet useful in futher improvement of the crop. The evalution of levels

of osmolytes and the activities of certain antioxidant enzymes in pearl millet throw some insight into the mechanism which can exploited as a source of enzyme for future genitic manipulation studies in drought tolerance.

Several hybrid varieties have been developed to suite climatic condition by using different approaches. However, the information on osmotic accumulation, antioxidant levels and activity of antioxidative enzymes in relation to water stress tolerance in CMS, restorers and hybrids of pearl millet is lacking. Keeping this view in mind the research is proposed on Biochemical evaluation of CMS (cytoplasmic male sterility), restorers and hybrids of pearl millet (*Pennisetum glaucum* L.) against drought tolerance with following objective.

Objective:

1) To evaluate the chlorophyll, photosynthetic rate, chlorophyll stability index, relative leaf water content and the levels of osmolytes and activities of antioxidant enzymes in pearl millet genotypes under drought stress.

2. REVIEW OF LITERATURE

The available information on relative leaf water content, total chlorophyll, photosynthetic rate, chlorophyll stability index, accumulation of proline, glycine betaine, lipid peroxidation and antioxidative enzymes superoxide dismutase during stress of crop plants in general and pearl millet in particular is briefly reviewed in this section. The information on the drought tolerance mechanism of major crop has been attempted in this section.

2.1 Physio-biochemical Traits:

2.1.1 Relative leaf water content (RLWC)

Relative leaf water content represents a useful indicator of the state of water balance of a plant, essentially because it express absolute amount of RLWC each artificial full saturation (Gonzalez, 2001). The balance between water supply to the leaf tissue and transpiration rate is reflected in the relative water content (RWC) of leaves, which is an essential measure of water status in plants (Lugojan and Ciulca 2011). RWC is an integrative indicator of plant water status that is used to assess water stress resistance. Drought stress causes RWC to drop, which causes stomatal closure, reduces CO_2 absorption (Gindaba *et al*., 2004). Water retention decreases under stress, relative leaf water content (RLWC) is one of the agricultural measures used to screen genotypes and varieties of different crops for saline and drought tolerance (Boutraa *et al*., 2010). Decrease in RWC in plants under drought stress may depend on plant vigour reduction and have been observed in many plants (Liu *et al.,* 2002).

The physiological effects of water stress on *Sesamum indicum* cultivars. In both cultivars, a lack of water resulted in a decrease in relative water content of leaves and an increase in proline. Drought-tolerant cultivars demonstrated a lower reduction in relative water content than drought-prone cultivars. Under mild stress, there is a positive correlation between a reduction in RWC and a higher proline concentration (Molaei *et al*., 2012). The increasing level of water stress in wheat significantly reduced relative leaf water content and the retention ability of the plant at different growth stages was different as plant progress toward maturity its water retention ability decreased (Zahoor *et al.,* 2000).

All chickpea cultivars have shown a significant reduction in RLWC when exposed to water stress. Under water stress, Pusa-362 and Pusa-1103 had the highest RLWC at blooming and pod formation phases, respectively, while SBD-377 and Flip 90-166 had the lowest RLWC at flowering and pod formation stages, respectively (Rizvi *et al*., 2014). Reduction in RWC values in loss of turgidity which leads to stomatal closure and in turn to reduce photosynthetic rates in foxtail millet (Vinothini *et al.*, 2016). Exposure to mild water stress resulted in minor decline in water relation, water potential, solute potential, turgor potential and RWC in both drought-tolerant and susceptible wheat genotypes as compared to control (Khanna and Selote, 2006). Relative water

content of barley leaves was decreased under drought stress as compared with the control (Jalali *et al*., 2012).

2.1.2 Photosynthetic rate

The rate of photosynthesis is decreases by drought stress. Drought-stricken plants have a decreased stomatal conductance to conserve water. As a result, CO_2 fixation and photosynthetic rate decline, resulting in lower assimilate generation for plant growth and yield. The factor limiting principal of photosynthesis under drought, stomatal closure (resulting in reduced leaf internal CO_2 concentration (Ci)) is undoubtedly the primary cause of reduced leaf photosynthetic rates during mild or severe drought stress (Chaves, 1991; Cornic, 2000). C_4 plant photosynthesis is most effective because to a CO_2 concentrating mechanism in bundle sheath cells and strong Rubisco activity, which results in less photorespiration (Sage and Zhu, 2011). However, moderate drought stress for 18 days during the grand development stage to panicle primordial initiation considerably reduced the mean photosynthetic rate by 16.6 per cent. (Mohanabharathi *et al.,* 2019). Both under control and stress conditions, significant genotypic differences in photosynthetic rate were detected. Under stress conditions, the lowest photosynthetic rate (31.5 u Mol. m-2 s -1) with just a 4.0 per cent decrease. Under stress circumstances, similar genotypic changes in photosynthetic rates have been documented (Subramanyam, 2000; Gupta *et al.,* 2011).

Greater stomatal conductance and transpiration rates resulted with a higher photosynthetic rate in PR-202 under mild drought stress. This suggested that CO_2 absorption per unit of water transpired was high, resulting in greater carboxylation and photosynthetic rate. Higher stomatal conductance in PR-202 indicates that it has superior water interactions in general. Under control conditions, photosynthetic rate was positively and significantly linked to stomatal conductance and transpiration rate (Anitha *et al.,* 2019; Mohanabharathi *et al.*, 2019; Maai *et al.*, 2020).

Photosynthetic rate is adversely affected by salinity stress with maximum reduction in net carbon dioxide assimilation at higher level of salinity. Net photosynthetic rate decreased substantially in mustard cultivars at 100Mm NaCl stress. (Khan *et al.,* 2010) observed linseed plants exposed to NaCl stress exhibited reduction in net photosynthetic rate.

2.1.3 Chlorophyll

Leaf chlorophyll concentration is an important parameter that is regularly measured as an indicator of chloroplast content, photosynthetic mechanism and of plant metabolism. Chlorophyll is an antioxidant compound which are present and stored in the chloroplast of green leaf plant and mainly it is present in the green area of leaves, stems (Mirza *et al*.,2013; Srichaikul *et al.,* 2011). Chlorophyll a and chlorophyll b are essential pigments of the plant photosystem (Richardson *et al*.,2002). Chlorophyll and carotenoid concentration in leaves, as well as pigment ratios like chlorophyll a, chlorophyll b and chlorophyll/carotenoid, are good indicators for stress detection and tolerance (Babani *et al*., 2003). A decrease of total chlorophyll with drought stress implies a

lowered capacity for light harvesting, since the production of ROS species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments (Herbinger *et al*., 2002). Drought stress lowered chlorophyll content consistently and considerably, and the degree of this loss was influenced by line as well as the interaction between line and treatments. The chlorophyll content in all lines returned to control levels after re-watering (Chen *et al*., 2016). The content of chlorophyll a, b, and total chlorophyll in sorghum plants reduced under stress levels increased. (Hinge *et al*., 2015). Chlorophyll content would either increase or decrease or even remain unchanged during drought stress depending on the duration and severity of the drought condition (Anjum *et al*., 2011).

Reduction in chlorophyll content were observed in the genotype exposed to drought. This reduction could be a typical symptom of oxidative stress may be the result of pigment photo-oxidative and chlorophyll degradation and significantly decrease of chlorophyll a and b caused by water deficit in six *Triticum aestivum* cultivars (Nyachiro *et al*., 2001). Drought stress lowers total chlorophyll in peanuts, (Reddy *et al*., 2003). Drought stress causes a decrease in total chlorophyll in *Vicia faba* cultivars, and that Giza, a drought-tolerant variety of *Vicia faba*, has a greater chlorophyll content than sensitive form of *Vicia faba* (Taybe *et.al*., 2006). Lower decrease in total chlorophyll content in drought tolerance varieties of *Phaseolus vulgaris* than drought stress. PEG induced drought stress imposed to plant significantly decreased total chlorophyll both the severe and mild stress in pigeonpea (Kumar *et al.,*2011). Drought stress imposed at the vegetative stage in chickpea, significantly decreased total chlorophyll content both at the vegetative and flowering stages, whereas drought stress imposed at anthesis also influenced these contents at flowering (Mafakheri *et al*., 2010).

2.1.4 Chlorophyll stability index

The parameters *viz.,* total chlorophyll content, chlorophyll stability Index, relative water content, proline accumulation, and protein synthesis are sufficient enough to provide reliable laboratory screening indictors to screen for drought tolerance in rice breeding programmes (Deivanai *et al*., 2010).

The stability of chlorophyll is a function of temperature, and it has been corelated to drought tolerance. The chlorophyll stability index is a measure of the membrane's integrity or the pigments' thermal stability under stress situations (Koleyoreas, 1958). (Mohan *et al*., 2000) suggested that higher CSI under stress leads to increased photosynthetic rate, more dry matter production and maximum productivity of the crop. The positive correlation between chlorophyll stability index and photosynthetic rate was observed under drought. A high CSI indicates that the stress had little influence on the chlorophyll content of the plants. A higher CSI helps plants survive stress by increasing the availability of chlorophyll. This results in enhanced photosynthetic rate, increased dry matter production, and improved productivity, this shows how well chlorophyll

performs under stress (Vijayan, 2017). (Todorov *et al*., 2003) Reported that drought and high temperature enhanced chlorophyllase activity and decreased photosynthetic pigments concentrations. The positive and significant association between grain yield and CSI was reported by (Raja Babu *et al.,* 2005).

The CSI is a single parameter used to assess a plant's cold (or drought) resistance. Drought stress and temperature stress both reduced membrane stability, chlorophyll content, and chlorophyll stability index in all wheat genotypes, (Sairam *et al.,* 1996). The effect of drought stress on the chlorophyll content of maize cultivars' leaves (*Zea Mays* L.), the chlorophyll stability index (CSI) measures a plant's ability to withstand stress.

2.2. Osmolytes Accumulation

2.2.1 Proline

Proline has osmoprotectant properties. It is an osmolyte that is widely distributed in plants and accumulates in greater amounts in drought-stressed plants than any other amino acid. Accumulation of proline in the leaves is a plant's physiological response to a lack of water. This makes it easier to keep the osmotic conditions in the cell stable and protect the activity of molecules like enzymes, allowing metabolic activity to continue even when the water tension is low. In response to diverse type of environmental challenges, proline accumulation occurs in a wide spectrum of plant species. Proline accumulation could be caused by a reduction in protein breakdown, consumption, or hydrolysis. Because of its segmented metabolism, extensive intercellular proline transfer occurs between the cytosol, chloroplasts, and mitochondria. Although the exact activities of proline in stress tolerance are unknown, it is thought that proline aids in the stabilization of subcellular structures, scavenging of free radicals, and buffering of the cellular redox potential (Mudasir *et al*., 2016). Proline, is an important amino acid, plays a crucial role in plants subjected to different stress conditions as it acts as a compatible osmotic molecule. Its accumulation has been shown to be enhanced during stress conditions (Tiwari *et al.,* 2016; Lata *et al.,* 2011).

Soluble sugars and proline were important osmotic adjustment substances in plant. (sharma and dietz, 2006). Plants accumulate metabolites such as proline, which are key protein precursors in plant metabolism and development. Proline is a good osmolyte, metal chelator, anti-oxidative defence molecule, and signalling molecule that accumulates in plant cells (Hayat *et al*., 2012). It also plays an important role in osmotic potential adjustment during water-deficit environment (Mafakheri *et al.,* 2010; Grover *et al.,* 2014). Besides, being a crucial osmolyte, proline has three other major functions during drought stress including antioxidative defense, metal chelation, and stress signaling (Haya *et al.,* 2012). Proline does not interfere with regular metabolic responses and help plants survive in stressful situations (Stewart, 1981). Proline buildup in plant tissues is also a strong indicator of environmental stress (Routley, 1966). The greater proline levels may

help cells retain water and prevent them from oxidative damage caused by drought (Wenli *et al*., 2016).

Enhanced level of proline in the leaves of drought stress plant may be due to the synthesis or breakdown of proline rich protein has been reported by several workers they also suggested that the genotype with high proline content manifest a high drought tolerance in pearl millet plant (Giancarla *et al.,* 2011 and Anjum *et al*., 2003). To elucidate further insights into the biochemical changes in response to drought stress, proline accumulation was studied at both early- and late-seedling growth stages and MDA accumulation at late-seedling growth stage in pearl millet.

There was a general increasing trend in the accumulation of free protein in all three parts with the addition of NaCl to the growth medium of plants of both species. The relatively higher accumulation of proline due to salt was observed in the leaves of *S.aculeanta* and in the nodules *of P.vulgaris* (Ashraf and Bashir,2003).The accumulation of osmoregulatory compound proline in shoot and root of the medicinal plant *Lepidium sativum* L. (commonly called garden cress) was greatly increased by NaCl stress (Manaa *et al.,* 2014). In response to dryness, the proline content of the leaf increase in all chickpea cultivars at growth stages. Drought stress caused a increase in proline concentration in the flowering stage than in the vegetative stage (Mafakheri *et al*., 2010).

2.2.2 Glycine betaine

Quaternary ammonium compound includes glycine betaine is the key osmolyte contributing toward osmotic adjustment (Haung *et al.,* 2000). Glycine betaine accumulation enhance plant salt tolerance as it preserves thylakoid and plasma membrane integrity. It is mainly localized in chloroplast and plays a vital role in chloroplast adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency and plasma membrane integrity (Yokoi *et al.,* 2002).

The role of abscisic acid on plant growth, water relations and glycine betaine metabolism in the leaves of maize cultivars, The Integrated Root-zone Drought Stress increased betaine aldehyde dehydrogenase (BADH) activity and choline content which act as the key enzyme and initial substrate, respectively, in GB biosynthesis (Lixin *et al*., 2012). Plants development and productivity are limited by high temperatures. In vitro tests revealed that GB protects several enzymes and protein complexes from heat instability (Gorhame *et al.,*1995). As a result, it's been proposed that GB increase resistance to high-temperature stress. Recent investigations revealed that GB-accumulating Arabidopsis displayed increased to high temperatures during seed imbibition and germination, as well as during the growth of young seedlings (Alia *et al*., 1998).

Plants subjected to a variety of abiotic stimuli, such as drought, temperature, and salt, have been found to benefit from proline and glycine betaine (GB) amino acids, which function as osmoprotectants. Plants undergo biochemical, physiological, and morphological changes in response to water deficits, (Basu *et al.,* 2010; Chaum *et al*., 2010).GB content showed a

significantly increase in linseed plant under NaCl stress compared to the control. The level of GB content was lower in unstressed plants supplied with $CaCl_2$ or GA_3 alone when compared with sail-stressed plant (Khan *et al.*, 2010). (Nayyar *et al.*, 2003) Stressed plants increased their glycine betaine content more than two fold on the 4^{th} day of stress compared with the1^{st} day of stress but it began to decline rapidly thereafter. Foliar application of glycine betaine raised its endogenous level more than threefold over controls on the 7^{th} day of stress, indicating its substantial uptake from leaves. Control plants showed very low values of glycine betaine (2.7–3.1 μmol g dW^{-1}).

The content of QAC's increased by two and three times in stressed plants of maize and wheat, respectively. Ca^{2+}, further, stimulated their accumulation to a significantly higher extent in wheat than maize (Nayyar, 2003).GB levels increased with salinity in both the varieties of wheat when compared with control and wheat variety, Kharchia 65 showed significantly higher GB content in control ana salinity levels at all the stages than KRL19 (Sairam *et al.,*2003).

2.3 Lipid Peroxidation

The peroxidation of lipid is the membrane damaging process known to occur in every living organism. The membrane damage is taken as a single parameter to determine the level of lipid destruction under various stresses. During lipid peroxidation, products are formed that include small hydrocarbon fragment such as ketones, malondialdehyde (MDA) etc. and compound which react with thiobarbutaric acid (TBA) to form coloured products called thiobarbutiric acid reactive substances (TBARS). Plant cell respond defensively to oxidative stress by removing the ROS and maintaining antioxidant defence compounds at levels that reflect ambient environmental conditions (Scandalios, 1997).

MDA is regarded as a marker for evaluation of lipid peroxidation or damage to plasmalemma and organelle membranes that increases with environmental stresses. Drought and salt stress induces lipid peroxidation through ROS production (Li *et al.*, 2008). The cell membrane integrity has been widely used as criterion to differentiate between stress tolerant and susceptible genotype and sometimes plants capacity to ovoid membrane damage has been directly correlated with abiotic stress tolerance (Gill and Tuteja, 2010). Stress-induced impairment in pigment biosynthetic pathways or pigment degradation, loss of the chloroplast membrane, and increased lipid peroxidation may cause a decrease in chlorophyll content, resulting in the generation of reactive oxygen species, which are potentially dangerous under drought stress conditions (Reddy *et al.,* 2004). 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).

(Mohammadi *et al.,* 2014) revealed that MDA content in chickpea was lowest under normal irrigation and highest under drought stress. The decomposition of lipid and production

MDA as result of drought. A significant increase in MDA content was detected for Galia, whereas MDA concentration rise significantly in the shoot tissues of drought-stressed Kirkagc seedlings at–0.4 MPa osmotic potential compared to the control. The lower values of MDA in Galia indicate that at cellular levels this cultivar has an efficient free radical quenching system (Kavas *et al.,* 2013).Lipid peroxidation levels of two alfalfa cultivars, as measured by the content of MDA in the leaves of both cultivars which were growing under normal growth conditions, a small level of lipid peroxidation was apparent however, under salt stress, the levels of MDA content in both alfalfa cultivars increased significantly with the increase in NaCl concentration (Babakhani *et al*., 2011). The increase in the MDA content in alfalfa under drought conditions indicates that some degree of damage to cell membranes is unavoidable (Yang *et al*., 2012).

2.4 Antioxidative Enzymes

2.4.1 Superoxide dismutase

Superoxide dismutase (SOD, EC 1.15.1.1) are a class of enzymes that dismutase of superoxide into oxygen and hydrogen peroxide, superoxide dismutase autocompetes damaging reaction of superoxide, thus protecting the cell form superoxide toxicity. It is known that plants have a well-organised defence system against ROS under stress defence. There is also a clear link between increasing cellular proline levels and the ability to increase a water shortage in higher plants. One of the most common types of antioxidant enzymes, which protect cellular and subcellular components from the detrimental effects of reactive oxygen species.

$$Mn^{3+}\,SOD\,(H_2O) + O_2^{-} \longrightarrow Mn^{3+}\,SOD\,(OH^{-}) + H_2O_2$$

Water stress causes the generation of reactive oxygen species at the cellular level, such as singlet oxygen (O_2), superoxide radicals, hydrogen peroxide (H_2O_2), and hydroxyl radical, which cause membrane damage (Tambussi *et al*., 2000). SOD is one of the most significant scavenging enzymes, and catalyses the dismutation of superoxide radicals to hydrogen peroxide (Bowler *et al*., 1992). SOD being one of the protective enzymes during water stress an increase in its activity under water stress tolerant varieties (Blum and Eberun, 1981).

Water stress induced increase in SOD activity has been reported in maize (Jhagtap and Bhargava 1995). The peroxidase, catalase increased at different osmotic gradients in comparison to control. The similar result of superoxide dismutase enzyme in three chickpea cultivars have been reported (Mohammadi *et al.,* 2011). (Pan *et al*., 2006) reported that the activities of Catalase decreased by increasing the PEG concentration, whereas increase in SOD activity were observed in both progressive stresses induced by PEG as compare to the control. An increase in SOD activity and decrease in CAT activity was also reported during drought stress in Liquorice. (Sairam *et al.,* 2001) reported that positive correlation between water stress tolerance with increase in SOD activity in wheat and salt stress induced increased of SOD activity is directly correlation with salt stress tolerance of all the genotype of pigeon pea.

3. MATERIAL AND METHODS

3.1 Material

3.1.1 Seeds

Seeds of thirty-one pearl millet genotypes were obtained from the Bajra Research Scheme, College of Agriculture, Dhule, as part of the All India Co-ordinated Pearl Millet Improvement Project.

Table 1 List of CMS, restorer line, maintainer lines and hybrids of pearl millet.

Sr. No	Restores (R-Line)	Sr. No	Maintainer (B-Line)
1	DHLBI 967	1	DHLBI-K-20/861
2	DHLBI 1035	2	DHLBI-K-20/862
3	DHLBI 1708	3	DHLBI-K-20/863
4	DHLBI 1822	4	DHLBI-K-20/866
5	DHLBI 1013	5	DHLBI-K-20/868
6	DHLBI 1074	6	DHLBI-K-20/871
7	DHLBI 1103	7	DHLBI-K-20/874
8	DHLBI 1603	8	DHLBI-K-20/877
9	DHLBI 1609	9	DHLBI-K-20/881
10	DHLBI 1806	10	DHLBI-K-20/883
11	DHLBI 1201	11	DHLBI-K-20/537
	Male Sterile (A-Line)		**Hybrid variety**
1	DHLB-8A	1	Dhanshakti(variety)
2	DHLB-16A	2	Phule Adishakti(Hybrid)
3	DHLB-17A		
4	DHLB-23A		
5	DHLB-24A		
6	DHLB-31A		
7	DHLB-35A		

3.1.2 Chemicals

The majority of the chemicals utilised in this study were of analytical grade and were obtained from (Sd. Fine Chemical Ltd., SRL Mumbai, E. Merck, Qualigen Fine Chemical Pvt., Ltd., Mumbai, Bangalore, Genei Pvt. Ltd., Bangalore).

3.2 Methods

3.2.1 Growing of seedlings.

Ten seeds of each cultivar were sown in duplicate in separate earthen pots holding about 10kg soil, and the crop was grown in natural daylight. Both sets of plants were irrigated on a regular basis until they reached the age of 40 days. After 40 days one set of plant was subjected to

water stress by withholding irrigation till plant reached wilting (10 days) while other set was irrigated as per the requirement of 50 days of sowing, the stressed condition was noticed as a transient wilting of growing plant. leaf sample collected from both sets for analysis.

3.2.2 Relative leaf water content

Relative leaf water content of leaves was measured by the method of Henderson and Davies (1990).

Procedure

The third leaf from the top of the main stem was detached from 5 randomly selected plants and kept in sealable plastic bag in an ice box. The leaf samples were brought to a laboratory where fresh weight was recorded immediately. The leaf samples were then immediately hydrated to full turgidity for 2 hours by floating on de-ionized water in a close petri-dish under room temperature. After 2 hours the samples were taken out of water and were well dried with a filter paper. They were immediately weighted to obtain fully turgid weight (TW). Samples were then dried at 80 °c for 36 h and dry weight (DW) was determined. The RLWC was calculated by using the following formula.

$$RWC\ (\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Where, RWC = Relative water content,

DW = Dry weight (g),

FW = Fresh weight (g)

TW = Turgid weight (g).

3.2.3 Photosynthetic rate

IRGA (Infrared Gas Analyzer, Model LI-COR ® Inc. Lincoin, Nebraska, USA) was used to determine the photosynthetic rate.

Growth stage: 40 to 65 days after sowing are very sensitive to drought stress for recording observation.

3.2.4 Chlorophyll

Chlorophyll content of leaves was measured by the method of Arnon's (1949). Chlorophyll is extracted in 80 % acetone and the absorbance at 663 nm and 645 nm are read in the spectrophotometer using the absorbance coefficient, the amount of chlorophyll a, chlorophyll b and total chlorophyll is calculated.

Reagent

1. Dilute analytical grade acetone 80 % (prechilled)

496 ml of pure acetone dissolved in 120 ml of distilled water. Total volume is 620 ml.

Procedure

The leaf samples of pearl millet were cut into small pieces and known weight (0.2g) of fresh leaf sample was macerated in a mortar and pestle and extracted with 10 ml of 80 % acetone. The contents were centrifuged at 5000 rpm for 10 min and the supernatant was collected. The above steps were repeated until the residue became colourless. The final volume of extract was made to 50 ml. The extinction of chlorophyll extract was recorded at 645 and 663 nm on a Spectrophotometer and a blank was run with 80 % acetone.

Calculation

The amount of total chlorophyll content was calculated by using the following formula and expressed in mg g^{-1} FW.

Total chlorophyll, mg g^{-1} FW = 22.2 (A645) + 8.02 (A 663) x V/ (1000 x W)

Where, A= absorbance at specific wavelength

V= Final volume of chlorophyll extract in 80 % acetone

W= Fresh weight of tissue extracted in g.

3.2.5 Chlorophyll stability index

Chlorophyll stability index of leaf measured by the method of Koleyoreas (1958).

Procedure

0.2 gm of fresh sample of uniform size were used for the study. Leaves were put into the ready test tube maintaining two sets, one set was kept in control under room temperature and other set was kept in water bath at 80 ^{0}C for 30 minutes. then spectral absorbance of the water recorded at 652 nm for each tube.

$$\text{CSI (\%)} = \frac{\text{OD of heated sample}}{\text{OD of control sample}} \times 100$$

3.2.6 Osmolytes

3.2.6.1. Proline

Proline content was determined by using acid ninhydrin reagent as per the method described by Bates *et al* (1973). The proline concentration was measured in moles µg-1 FW.

Reagents

1. **Acid ninhydrin:**

   Ninhydrin (1.25g) was dissolved in 30 ml of glacial acetic acid and 20 ml of 6 M phosphoric acid with agitation.

2. **Sulphosalicylic acid (3% w/v):**

   Three grams of Sulphosalicylic acid was dissolved in distilled water and the volume made to 100 ml.

3. **Stock solution of L-proline (100 mM):**

   Standard L-proline (1.15g) was dissolved in distilled water and the final volume made to

100 ml.

4. Working standard solution of L-proline (1 mM):

From the stock solution, 1 ml was again diluted to 100 ml with distilled water. This diluted working solution contained 1µmole of proline per ml.

Procedure

Leaf samples (0.2g) were homogenized with 10 ml of 3% (w/v) aqueous solution of sulphosalicylic acid and the homogenate filtered through Whatman No. 1 filter paper. A suitable volume of the filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100 ^{0}C and the reaction was terminated by placing the tubes in ice bath. The reaction mixture was extracted with 4 ml toluene and mixed vigorously for 15 to 20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from the standard curve and calculated on a fresh weight basis.

Calibration of standard curve

In a triplicate set of test tubes, the working standard solution of proline was pipetted as 0, 0.l. 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1ml having the corresponding proline concentration of 0 to 1µmoles. The volume was made to 1 ml with distilled water. The colour was developed as described above. The absorbance was recorded at 520 ηm and a standard graph of absorbance against concentration of proline was plotted (Fig. 1).

3.2.6.2 Glycine betaine

The glycine betaine content in the leaves of pearl millet was measured by using the Dragendorff reagent according to Stumf's procedure (1984). The content of glycine betaine was measured in µmoles g-1 DW.

Reagents

1. Dragendroff reagent:

This reagent was prepared by mixing the equal volume of 0.35 M bismuth nitrate in 20% acetic acid (v/v) and 2.45 M sodium iodide (NaI) in distilled water.

2. Betaine aldehyde solution:

The solution was prepared by dissolving 0.5g of betaine aldehyde in 1 ml distilled water.

3. Bismuth nitrate solution (0.35 M):

The solution was prepared by dissolving bismuth nitrate in distilled water and 20 per cent acetic acid. The volume was made to 25 ml with distilled water.

4. Sodium iodide solution (2.45 M):

The solution was prepared by dissolving NaI in distilled water and the volume was made to 25 ml.

5. Working solution of sodium iodide (0.49 M):

Five ml NaI (2.45 M) solution was diluted in distilled water and the volume made to 25 ml.

Procedure

0.2 g of leaf sample was crushed in a mortar and pestle in 2 ml of 80% (v/v) ethanol. It was transferred into the eppendorf tubes which were kept in the hot water bath for 20 min. The tubes were then removed from water bath and cooled at room temperature. It was centrifuged at 10,000xg for 20 min and the supernatant was collected in clean eppendorf tubes.

The supernatant (0.2 ml) was pipetted in the microfuge tubes to which 100 µl Dragendroff reagent was added. The solution was then centrifuged at 7000xg for one min. After centrifugation, the supernatant was drawn with the help of a syringe and the orange pellet was dried. The pellet was dissolved in one ml solution of 2.45 M NaI. From this 200 µl. aliquot was pipetted in test tubes containing 3 ml diluted 0.49 M NaI solution and mixed. The absorbance was read at 467 nm using 0.49 M NaI solution as a blank.

Calibration of standard curve

A standard curve for glycine betaine was made by placing 2.5 to 25 µl of a working solution of betaine aldehyde in a set of 2 ml micro centrifuge tubes (50 mg/ml) to which 100 µl of Dragendorff reagent was added causing the precipitation of the QAC. It was then centrifuged at 7000 rpm for one min. The supernatant was completely removed. The pellet was dissolved in 1 ml of 2.45 M NaI solution A known quantity of aliquot (30 ul) was then added to three ml of 0.49 M NaI solution. The absorbance was read at 467 nm using 0.49 M NaI solution as a blank and a standard curve of absorbance vs concentration of glycine betaine was plotted.

3.2.7 Lipid peroxidation

Lipid peroxidation is oxidative degradation of lipid fatty acid by reactive oxygen species. The level of lipid peroxidation is measured in terms of thiobarbituric acid reactive substance (TBARS content) (Heath and Packer, 1968).

Reagents

1. Trichloroacetic acid (0.1%):

A trichloroacetic acid solution was produced by dissolving 0.1 g TCA in water and then diluting to 100 ml.

2. Thiobarbituric acid reagent:

Five grams of TBA was dissolved in a 20% TCA, and then the volume was made 100 ml with 20% TCA.

Procedure

1. **Sample extraction**: Leaf sample 0.2 g was homogenized in 4 ml of 0.1 % TCA. The homogenate was centrifuged at 15000 rpm for 15 min and the supernatant was used for the

estimation of TBARS content.

2. Assay Four ml of 0.5 % TBA in 20 % TCA was added in 10 ml aliquot of the supernatant. The mixture was heated at 95^0C for 30 min in the lab electric oven and then cooled in an ice bath. After cooling the aliquot was centrifuged at 10,000 rpm for 10 min. The absorbance of the clear supernatant was recorded at 532 nm. Values of non-specific absorption recorded at 600 nm and were subtracted from the values recorded at 532 nm. The TBARS content was calculated by using extinction coefficient E = 155 m $M^{-1}cm^{-1}$.

3.2.8 Antioxidative enzymes

3.2.8.1 Estimation Soluble proteins

Soluble proteins in the leaves were determined by the colorimetric method of Lowry *et. al.,* (1951) using bovine serum albumin as the standard protein.

Reagents

1. Reagent 'A' (alkaline sodium carbonate)

Sodium carbonate (2g) was dissolved in 0.1 M sodium hydroxide and the volume made to 100 with M NaOH.

2. Reagent 'B' (copper sulphate-sodium potassium tartarate solution, 0.5% w/v):

Copper sulphate (0.5 g) dissolved in 1% (w/v) sodium potassium tertarate and the volume made to 100ml with 1% Na-K tartrate solution.

3. Alkaline copper tartarate reagent:

Fresh alkaline copper tartrate reagent was prepared just before use by mixing 50 ml of reagent 'A' and 1 ml of reagent 'B'.

4. Folin-Ciocalteau (Phenol) reagent:

It was prepared by diluting one part of commercial grade reagent with one part of distilled water on the day of use.

5. Stock solution of bovine serum albumin (BSA):

BSA (100 mg) was weighed accurately and dissolved in distilled water and the volume made to 100 ml with distilled water.

6. Working solution of BSA:

10 ml of the stock solution was pipetted into 100ml volumetric flask and the volume made with distilled water. The concentration of this solution was 100µg/ml.

A. Extraction of soluble proteins

0ne gram of leaf sample was homogenized with 10ml 0.1 M potassium phosphate buffer. It was then centrifuged at 10,000 × g for 20 min and the supernatant used for the estimation of soluble proteins.

B. Colour development

The supernatant (100µl) was pipetted in series of test tubes in triplicate and volume made

to 1 ml. it was mixed with 5 ml of alkaline copper reagent and kept for 10 min at room temperature. To this, 500µl of diluted Folin-Ciocalteau reagent was rapidly with immediate mixing. The intensity of the purple colour was measured after 30 min at 660 nm on spectronic-20 spectrophotometer. The protein was estimated from the standard curve and expressed as mg g^{-1} FW.

C. Calibration of a standard curve for the determination of soluble proteins

The standard solution of BSA (100, 200, 300, …. µl) was pipetted series of test tubes In triplicate having corresponding BSA concentration of 10, 20, 30, …. And 100µg. the volume was made to 1000 µl with distilled water and the colour developed as above. The absorbance was read at 660 nm and the standard curve was prepared by plotting absorbance Vs concentration of the standard protein (fig.6).

3.2.8.2 Superoxide dismutase

Activities of superoxide dismutase was measured by the method of Dhindsa (1981) .

Reagents

1. Nitro Blue Tetrazolium (NBT) (2.25mM):

Tetrazolium (0.183mg) was dissolve in 100 ml distilled water.

2. Riboflavin(60µM):

Riboflavin 0.022mg dissolved in 100ml distilled water.

3. Methionine (200mM):

Dissolve 2.984g methionine in 100ml distilled water.

4. Ethylene diamine tetra acetic acid (3mM):

It was prepared by dissolving 0.037 g EDTA in 100 ml distilled water.

5. Sodium carbonate (1.5 M):

Dissolve 7.947 gm Sodium carbonate in 50ml distilled water.

6. Potassium Phosphate buffer (0.1 M, pH 7.5):

It was prepared by mixing 180 ml of 0.2 M KOH and 300 ml of 0.2 M KH_2PO_4 containing 120 ml distilled water and final volume was made to 600ml after adjusting pH to 7.5.

Procedure

A dilution series of the enzyme extract was pipetted in test tube. To all test tubes 3ml of a solution containing 0.1M potassium phosphate buffer pH 7.5 (1.5 ml), 200mM L- methionine (200 µl), 2.25 mM NBT (100 µl), 3mM EDTA (100 µl), 60 µM Riboflavin (100 µl), 1.5 M Sodium carbonate (100 µl), and enzyme extract (100 µl) was added, to start the reaction 60 µM Riboflavin (100µl) was added at the same time placing tubes under fluorescent light for 1.5min.

After this period tubes were transferred to the dark, riboflavin was added to the tube containing the dark, riboflavin was added to the tube containing the blank and absorbance was determined at

560 nm on Spectronic-20 spectrophotometer, Superoxide dismutase activity was determined by calculation.

3.2.9 Statistical evaluation

The data on biochemical constituents was examined using a factorial completely randomized design (Panse and Sukhatme, 1985).

4. RESULT AND DISCUSSION

In the present investigation experiment was conducted with thirty-one genotype of pearl millet on effect of drought (water) stress by withholding irrigation and on biochemical constituents, antioxidative enzymes and some physiological parameter. The results of these studies are presented and discussed with following heading.

4.1 The Effect of Leaf Water Content (RLWC) in the Leaves of Pearl Millet during Drought Stress.

Relative leaf water content in seedling of pearl millet genotype under control and stressed condition presented in Table 2.

There was decrease in water content when plants were exposed to water stress. The RLWC in the leaves of control plant of pearl millet ranged from 74.40 to 84.65 per cent in restorers, 72.82 to 87.03 per cent in male sterile line, 78.63 to 86.23 per cent in maintainer line and 87.21 to 89.25 per cent in hybrid variety. During water stress it was found to the tune of 65.14 to 78.84 per cent in restorers, 65.1 to 78.12 per cent in male sterile, 69.91 to 79.34 per cent in maintainer and 79.33 to 81.64 per cent in hybrid Variety.

The lowest per cent decrease in RLWC (4.04 %) was recorded in Dhanshakti Variety (4.62 %),Hybrid Phule Adishakti (4.44%), in maintainer DHLBI-K-20/883(4.62 %), followed by restorers DHLBI-1708 (5.53 %) and male sterile DHLB-24A (10.23 %) this seen to be promising for water stress. Similar types of observation were recorded in (Divya *et al.*, 2019) in barnyard millet, (Vinothini *et al.,* 2016) in foxtail millet, (Deshmukh *et al*., 2012; Addisie and Yamane, 2011; Vijayalakshmi *et al*., 2012; Ukani *et al*., 2019) in pearl millet, (Divya *et al.,* 2019) in barnyard millet. Hence present results are in agreement with earlier reports of different authors.

4.2 Photosynthetic Rate of Pearl Millet during Drought Stress.

Photosynthetic rate in seedling of pearl millet genotype under control and stressed condition presented in Table 3.

There was decrease in photosynthetic rate when plants were exposed to water stress. The photosynthetic rate in the leaves of control plant of pearl millet ranged from 27.23 to 33.23 µmole CO_2 m^{-2} s^{-1} in restorers, 26.47 to 28.28 µmole CO_2 m^{-2} s^{-1} in male sterile, 27.33 to 33.41 µmole CO_2 m^{-2} s^{-1} in maintainer line, and 33.38 to 35.61 µmole CO_2 m^{-2} s^{-1} in hybrids. During water stress it was found to the tune of 23.22 to 30.23 µmole CO_2 m^{-2} s^{-1} in restorers, 22.23 to 24.88 µmole CO_2 m^{-2} s^{-1} in male sterile line, 23.25 to 29.6 µmole CO_2 m^{-2} s^{-1} in maintainer, and 29.50 to 29.60 µmole CO_2 m-2 s-1 in hybrid Variety.

The lowest per cent decrease in Dhanshakti Variety (5.08 %),Hybrid Phule Adishakti (9.97 %), in maintainer (4.62 %) in DHLBI-K-20/883, followed by restorers (9.02 %) in DHLBI-1708, male sterile DHLBI-24A (8.21%), this seen to be promising for water stress. Similar types of observation were recorded in (Divya *et al*., 2019) in barnyard millet, Nanja *et al.* (2020) in finger

Table 2 Effect of drought stress on relative leaf water content in the leaves of cytoplasmic male sterile, restorer lines, maintainer lines and hybrids of pearl millet.

Sr. No	Genotype	Relative leaf water content (%)		
		Control	Stress	Percent decrease
	Restorers (R-Line)			
1	DHLBI 967	76.37	66.32	13.15
2	DHLBI 1035	74.40	65.14	12.44
3	DHLBI 1708	83.46	78.84	5.53
4	DHLBI 1822	76.90	67.35	12.41
5	DHLBI 1013	76.69	66.90	12.76
6	DHLBI 1074	75.57	68.51	9.34
7	DHLBI 1103	75.90	69.56	8.35
8	DHLBI 1603	78.90	70.35	10.83
9	DHLBI 1609	84.65	76.20	9.98
10	DHLBI 1806	75.40	64.33	14.61
11	DHLBI 1201	78.15	70.24	10.12
	Mean	77.84	69.43	10.86
	Range	74.40-84.65	64.33-78.84	
	Male Sterile (A-Line)			
1	DHLB-8A	76.04	67.12	11.73
2	DHLB-16A	83.25	73.56	11.63
3	DHLB-17A	84.75	76.21	10.07
4	DHLB-23A	77.26	69.90	9.52
5	DHLB-24A	87.03	78.12	10.23
6	DHLB-31A	85.36	78.12	8.48
7	DHLB-35A	75.82	65.10	14.13
	Mean	81.35	72.59	10.82
	Range	75.82-87.03	65.10-78.12	
	Maintainer (B-Line)			
1	DHLBI-K-20/861	84.65	79.30	6.32
2	DHLBI-K-20/862	85.34	76.25	10.65
3	DHLBI-K-20/863	84.30	79.34	9.11
4	DHLBI-K-20/866	85.50	76.85	10.11
5	DHLBI-K-20/868	81.22	73.66	9.30
6	DHLBI-K-20/871	78.63	72.35	7.98
7	DHLBI-K-20/874	79.64	74.18	6.85
8	DHLBI-K-20/877	84.62	77.65	8.23
9	DHLBI-K-20/881	86.23	78.81	8.60
10	DHLBI-K-20/883	82.31	75.64	4.62
11	DHLBI-K-20/537	81.91	69.91	9.34
	Mean	82.5	75.81	8.10
	Range	78.63-86.23	69.91-79.34	
	Hybrid variety			
	Dhanshakti(Variety)	89.25	85.64	4.04
	Phule Adishakti(Hybrid)	87.21	83.33	4.44
	Mean	88.23	80.48	4.24
	Range	87.21-89.25	83.33-85.64	
	Comparison	SE±	CD at 5%	
1	**Genotype**	0.16	0.29	
2.	**Treatment**	0.10	0.47	

Table 3 Effect of drought stress on photosynthetic rate in the leaves of cytoplasmic male sterile, restorer lines, maintainer lines and hybrids of pearl millet.

Sr. No	Genotype	Photosynthetic rate (µmole CO_2 m^{-2} s^{-1})		
		Control	Stress	Percent decrease
	Restorers (R-Line)			
1	DHLBI 967	29.36	25.45	13.31
2	DHLBI 1035	30.25	26.36	12.85
3	DHLBI 1708	33.23	30.23	9.02
4	DHLBI 1822	29.30	24.76	15.49
5	DHLBI 1013	31.11	26.12	16.03
6	DHLBI 1074	32.55	29.30	9.98
7	DHLBI 1103	31.17	26.33	15.52
8	DHLBI 1603	28.64	24.25	15.32
9	DHLBI 1609	28.55	24.80	13.13
10	DHLBI 1806	31.79	23.22	26.95
11	DHLBI 1201	27.23	23.95	12.04
	Mean	30.28	25.88	14.51
	Range	27.23-33.23	23.22-30.23	
	Male Sterile (A-Line)			
1	DHLB-8A	27.62	24.88	9.92
2	DHLB-16A	27.27	22.94	15.87
3	DHLB-17A	26.60	23.55	11.46
4	DHLB-23A	26.92	22.84	15.15
5	DHLB-24A	25.32	23.24	8.21
6	DHLB-31A	28.47	23.66	16.89
7	DHLB-35A	27.20	22.23	18.27
	Mean	27.05	23.47	13.11
	Range	26.47-28.28	22.23-24.88	
	Maintainer (B-Line)			
1	DHLBI-K-20/861	30.20	25.32	16.15
2	DHLBI-K-20/862	32.55	29.30	9.98
3	DHLBI-K-20/863	32.20	25.32	21.36
4	DHLBI-K-20/866	32.47	27.24	16.10
5	DHLBI-K-20/868	30.25	26.36	12.85
6	DHLBI-K-20/871	29.79	25.45	14.56
7	DHLBI-K-20/874	29.30	24.76	15.49
8	DHLBI-K-20/877	28.27	24.25	14.22
9	DHLBI-K-20/881	27.33	23.87	12.66
10	DHLBI-K-20/883	33.41	29.60	8.67
11	DHLBI-K-20/537	29.25	23.25	20.21
	Mean	30.36	25.88	14.75
	Range	27.33-33.41	23.25-29.60	
	Hybrid variety			
	Dhanshakti(Variety)	35.61	33.80	5.08
	Phule Adishakti(Hybrid)	33.38	30.05	9.97
	Mean	34.49	29.59	9.35
	Range	33.38-35.61	30.05-32.50	
	Comparison	SE±	CD at 5%	
1	**Genotype**	0.16	0.45	
2.	**Treatment**	0.10	0.28	

millet, (Ukani *et al.,* 2019) in pearl millet. Hence present results are in agreement with earlier reports of different authors.

4.3 Effect of Drought Stress on Chlorophyll Contents in the Leaves of Pearl Millet.

Total chlorophyll in seedling of pearl millet genotype under control and stressed condition presented in Table 4.

There was decrease in total chlorophyll when plants were exposed to water stress. The total chlorophyll in the leaves of control plant of pearl millet ranged from 1.41 to 2.89 mg g^{-1} in restorers, 1.49 to 2.80 mg g^{-1} in male sterile, 1.65 to 3.10 mg g^{-1} in maintainer and 2.11 to 3.73 mg g^{-1} in hybrids variety and During water stress it was found to the tune of in restorers 1.14 to 2.47 mg g^{-1} in restorers, 1.02 to 1.82 mg g^{-1} in male sterile, 1.23 to 2.50 mg g^{-1} in maintainer and 1.90 to 3.45 mg g-1 FW in hybrid Variety.

The lowest per cent decrease in total chlorophyll (7.5 %) was recorded in Dhanshakti Variety (7.50 %), Hybrid Phule Adishakti (9.95 %), in maintainer (4 %) in DHLBI-20/883, followed by restorers DHLBI-1708 (11.46 %) and male sterile DHLBI-24A (21.42 %), this seen to be promising for water stress. Similar types of observation were recorded (Kathare *et al.,* 2019; Sushmitha *et al*., 2018; Dubey *et al*., 2018) in little millet, (Menna *et al*., 2013; Mamta *et al*., 2019) in pearl millet. Hence present results are in agreement with earlier reports of different authors.

4.4 Effect of Drought Stress on Chlorophyll Stability Index in the Leaves of Pearl Millet.

Chlorophyll stability index in seedling of pearl millet genotype under control and stressed condition presented in Table 5.

There was decrease in chlorophyll stability index when plants were exposed to water stress. The chlorophyll stability index in the leaves of control plant of pearl millet ranged from 73.14 to 83.11 per cent in restorers, 74.50 to 87.65 per cent in male sterile, 74.50 to 88.86 per cent in maintainer and 90.81 to 92.86 per cent in hybrids variety and During water stress it was found to the tune of 54.15 to 69.58 per cent in restorers, 54.9 to 77.5 per cent in male sterile, 54.9 to 79.23 per cent maintainer and 82.26 to 84.35 per cent in hybrid variety.

The lowest per cent decrease in chlorophyll stability index (9.16 %) was in Dhanshakti Variety (9.16 %),Hybrid Phule Adishakti (9.41 %), in maintainer (10.42 %) in DHLBI-20/883, followed by restorers DHLBI-1708 (16.27 %) in DHLBI-1708 and male sterile DHLBI-24A (11.58%), this seen to be promising for water stress. Similar types of observation were recorded in (Divya *et al* 2019) in barnyard millet and (Mohan *et al* 2019) in rice and hence present results are in agreement with earlier reports of different authors.

Table 4 Effect of drought stress on total chlorophyll in the leaves of cytoplasmic male sterile, restorer lines, maintainer lines and hybrids of pearl millet.

Sr. No	Genotype	Total chlorophyll (mg g^{-1} FW)		
		Control	Stress	Percent decrease
	Restorers (R-Line)			
1	DHLBI 967	1.41	1.08	23.40
2	DHLBI 1035	1.56	1.24	20.51
3	DHLBI 1708	2.79	2.47	11.46
4	DHLBI 1822	1.59	1.07	32.70
5	DHLBI 1013	1.52	1.03	32.23
6	DHLBI 1074	1.48	1.10	25.67
7	DHLBI 1103	1.51	1.15	23.84
8	DHLBI 1603	1.62	1.21	25.30
9	DHLBI 1609	2.89	1.95	32.52
10	DHLBI 1806	1.70	1.14	33.52
11	DHLBI 1201	1.49	1.15	22.81
	Mean	1.77	1.32	25.81
	Range	1.41-2.89	1.14-2.47	
	Male Sterile (A-Line)			
1	DHLB-8A	1.51	1.10	27.15
2	DHLB-16A	1.49	1.15	22.81
3	DHLB-17A	1.50	1.03	31.33
4	DHLB-23A	1.55	1.12	27.74
5	DHLB-24A	2.80	1.82	21.42
6	DHLB-31A	2.75	1.85	32.72
7	DHLB-35A	1.58	1.02	35.44
	Mean	1.88	1.29	28.37
	Range	1.49-2.80	1.02-1.82	
	Maintainer (B-Line)			
1	DHLBI-K-20/861	3.04	2.02	33.52
2	DHLBI-K-20/862	2.92	1.86	36.30
3	DHLBI-K-20/863	2.69	1.80	33.08
4	DHLBI-K-20/866	3.10	2.05	33.87
5	DHLBI-K-20/868	1.85	1.45	21.62
6	DHLBI-K-20/871	1.65	1.33	19.38
7	DHLBI-K-20/874	1.72	1.40	18.60
8	DHLBI-K-20/877	2.85	1.82	36.14
9	DHLBI-K-20/881	2.95	1.95	33.89
10	DHLBI-K-20/883	2.98	2.50	16.10
11	DHLBI-K-20/537	1.90	1.23	35.26
	Mean	2.39	1.72	28.88
	Range	1.65-3.10	1.23-2.50	
	Hybrid variety			
	Dhanshakti(Variety)	3.73	3.45	7.50
	Phule Adishakti(Hybrid)	2.11	1.90	9.95
	Mean	3.73	3.45	7.50
	Range	2.11-3.73	1.90-3.45	
	Comparison	SE±	CD at 5%	
1	**Genotype**	0.019	0.05	
2.	**Treatment**	0.012	0.030	

Table 5 Effect of drought stress on chlorophyll stability index in the leaves of cytoplasmic male sterile, restorer lines, maintainer lines and hybrids of pearl millet.

Sr. No	Genotype	Chlorophyll stability index (%)		
		control	Stress	Percent decrease
	Restorers (R-Line)			
1	DHLBI 967	74.48	56.25	24.47
2	DHLBI 1035	75.80	55.80	26.38
3	DHLBI 1708	83.11	69.58	16.27
4	DHLBI 1822	76.53	58.23	23.91
5	DHLBI 1013	74.83	54.75	26.83
6	DHLBI 1074	74.20	54.88	26.03
7	DHLBI 1103	74.90	54.91	26.68
8	DHLBI 1603	79.44	58.50	26.35
9	DHLBI 1609	84.85	64.35	24.16
10	DHLBI 1806	74.25	54.15	27.07
11	DHLBI 1201	73.14	56.64	22.59
	Mean	76.86	58.00	24.53
	Range	73.14-83.11	54.15-69.58	
	Male Sterile (A-Line)			
1	DHLB-8A	76.35	57.35	24.88
2	DHLB-16A	82.90	59.86	27.79
3	DHLB-17A	74.50	54.90	26.30
4	DHLB-23A	74.85	54.86	26.70
5	DHLB-24A	87.65	77.50	11.58
6	DHLB-31A	84.13	62.85	25.59
7	DHLB-35A	79.85	55.90	29.99
	Mean	79.46	60.46	23.91
	Range	74.5-87.65	54.9-77.5	
	Maintainer (B-Line)			
1	DHLBI-K-20/861	80.18	62.18	22.44
2	DHLBI-K-20/862	83.50	66.01	20.94
3	DHLBI-K-20/863	77.25	64.83	16.07
4	DHLBI-K-20/866	88.86	79.15	10.92
5	DHLBI-K-20/868	74.82	65.10	12.99
6	DHLBI-K-20/871	76.35	59.25	22.39
7	DHLBI-K-20/874	74.50	54.90	26.30
8	DHLBI-K-20/877	84.76	71.60	15.52
9	DHLBI-K-20/881	85.77	72.16	15.86
10	DHLBI-K-20/883	88.45	79.23	10.42
11	DHLBI-K-20/537	74.90	54.20	28.97
	Mean	80.84	66.14	18.43
	Range	74.50-88.86	54.20-79.23	
	Hybrid variety			
	Dhanshakti(Variety)	92.86	84.35	9.16
	Phule Adishakti(Hybrid)	90.81	82.26	9.41
	Mean	91.83	83.30	9.28
	Range	90.81-92.86	82.26-84.35	
	Comparison	SE±	CD at 5%	
1	**Genotype**	0.16	0.45	
2	**Treatments**	0.10	0.28	

4.5 Effect of Drought Stress on Osmolytes Accumulation in Pearl Millet Leaves.

4.5.1 Proline

Proline content in seedling of pearl millet genotype under control and stressed condition presented in Table 6.

Plant accumulate organic osmoprotectants through biochemical mechanisms which improve their ability to withstand stress. The leaf proline content in control plants was found to be ranged from 4.39 to 5.80 μmoles g^{-1} in restorers, 4.17 to 5.89 μmoles g^{-1} in male sterile, 4.42 to 6.05 μmoles g^{-1} in maintainer and 6.25 to 6.50 μmoles g^{-1}in hybrid variety after exposing to water stress, it was found to be increase to tune of 7.10 to 11.55 μ moles g-1 in restorers ,7.52 to 11.65 μmoles g-1 in male sterile, 8.35 to 11.95 μ moles g-1 in maintainer and 14.90 to 15.60 μ moles g-1 FW in hybrid variety.

The maximum per cent increase of in restorers DHLBI-1708 was to be 90.13 percent in DHLBI-1708, in male sterile DHLBI-24A it was 135.35 per cent, in maintainer line DHLBI-K-20/883 it was 130.82 per cent and it was highest that is 140.00 percent variety Dhanshakti. When compared to all line both the variety Dhanshakti and Hybrid Phule adishakti accumulated highest proline content is 140.00 and 138.40 per cent respectively, among the male sterile, maintainer and restorers DHLB-24A, DHLBI-K-20/883 and DHLBI-1708 has recorded maximum accumulation during water stress. Similar types of were recorded in (Tiwari *et al* 2019) in finger millet, (Ukani *et al.,* 2019) in pearl millet, (Keyvan *et al*., 2010; Akhkha *et al.,* 2011) in Wheat, (Mafakheri *et al.,* 2010) in chickpea, (Kabir *et al*., 2015) in barley. Hence present results are in agreement with earlier reports of different authors.

4.5.2 Glycine betaine

Glycine betaine content in seedling of pearl millet genotype under control and stressed condition presented in Table 7.

There was increase in glycine betaine content when plants were exposed to water stress. The leaf glycine betaine content in control plants was found to be ranged from 209.26 to 292.38 μg g^{-1} in restorers, 211.28 to 317.99 μg g^{-1} in male sterile, 227.76 to 386.88 μg g^{-1}in maintainer and 391.75 to 399.69 μg g^{-1}in hybrid variety and after exposing water stress it was found to be increase to tune of 236.12 to 374.42 μg g^{-1} in restorers, 235.18 to 378.60 μg g^{-1}male sterile, 266.77 to 460.81 μg g^{-1}maintainer and 501.62 to 521.00 μg g^{-1}in hybrid variety.

The maximum per cent of glycine betaine increases in restorers DHLBI-1708 was found to be 28.05 per cent in DHLBI-1708, in male sterile DHLBI-24A it was 22.96 per cent, maintainer DHLBI-K-20/883 it was 19.10 per cent and it was highest that is 30.35 per cent in Dhanshakti. When compared to be all lines both the variety Dhanshakti and hybrid Phule Adishakti accumulate highest glycine betaine content is 30.28 and 28.04 per cent respectively, among the male sterile, maintainer and restorers DHLB-24A, DHLBI-K-20/883 and DHLBI-1708 has recorded maximum accumulation during water stress. Similar types of observation were recorded in (Deshamukh *et*

al., 2017) in pearl millet, (Ajithkumar and panneerselvam, 2013) in finger millet, (Zhang *et al*., 2014) in maize, (Neto *et al*., 2009) in sorghum. Hence present results are in agreement with earlier reports of different authors.

Table 6 Effect of drought stress on proline content in the leaves of cytoplasmic male sterile, restorer lines, maintainer lines and hybrids of pearl millet.

Sr. No	Genotype	Proline (µmoles g^{-1} FW)		
		Control	Stress	Percent increase
	Restores (R-Line)			
1	DHLBI 967	4.60	7.22	56.95
2	DHLBI 1035	4.45	7.91	77.75
3	DHLBI 1708	5.80	11.55	90.13
4	DHLBI 1822	4.65	7.10	52.68
5	DHLBI 1013	4.30	7.90	83.72
6	DHLBI 1074	4.39	7.80	77.67
7	DHLBI 1103	4.43	7.50	69.30
8	DHLBI 1603	4.65	7.90	69.89
9	DHLBI 1609	4.59	7.10	54.68
10	DHLBI 1806	4.65	7.10	52.68
11	DHLBI 1201	4.50	7.65	70.00
	Mean	4.61	7.53	69.15
	Range	4.39-5.80	7.10-11.55	
	Male Sterile (A-Line)			
1	DHLB-8A	4.75	8.90	87.36
2	DHLB-16A	4.50	8.55	90.00
3	DHLB-17A	4.17	8.42	101.89
4	DHLB-23A	4.70	9.65	105.31
5	DHLB-24A	4.95	11.65	135.35
6	DHLB-31A	4.37	7.85	79.63
7	DHLB-35A	4.20	7.52	79.04
	Mean	4.52	8.93	96.94
	Range	4.17-5.89	7.52-11.65	
	Maintainer (B-Line)			
1	DHLBI-K-20/861	5.15	10.85	110.67
2	DHLBI-K-20/862	5.19	10.10	94.60
3	DHLBI-K-20/863	4.42	8.47	91.62
4	DHLBI-K-20/866	5.24	11.20	113.70
5	DHLBI-K-20/868	5.25	11.85	125.71
6	DHLBI-K-20/871	5.13	11.55	125.14
7	DHLBI-K-20/874	4.60	9.90	115.21
8	DHLBI-K-20/877	5.25	11.85	125.71
9	DHLBI-K-20/881	6.05	11.95	97.52
10	DHLBI-K-20/883	5.32	12.28	130.82
11	DHLBI-K-20/537	4.45	8.35	87.64
	Mean	5.09	10.75	110.75
	Range	4.42-6.05	8.35-11.95	
	Hybrid variety			
	Dhanshakti(Variety)	6.5	15.60	140.00
	Phule Adishakti(Hybrid)	6.25	14.90	138.40
	Mean	6.37	15.25	139.20
	Range	6.25-6.50	14.90-15.60	
	Comparison	**SE±**	CD at 5%	
1	**Genotype**	0.01	0.03	
2	**Treatments**	0.008	0.02	

Table 7 Effect of drought stress on glycine betaine in the leaves of cytoplasmic male sterile, restorer lines, maintainer lines and hybrids of pearl millet.

Sr. No	Genotype	Glycine betaine (µg g^{-1} FW)		
		Control	stress	Percent increase
	Restorers (R-Line)			
1	DHLBI 967	269.07	317.82	18.11
2	DHLBI 1035	242.61	275.14	13.40
3	DHLBI 1708	292.38	374.42	28.05
4	DHLBI 1822	250.29	297.67	18.92
5	DHLBI 1013	233.22	266.34	14.20
6	DHLBI 1074	209.26	243.55	16.35
7	DHLBI 1103	250.12	302.37	20.88
8	DHLBI 1603	276.76	328.06	18.53
9	DHLBI 1609	292.38	334.64	14.45
10	DHLBI 1806	209.66	236.12	12.62
11	DHLBI 1201	280.94	328.15	16.80
	Mean	255.15	300.38	17.48
	Range	209.26-292.38	236.12-374.42	
	Male Sterile (A-Line)			
1	DHLB-8A	249.70	292.81	17.26
2	DHLB-16A	297.59	335.06	12.59
3	DHLB-17A	310.91	352.74	13.45
4	DHLB-23A	227.50	285.83	13.77
5	DHLB-24A	217.43	267.37	22.96
6	DHLB-31A	317.99	378.60	19.06
7	DHLB-35A	211.28	235.18	11.31
	Mean	261.77	306.79	15.19
	Range	211.28-317.99	235.18-378.60	
	Maintainer (B-Line)			
1	DHLBI-K-20/861	317.56	369.81	16.45
2	DHLBI-K-20/862	331.56	369.30	11.38
3	DHLBI-K-20/863	340.36	376.04	10.48
4	DHLBI-K-20/866	352.14	385.09	9.35
5	DHLBI-K-20/868	342.49	394.39	15.15
6	DHLBI-K-20/871	243.81	283.84	16.42
7	DHLBI-K-20/874	227.76	267.37	17.39
8	DHLBI-K-20/877	361.10	404.21	11.93
9	DHLBI-K-20/881	380.14	446.55	17.47
10	DHLBI-K-20/883	386.88	460.81	19.10
11	DHLBI-K-20/537	246.71	266.77	14.67
	Mean	328.38	375.74	14.43
	Range	227.76-386.88	266.77-460.81	
	Hybrid variety			
	Dhanshakti(Variety)	399.69	521.00	30.35
	Phule Adishakti(Hybrid)	391.75	501.62	28.04
	Mean	395.72	511.31	29.15
	Range	391.75-399.69	501.62-521.00	
	Comparison	SE±	CD at 5%	
1	**Genotype**	0.18	0.52	
2	**Treatments**	0.17	0.32	

4.6 Effect of Drought Stress on Lipid Peroxidation in Pearl Millet Leaves.

Lipid peroxidase content in seedling of pearl millet genotype under control and stressed condition presented in Table 8.

Table 8 Effect of drought stress on lipid peroxidation in the leaves of cytoplasmic male sterile, restorer lines, maintainer lines and hybrids of pearl millet.

Sr. No	Genotype	Lipid peroxidation (nmol MDA g^{-1} FW)		
		control	stress	Percent decrease
	Restorers (R-Line)			
1	DHLBI 967	158.01	178.40	12.90
2	DHLBI 1035	158.91	175.70	10.56
3	DHLBI 1708	172.65	196.65	13.9
4	DHLBI 1822	161.31	183.30	13.62
5	DHLBI 1013	154.41	175.60	13.72
6	DHLBI 1074	155.44	176.80	13.74
7	DHLBI 1103	160.31	180.30	12.46
8	DHLBI 1603	167.32	189.30	13.13
9	DHLBI 1609	169.53	189.50	11.77
10	DHLBI 1806	156.23	171.30	9.64
11	DHLBI 1201	173.24	191.25	10.39
	Mean	162.48	182.55	12.34
	Range	154.41-172.65	171.3-196.65	
	Male Sterile (A-Line)			
1	DHLB-8A	151.43	171.53	13.27
2	DHLB-16A	179.30	198.53	10.72
3	DHLB-17A	173.96	192.60	10.71
4	DHLB-23A	155.07	173.53	11.90
5	DHLB-24A	176.56	202.50	14.69
6	DHLB-31A	174.16	198.60	14.03
7	DHLB-35A	151.90	166.49	9.60
	Mean	166.05	186.25	12.13
	Range	151.43-179.30	166.49-202.50	
	Maintainer (B-Line)			
1	DHLBI-K-20/861	183.25	209.06	14.08
2	DHLBI-K-20/862	187.31	215.96	15.29
3	DHLBI-K-20/863	167.90	189.30	12.74
4	DHLBI-K-20/866	177.30	198.26	11.82
5	DHLBI-K-20/868	155.98	179.40	15.01
6	DHLBI-K-20/871	170.20	193.40	13.63
7	DHLBI-K-20/874	156.91	182.60	16.37
8	DHLBI-K-20/877	190.91	220.50	15.49
9	DHLBI-K-20/881	189.23	215.20	13.72
10	DHLBI-K-20/883	191.98	225.00	17.19
11	DHLBI-K-20/537	153.43	171.53	11.79
	Mean	174.94	200.01	14.28
	Range	153.43-191.98	171.53-225.00	
	Hybrid variety			
	Dhanshakti(Variety)	204.50	240.66	17.68
	Phule Adishakti(Hybrid)	201.30	234.91	16.69
	Mean	202.90	237.78	17.19
	Range	201.3-204.5	234.91-240.66	
	Comparison	SE±	CD at 5%	
1	**Genotype**	0.15	0.042	
2	**Treatments**	0.09	0.26	

There was increase in lipid peroxidase content when plants were exposed to water stress. The leaf lipid peroxidase content in control plant of pearl millet ranged form in restorers line 154.41 to 172.65 nmol MDA g^{-1} in restorers line, 151.43 to 179.30 nmol MDA g^{-1} in male sterile, 153.43 to 191.98 nmol MDA g^{-1} in maintainer and 201.30 to 204.50 nmol MDA g^{-1} in hybrid variety and after exposing water stress, it was found to be increase to tune of 171.3 to 196.65 nmol MDA g^{-1} in restorers ,166.49 to 202.50 nmol MDA g^{-1} male sterile, 171.53 to 225.00 nmol MDA g^{-1} in maintainer and 234.91 to 240.66 nmol MDA g^{-1} in hybrid variety.

The maximum per cent lipid peroxidase increase in restorers line DHLBI-1708 was found to be 13.90 per cent in DHLBI-1708, in male sterile DHLBI-24A it was 14.69 per cent, in maintainer DHLBI-K-20/883 it was 17.19 per cent and it was highest that is hybrids 17.68 percent was variety Dhanshakti .When compared to be all lines both the variety Dhanshakti and hybrid Phule adishakti content highest glycine betaine is 20.12 and 18.68 per cent respectively, among the male sterile ,maintainer and restorers DHLB-24A, DHLBI-K-20/883 and DHLBI-1708 has recorded maximum damage during water stress. Similar types of observation were recorded in (Deshamukh *et al*., 2017) in pearl millet, (Lata *et al*., 2010) in foxtail millet, (Moussa *et al*.,2011; Chugh *et al* 2011) in maize and hence present results are in agreement with earlier reports of different authors.

4.7 Effect of Drought Stress on Antioxidative Enzymes in Pearl Millet Leaves.

4.7.1 Superoxidase dismutase

The change in superoxide dismutase activity due to water stress is presented in Table 9. The water stress has caused significant increase in superoxide dismutase activity in water stressed seedlings of pearl millet.

There was increase in superoxide dismutase activity when plants were exposed to water stress. The superoxide dismutase under control condition ranged from 89.65 to 91.71 units mg-1 protein in restorers, 88.92 to 90.93 units mg-1 protein in male sterile, 88.61 to 91.89 units mg-1 protein in maintainer and 91.78 to 92.10 units mg-1 protein in hybrid variety, and after exposing to water stress ,it was found to be increase to tune 110.63 to 123.54 units mg-1 protein in restorers, 111.36 to 119.91 mg-1 protein in male sterile,109.6 to 121.96 units mg-1 protein in maintainer and 125.85 to 127.95 units mg-1 protein in hybrid variety. The maximum per cent of superoxide dismutase activity increase in restorers' line DHLBI-1708 was found to be 36.44 per cent, in male sterile DHLBI-24A it was 31.87 per cent, in maintainer DHLBI-K-20/883 it was 32.72 per cent and it was highest that is 38.92 per cent in variety Dhanshakti. When compared to be all lines both the variety Dhanshakti and Hybrid Phule Adishakti highest superoxide dismutase activity is 38.92 and 37.12 per cent respectively, among the male sterile, maintainer and restorers DHLB-24A, DHLBI-K-20/883 and DHLBI-1708 has recorded maximum activity of superoxide dismutase during water stress.

Table 9 Effect of drought stress on superoxide dismutase in the leaves of cytoplasmic male sterile, restorer lines, maintainer lines and hybrids of pearl millet.

Sr. No	Genotype	Superoxidase mutase (units mg^{-1} protein)		
		Control	Stress	Percent increase
	Restorers (R-Line)			
1	DHLBI 967	91.04	118.02	29.63
2	DHLBI 1035	91.71	122.69	33.78
3	DHLBI 1708	90.54	123.54	36.44
4	DHLBI 1822	89.98	121.01	34.48
5	DHLBI 1013	91.53	122.58	33.92
6	DHLBI 1074	90.85	111.76	23.01
7	DHLBI 1103	90.65	112.61	24.22
8	DHLBI 1603	91.61	113.63	24.03
9	DHLBI 1609	90.94	117.86	29.60
10	DHLBI 1806	89.95	110.63	22.99
11	DHLBI 1201	90.57	113.64	25.47
	Mean	90.85	117.08	28.87
	Range	89.65-91.71	110.63-123.54	
	Male Sterile (A-Line)			
1	DHLB-8A	89.58	117.55	31.22
2	DHLB-16A	90.52	113.47	25.35
3	DHLB-17A	89.92	111.89	24.43
4	DHLB-23A	89.10	110.04	23.50
5	DHLB-24A	90.93	119.91	31.87
6	DHLB-31A	90.04	111.95	24.33
7	DHLB-35A	90.95	111.36	22.44
	Mean	89.85	113.73	26.57
	Range	88.92-90.93	111.36-119.91	
	Maintainer (B-Line)			
1	DHLBI-K-20/861	89.36	110.34	23.47
2	DHLBI-K-20/862	88.70	111.66	25.88
3	DHLBI-K-20/863	89.40	113.36	26.80
4	DHLBI-K-20/866	88.77	111.15	25.21
5	DHLBI-K-20/868	89.53	112.48	25.63
6	DHLBI-K-20/871	90.53	113.52	25.39
7	DHLBI-K-20/874	91.57	113.60	24.05
8	DHLBI-K-20/877	90.90	118.83	30.72
9	DHLBI-K-20/881	91.39	121.72	33.18
10	DHLBI-K-20/883	91.89	121.96	32.72
11	DHLBI-K-20/537	88.61	109.60	23.68
	Mean	90.05	114.38	26.98
	Range	88.61-91.89	109.6-121.96	
	Hybrid variety			
	Dhanshakti(Variety)	92.10	127.95	38.92
	Phule Adishakti(Hybrid)	91.78	125.85	37.12
	Mean	95.31	126.90	33.14
	Range	91.78-92.10	125.85-127.95	
	Comparison	SE±	CD at 5%	
1	**Genotype**	1.23	3.45	
2	**Treatments**	0.76	2.15	

Similar types of observation were recorded in (Kumar *et al*., 2008; Kaur *et al*., 2013; Moran *et al.,* 1994) in chickpea and (Vijayalakshmi *et.al*., 2012; Shivhare *et al*., 2019) in pearl millet and hence present results are in agreement with earlier reports of different authors.

5. SUMMARY AND CONCLUSION

Drought is one of the major abiotic stress factors, which affect the productivity of crops. Drought results in generation of reactive oxygen species. Accumulation of osmolytes, antioxidative enzymes and biochemical constitutions in plants under drought are regarded as indicators of the stress tolerance.

Pearl millet (*Pennisetum glaucum* L.) is an ideal candidate for mining genes for abiotic stress tolerance, since it is relatively tolerant to drought salinity and heavy metals, hence it felt necessary to estimate the level of osmolytes, antioxidative enzymes and biochemical constitutions in order to understand drought tolerant mechanism in this crop. Keeping this in view, the present study, entitled "Biochemical evaluation of CMS (cytoplasmic male sterility), restorers and hybrids of pearl millet (*Pennisetum glaucum* L.) against drought tolerance," was carried out during the rabi season of 2020-21. Drought stress was imposed on 50-day-old seedlings of CMS, restorers, maintainers and hybrids of pearl millet grown in pots. Biochemical parameters such as RLWC, total chlorophyll, chlorophyll stability index, photosynthetic rate, proline, glycine betaine, activity of antioxidative enzymes such as superoxide dismutase and lipid peroxidation were measured in leaf samples from control and stressed seedlings. The results obtained during this investigation are briefly summarized in this section.

1. Relative leaf water content in control plants recorded mean RLWC of 77.84 per cent in restorer, 81.35 per cent in male sterile, 82.50 per cent in maintainers and 88.23 per cent in hybrid variety. During water stress the mean RLWC was 69.43 per cent in restorer,72.59 per cent in male sterile, 75.81 per cent in maintainers and 80.48 per cent in hybrid variety. The lowest per cent decrease in RLWC (4.04 %) in Dhanshakti variety (4.62 %), Hybrid Phule Adishakti (4.44%),in maintainer DHLBI-K-20/883 (4.62%), followed by restorer DHLBI-1708(5.53%) and male sterile DHLB-24A (10.23 %).

2. Photosynthetic rate in control plant recorded mean photosynthetic rate of 30.28 µmole CO_2 m^{-2} s^{-1} in restorer, 27.05 µmole CO_2 m^{-2} s^{-1} in male sterile, 30.36 µmole CO_2 m^{-2} s^{-1} in maintainers, and 34.49 µmole CO_2 m^{-1} s^{-1} in hybrid variety. During water stress the mean photosynthetic rate was 25.88 µmole CO_2 m^{-1} s^{-1} in restorer, 23.47 µmole CO_2 m^{-1} s^{-1} in male sterile line, 25.88 µmole CO_2 m^{-1} s^{-1} in maintainer, and 29.59 µmole CO_2 m^{-1} s^{-1} in hybrid variety. The lowest per cent decrease in photosynthetic rate (5.08 %) was recorded in Dhanshakti variety (5.08 %),Hybrid Phule Adishakti (9.97 %),in maintainer DHLBI-K-20/883(8.67%), followed by restorer (9.02 %) in DHLBI-1708, male sterile DHLBI-24A (8.21%).

3. Total chlorophyll in the leaves of control plants recorded mean total chlorophyll of 1.77 mg g-1 in restorer, 1.88 mg g^{-1} in male sterile, 2.39 mg g-1 in maintainers and 3.73 mg g-1 in hybrid variety and During water stress the mean total chlorophyll was restorer 1.32 mg g-1 in restorer, 1.29 mg g-1 in male sterile, 1.72 mg g-1 in maintainer and 3.45 mg g-1 FW in hybrid variety. The

lowest per cent decrease in total chlorophyll (7.5 %) was recorded in Dhanshakti variety (7.50 %),Hybrid Phule Adishakti (9.95 %),in maintainer DHLBI-K-20/883, followed by restorers DHLBI-1708 (11.46 %) and male sterile DHLBI-24A (21.42 %),

4. The chlorophyll stability index in the leaves of control plant recorded mean Chlorophyll stability index of 76.86 per cent in restorer, 79.46 per cent in male sterile, 80.84 per cent in maintainer and 91.83 per cent in hybrid variety and During water stress the mean CSI was 58.60 per cent in restorer, 60.46 per cent in male sterile, 66.14 per cent maintainer and 83.30 per cent in hybrid variety. The lowest per cent decrease in CSI (9.16 %) was recorded in Dhanshakti variety (9.16 %),Hybrid Phule Adishakti (9.41 %), maintainer DHLBI-K-20/883, followed by restorer DHLBI-1708 (16.27 %) in DHLBI-1708 and male sterile DHLBI-24A (11.58%).

5. The proline content in control plants recorded mean proline of 4.61 μ moles g^{-1} in restorer, 4.52 μmoles g^{-1} in male sterile, 5.09 μmoles g^{-1} in maintainer and in hybrid variety 6.37 μmoles g^{-1} in hybrids and During water stress the mean proline was 7.53 μmoles g^{-1} in restorers, 8.93 μmoles g^{-1} in male sterile, 10.75 μmoles g^{-1} in maintainer and 15.25 μ moles g^{-1} in hybrid variety. The maximum per cent increase in proline restorer 90.13 percent was recorded in DHLBI-1708, 135.35 per cent male sterile DHLBI-24A, 130.82 per cent maintainer DHLBI-K-20/883 and it was highest that is 140.00 per cent Dhanshakti variety.

6. The glycine betaine content in control plants recorded mean glycine betaine of 255.15 μg g^{-1}in restorer, 261.77 μg g^{-1} in male sterile, 328.38 μg g^{-1} in maintainer and 395.72 μg g^{-1} in hybrid variety and During water stress the mean glycine betaine was 300.38 μg g^{-1} in restorer, 306.79 μg g^{-1} male sterile, 375.74 μg g^{-1}maintainer and 511.31 μg g^{-1} in hybrid variety. The maximum per cent increase in glycine betaine restorer 28.05 per cent was recorded in DHLBI-1708, 22.96 per cent male sterile DHLBI-24A, 19.10 per cent maintainer DHLBI-K-20/883 and it was highest that is 30.35 per cent was Dhanshakti variety.

7. The lipid peroxidation content in control plants recorded mean lipid peroxidation of 162.48 nmol MDA g^{-1} in restorer line, 166.05 nmol MDA g^{-1} in male sterile, 174.94 nmol MDA g^{-1}in maintainer and 202.90 nmol MDA g^{-1} in hybrid variety and During water stress the mean lipid peroxidase was 182.55 nmol MDA g^{-1} in restorer, 182.55 nmol MDA g^{-1} male sterile ,186.25 nmol MDA g^{-1} in maintainer and 237.38 nmol MDA g^{-1} FW in hybrid variety. The maximum per cent increase in lipid peroxidase restorer 13.90 per cent was recorded DHLBI-1708, 14.69 per cent male sterile DHLBI-24A, 17.19 per cent maintainer DHLBI-K-20/883 and it was highest that is 17.68 per cent was Dhanshakti variety.

8. The superoxide dismutase activity in control plants recorded mean superoxide dismutase of 90.85 units mg^{-1} protein in restorer, 89.85 units mg^{-1}protein in male sterile, 90.05 units mg^{-1} protein in maintainer and 95.31 units mg^{-1} protein in hybrid variety, and During water stress the mean SOD 117.08 units mg^{-1} protein in restorer, 113.73 mg^{-1} protein in male sterile, 114.38 units mg^{-1} protein

in maintainer and 126.90 units mg^{-1} protein in hybrid variety. The maximum per cent increase in superoxide dismutase activity restorer 36.44 per cent was recorded DHLBI-1708, 31.87 per cent male sterile DHLBI-24A, 32.72 per cent maintainer DHLBI-K-20/883 and it was highest that is 38.92 per cent Dhanshakti variety.

Form the above results it can be concluded that restorers DHLBI-1708, male sterile DHLB-24A, maintainer DHLBI-K-20/883, were found to be superior for water stress tolerance over others. Therefore, it can be concluded that above lines may be used to stress and then the breeding programme for drought tolerance.

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7. VITAE

Mr. HARIHAR BABAN LANDAGE
MASTER OF SCIENCE (AGRICULTURE)
IN
BIOCHEMISTRY

2022

Title of thesis		**:**	"Biochemical Evaluation of CMS (Cytoplasmic Male Sterile), Restorers and Hybrids of Pearl Millet (*Pennisetum glaucum*) against Drought Tolerance."
Major field		**:**	Biochemistry
Biographical information:			
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	Place of Birth	**:**	Born at Akluj, Dist. Solapur
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Educational	**Bachelor Degree Obtained**	**:**	Received B.Sc. (Agri.) degree from College of Agriculture, Kadegaon (Sangli)
	Class	**:**	I class
	Name of the University	**:**	Mahatma Phule Krishi Vidyapeeth, Rahuri.
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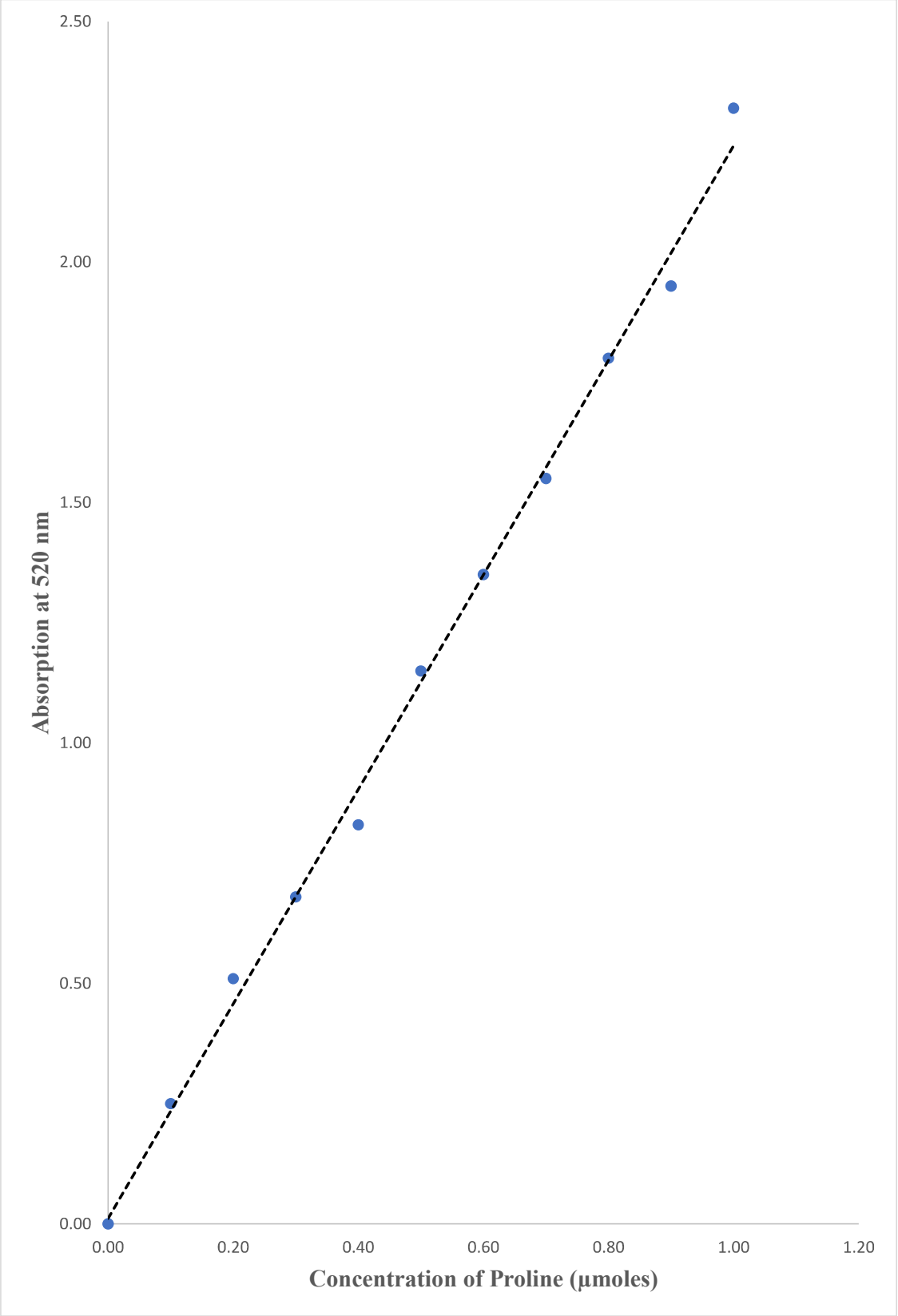

Fig. 4 Calibration of standard curve for estimation of proline.

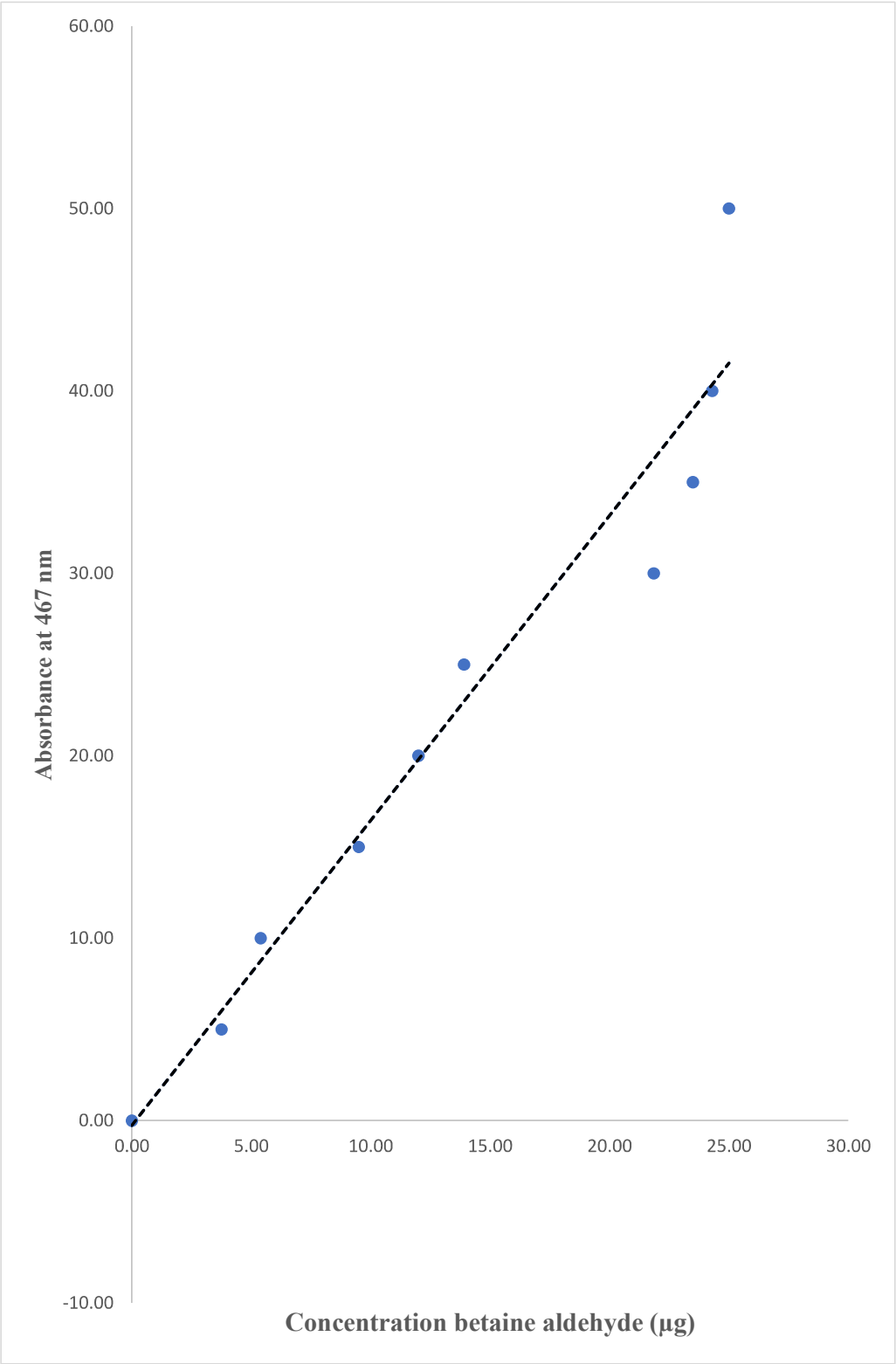

Fig. 5 Calibration of standard curve of glycine betaine

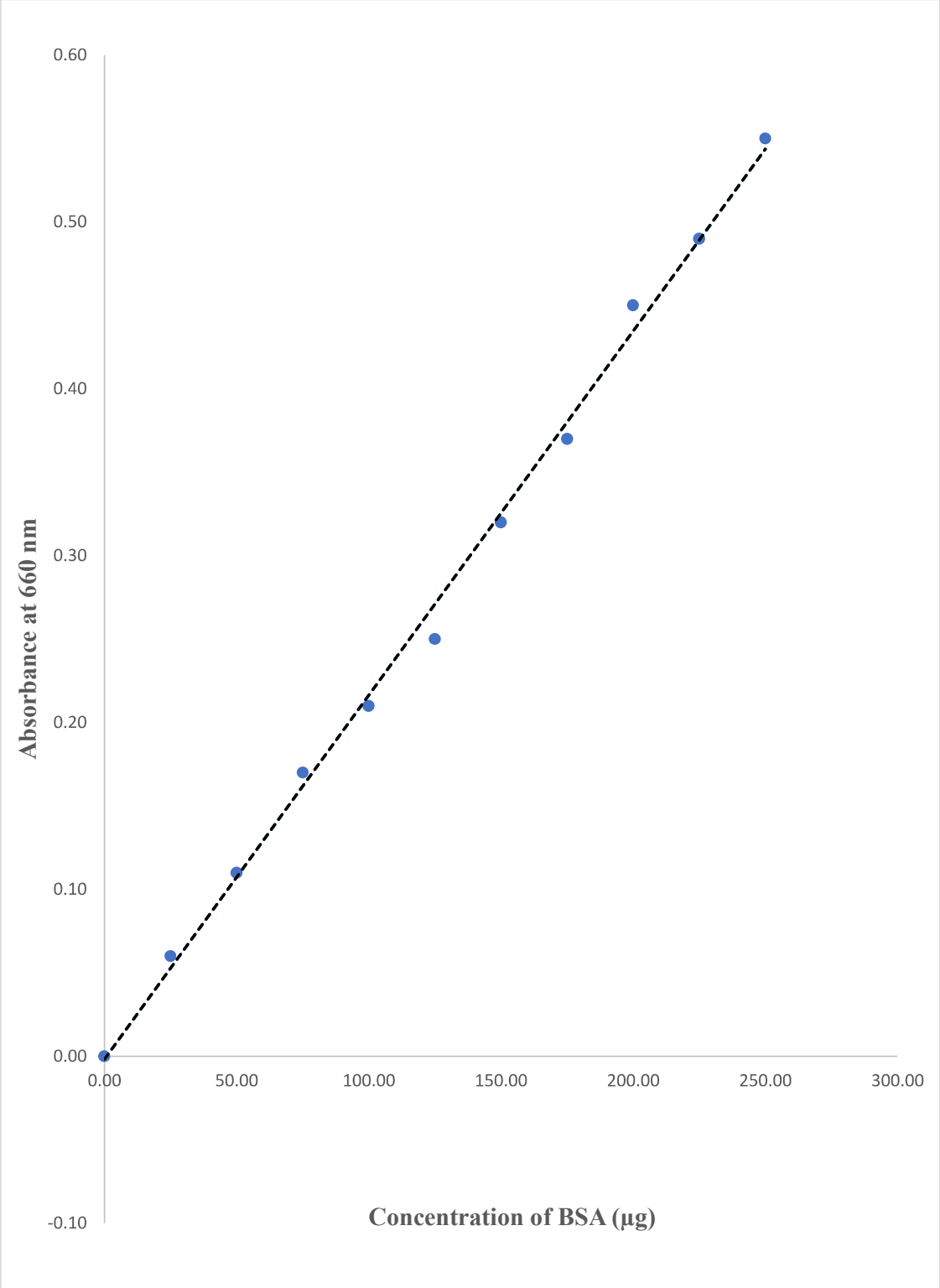

Fig. 6 Calibration of standard curve of soluble protein

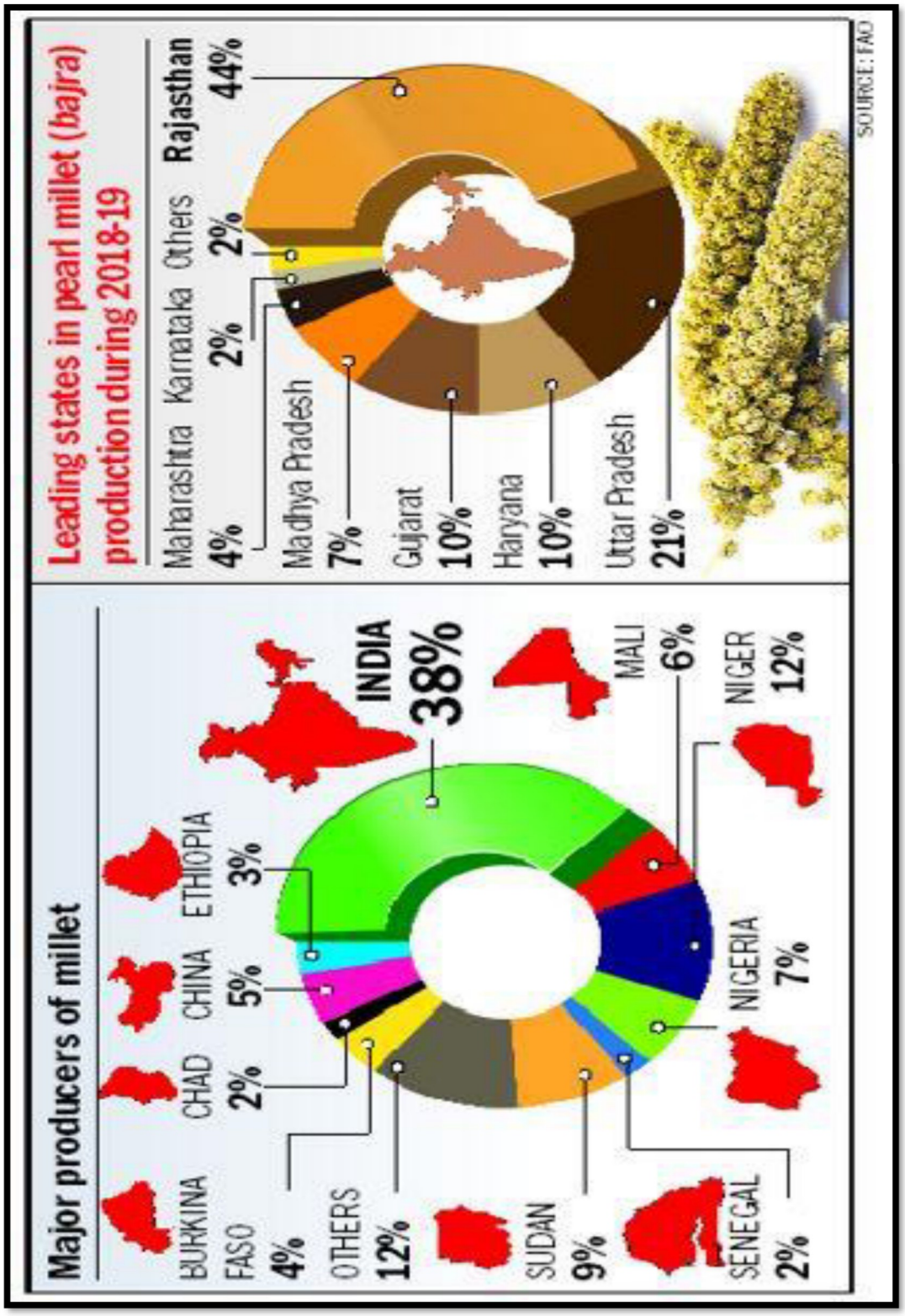

Fig. 1 Area and production of pearl millet in World and India

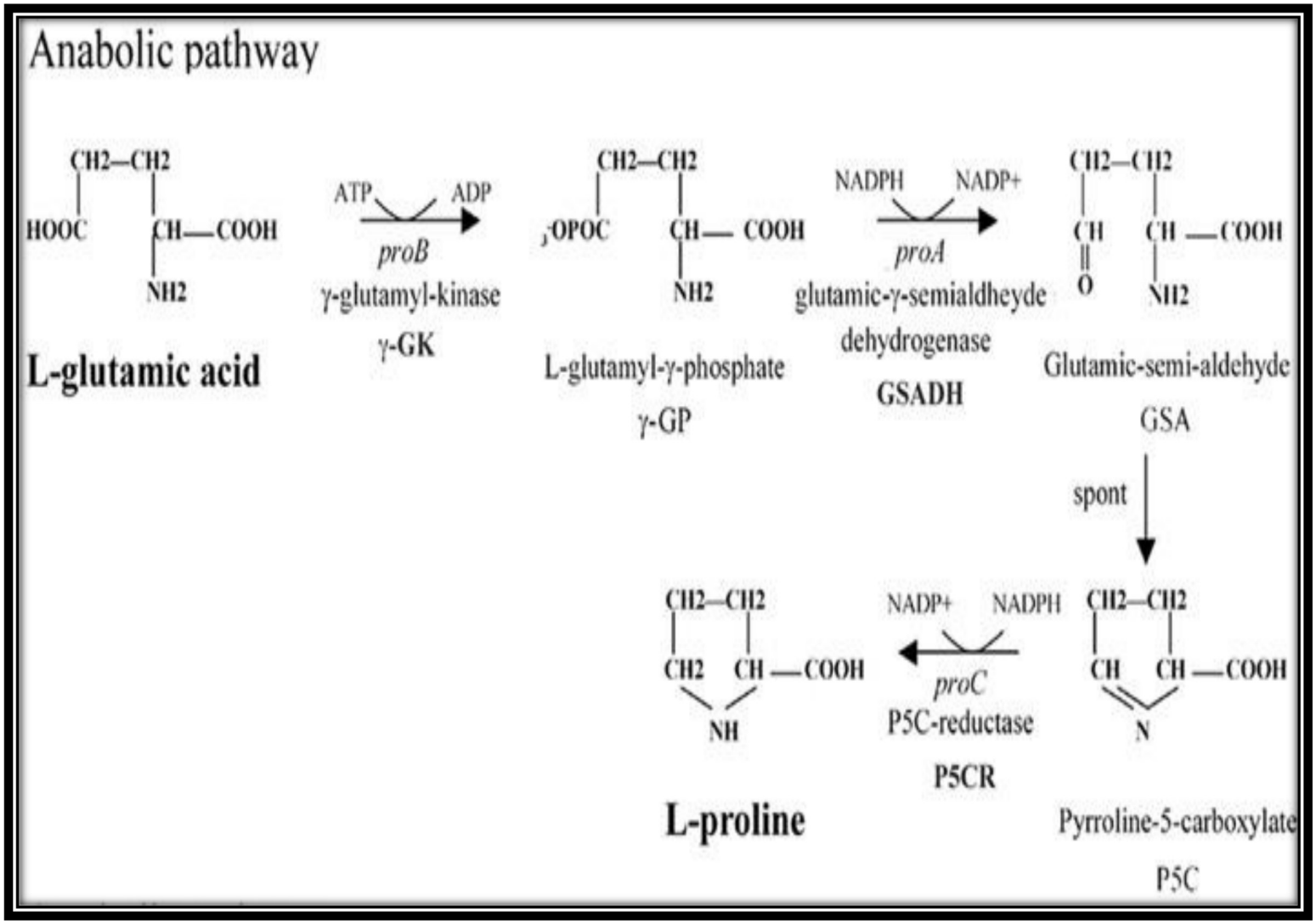

Fig. 2 Proline biosynthesis pathway of plant

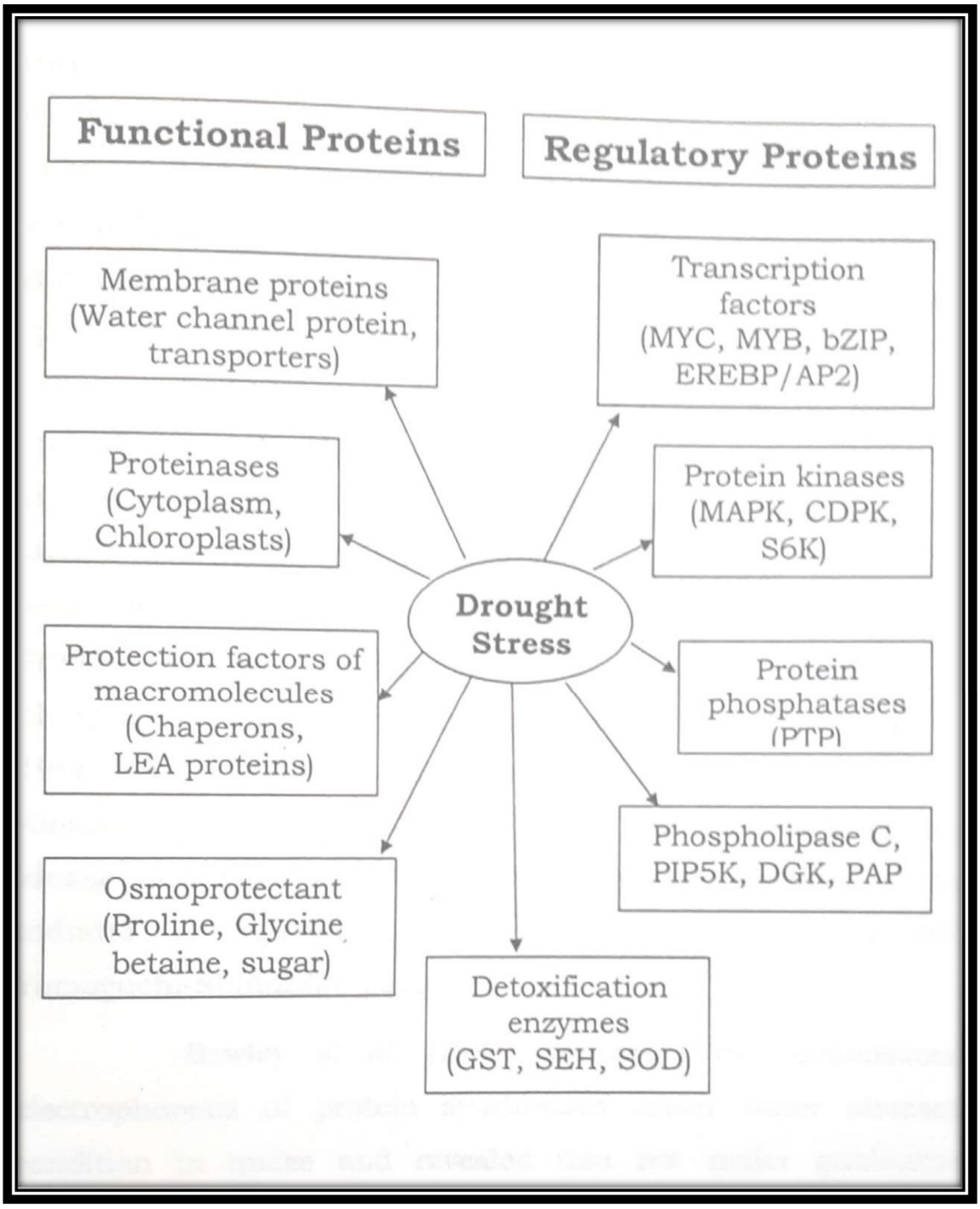

Fig. 3 Drought stress inducible genes and their possible functions in stress tolerance and response.

Fig- At sowing time

Fig- At irrigation time

Fig- 10 Days of after sowing

Fig- 25 Days of after sowing

Before stress

Fig - 40 Days after sowing

After stress

Fig 50 Days after sowing, (after 10 days stress)

Plate 1 Growth of pearl millet CMS, restorers, maintainer line and hybrids under normal and water stress condition.