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Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene-linked SSR markers

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By

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2020

RVSKVV/2169

CERTIFICATE – I

This is to certify that the thesis entitled **"Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene-linked SSR markers"** submitted in partial fulfilment of the requirement for the Degree of **MASTER OF SCIENCE** in **AGRICULTURE (Plant Molecular Biology and Biotechnology)** of **Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior** is a record of the bona-fied research work carried out by Mr. **Mohan Lal Choudhary** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and Director of Instruction.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of this investigation has been acknowledged by the scholar.

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CERTIFICATE – II

This is to certify that thesis the entitled **"Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene-linked SSR markers."** submitted by Mr. **Mohan Lal Choudhary** to the Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE** in **AGRICULTURE** in the department of **Plant Molecular Biology and Biotechnology, College of Agriculture, Gwalior** has been accepted after evaluation by the External Examiner and approved by the Student's Advisory Committee after an oral examination of the same.

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List of Abbreviations/Acronyms

Abbreviations/ Acronyms	Meaning
%	Percent
&	And
@	At the rate of
λ	Lambda
Ag.	Agriculture
bp	Base Pairs
cM	Centimorgan
CL	Chloroform: Isoamyl alcohol
Conc.	Concentration
°C	Degree Celsius
CD	Critical difference
Cv	Cultivar / Coefficient of variation
Cm	Centimetre (s)
CTAB	Cetyl trimethyl ammonium bromide
dNTPs	Deoxy Nucleotide Triphosphates
DNA	Deoxyribose Nucleic Acid
DAS	Days after sowing
e.g.	Exampli Gratia (for Example)
EDTA	Ethylene Diamine Tetra Acetic Acid
et al.	et alia (and others)
EtBr	Ethidium bromide
Fig.	Figure
gm	Gram
ha	Hectare
hr	Hour
HI	Harvest index
i.e.,	In reference to; that is
Kg	Kilogram
kg ha^{-1}	Kilogram per hectare
K	Potassium
LA	Leaf area
mb	Megabytes
Max.	Maximum
MgCl2	Magnesium chloride
mM	Milli Molar
Ml	Millilitre
mg	milligram
min	Minutes
M	Molar / Molarity / Meter
Mgm^{-3}	Mega gram per cubic meter
$mgkg^{-1}$	Milli gram per kilogram
MT	Metric tones
M.P.	Madhya Pradesh
N	Normality
ng	Nano gram

No.	Number
N	Nitrogen
PCI	Phenol Chloroform Isoamyl
PCR	Polymerase chain reaction
PIC	Polymorphic Information Content
qt	Quintal
$qtha^{-1}$	Quintal per hectare
rpm	Revolution per minute
RNase	Ribonuclease
RBD	Randomized Block Design
RNA	Ribonucleic acid
RT	Room Temperature
RVSKVV	Rajmata Vijayaraje Scindia Krishi Vishwa Vidhyalaya
S. E. m (d)	Standard error mean of difference
SE	Standard Error
Temp.	Temperature
$t\ ha^{-1}$	Tonnes per hectare
taq	*Thermus aquaticus*
TEB	Tris-Boric acid-EDTA
TE	Tris-EDTA
µl	Microliter
UPGMA	Unweighted pair group method with arithmetic mean
Viz.	Namely
v/v	Volume/ volume
Wt.	Weight
w/v	Weight/ volume

CHAPTER- I

INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a C_4, annual and diploid species (2n=2x=14) with a large genome (2450 Mbp). It belongs to poaceae family. The current officially accepted name of pearl millet is *Pennisetum glaucum* (L.) (Chase, 1921). It is believed to have originated from West Africa (Vavilov, 1950) from where it spread into India and other countries. It is a cereal grown in the arid tropical region and semi-arid areas of Asia and Africa. It's a primary food of most of the countries in these two regions. Pearl millet have common names including Bulrush millet, Babala, Ddukn (in the Sudan), and Bajra (in India). In India, (Kambu in Tamil nadu); ("Kambam" in Kerla); (Bajri or Bajro in Gujarat); (Bajri in Rajasthan and Maharashtra), (Sajje/kambu in Kannada); (Bajra in Hindi, Urdu and Punjabi and (sajjalu in Telugu) and ("ba: jra:" in Bengali). It is mainly used in poorest countries and by the poorest peoples. So, also known as the "Poor man's cereal crop" (Alam *et al*., 2010).

Pennisetum is largest genera in the tribe *Paniceae* with five sections and approximately 140 species that are widely distributed in the tropics and subtropics (Clayton, 1972). The taxonomical classification presently adopted for pearl millet is based on Clayton (1972). However, de Wet (1977) accepted *Pennisetum glaucum* for annual pearl millet species instead of *P. americanum* suggested by Brunken (1977). The present taxonomical classification accepted is presented in Table 1.1.

Pearl millet is one of the most important cultivated cereals in the world, ranking sixth after rice, wheat, maize, barley and sorghum in terms of area. It is grown on about 30 mha in more than 30 countries. The majority of this area is in Asia (>10 mha), Africa (about 18 mha) and Americas (>2 mha) (Gupta *et al.,* 2015). In India, pearl millet is the fourth most widely cultivated edibile crop after rice, wheat and maize. It occupies 7.48 million hectares with an average production of 9.21 million tonnes and the productivity of 1231 kgha^{-1} during 2017-18 (Agricultural Statistics at a glance, 2019; Govt. of India). The major pearl millet growing states are Rajasthan, Maharashtra, U.P., Gujarat and Haryana which produces 90% of total production of the country. Most of pearl millet in India is grown in rainy (kharif)

season (June/July-September/October). It is also cultivated during summer season (February– May) in parts of Gujarat, Rajasthan and Uttar Pradesh and during the post rainy (Rabi) season (November – February) at a small scale in Maharashtra and Gujarat.

In Madhya Pradesh, area is 4.14% of all India under pearl millet which is about 0.31 MH production (8.20%) of india. The major growing districts in M.P. are Morena, Bhind, Gwalior and some parts of Shivpuri and Jhabua.

Table 1.1 Taxonomical Classification of Pearl millet (*Pennisetum glaucum*)

Taxonomical Classification		
Kingdom	Plantae	Plants
Sub kingdom	Tracheobionta	Vascular plants
Super division	Spermatophyta	Seed plants
Division	Magnoliophyta	Flowering plants
Class	Liliopsida	Monocotlyedons
Subclass	Commelinidae	
Order	Cyperales	
Family	Poaceae	Grass family
Subfamily	Panicoideae	
Tribe	Paniceae	
Subtribe	Panicinae	
Section	Panicillaria	
Genus	*Pennisetum*	
Species	*glaucum*	

Nutritionally, pearl millet is a good source of energy and high levels of minerals (such as iron, zinc, calcium, magnesium, phosphorous), vitamins, lipids, crude fibres and high-quality protein 9-13% (Uppal *et al.,* 2015). Carbohydrates are the main component of *Pennisetum glaucum* grains varying from 71.82 to 81.02 per cent (Cheik *et al.,* 2006). Fat content of pearl millet varieties vary from 4.32 to 5.11 per cent (Abdalla *et al*., 2009). The total sugars in pearl millet ranges from 2.55 to 2.93 per cent, non-reducing sugars between 2.15 to 2.57 per cent and reducing sugars from 0.34 to 0.39 per cent (Rekha, 1997; Poonam, 2002). The amino acid and fatty acid composition are also better than other cereals. Pearl millet grain has considerably high level of phytic acid ranging from 603.33 to 678.33 mg100g^{-1} and polyphenols 502.78 to 658.30 mg100g^{-1} (Anju, 2005). Abdalla *et al*. (2009) analyzed pearl millet grain and reported 4.31-5.30 per cent crude fibre, 1.53-2.00 per cent ash, 450 -990 mg phosphorus, 10-80 mg calcium, 7-18.0 mg iron, 5.3-7.0 mg zinc, 1.0-1.8 mg copper and 1.8-2.3 mg manganese content. Pearl millet is equal or superior

to corn, sorghum and rice in protein and oil contents. It contains more iron and compares well in phosphorus and calcium contents to other cereals. Pearl millet has better mineral profile because of higher calcium but owing to certain inherent factors, the availability of these minerals to human system is low. Antinutrients (phytic acid and polyphenols), present in considerable amounts (Mahajan and Chauhan, 1987) limit protein and starch digestibility (Yoon *et al.,* 1983; Carnovale *et al.,*1988) hinder mineral bio-availability (Harland and Oberlease, 1987) and inhibit proteolytic (Knuckles *et al.,* 1985) and amylolytic enzymes (Sharma *et al.,* 1978). Due to the nutritional superiority and climate-resilient nature of pearl millet over other crops, it has given the tag of “nutricereals” by the Ministry of Agriculture and Farmers Welfare, Government of India.

Pearl millet is a highly cross-pollinated with protogynous nature crop leads to higher degree of out crossing rate ranging between 70 to 80% (Burton, 1974) with protogynous condition. Inflorescence is a cylindrical spike consisting of a central rachis on which the groups of spikelets are densely packed. Spikelets bear two types of florets, one being bisexual and the other is staminate. The unisexual staminate florets are sessile and born below the bisexual flowers. These have three anthers and lack the female organs. The bisexual floret consists of a single pistil with two feathery stigmas and three anthers enclosed between lemma and palea. The stigma remains receptive for one to two days. In hermaphrodite flowers, anthesis starts from apex of the panicle toward the base where as the anthesis of the staminate flowers starts 2-3 days after the anthesis of hermaphrodite flowers. Anthesis occurs throughout the day and night but maximum between 10 p.m. to mid night. Pollen grains remain viable for 5-7 hrs. Pearl millet is highly heterozygous and heterogenous being allogamous in nature. With the added advantage of commercially exploitable cytoplasmic-nuclear male-sterility systems, both open pollinated cultivars and hybrid cultivars (single cross, three-way cross, top cross and inter-population hybrids) are feasible.

Drought stress is the most important environmental constraint limiting factor for crop production worldwide (Ihsan *et al*., 2016). Drought is the most damaging abiotic stress affecting crop productivity, which is caused by insufficient rainfall and/or altered precipitation patterns (Toker *et al.,* 2007). The seriousness of drought stress depends on its timing, period and intensity (Serraj *et al.,* 2005). Moreover, it

has been causing global warming, which in turn is responsible for raising the earth's surface temperature and sea water level. As of today, climate–yield forecast is well captured in several important major crop species through simulations (Lobell *et al.,* 2011). Pearl millet is a drought tolerant cereal which can thrive well in the areas of low rainfall tracked by drought period and adapted to a wide range of ecological conditions (Maciel and Tabosa, 1982). Pearl millet is generally suitable for cultivation in arid and semi-arid regions of the country (Khairwal *et al.,* 2007).

Drought itself is a complex phenomenon and several parameters influencing it were found to be under genetic control. Drought is one of the most important abiotic stresses limiting global crop production. Plants display number of physiological and biochemical responses at cellular and whole organism level on account of water stress conditions. It induce reduction in leaf water potential, stomatal conductance, nitrate reduction and inhibits leaf enlargement while osmolytes such as total soluble sugars and proline are increased (Jafar *et al*., 2004; Adejare and Umebese, 2007). The root length, shoot length, total leaf area, fresh and dry weight, chlorophyll a, b, total chlorophyll and carotenoid were significantly reduced under water stress treatments. Water stress increased the proline (Manivannan *et al*., 2007). Reduction in RWC (%), Increase in proline which are closely linked with drought tolerance and have the potential to improve crop yield (Goyal *et al.,* 2001). Drought stress generally causes decrease in the total chlorophyll (Chl) content (Terzi and Kadioglu, 2006; Farooq *et al.,* 2009; Iwuala E., 2020) while the Chl a/b ratio usually increases (Ashraf *et al*., 2001). Drought elicits many different physiological responses in plants. The rate of photosynthesis is decreased mainly by stomatal closure, membrane damage and disturbed activity of many enzymes, especially those involved in ATP synthesis. Proline act as a cellular osmotic regulator between cytoplasm and vacuole for the protection of plants by detoxifying Reactive Oxygen Species (ROS), thus protecting membrane integrity and stabilizing antioxidant enzyme.

Drought impacts growth, yield, membrane integrity, chlorophyll content, osmotic adjustment, water relations, and photosynthetic activity (Benjamin and Nielsen, 2006; Praba *et al.*, 2009). Drought stress is influence from climatic, edaphic and agronomic factors. The susceptibility of plants to drought stress varies in dependence of level of stress, different accompanying stress factors, plant species and their developmental stages (Demirevska *et al.,* 2009). Acclimation of plants to

water deficit is the results of different events, which result in adaptive changes in plant growth and physio-biochemical processes, like changes in plant part, growth rate, tissue osmotic potential and antioxidant defenses (Duan *et al.,* 2007).

Drought limits the agricultural production by preventing the crop plants from expressing their full genetic potential (Mitra, 2001). Out of those, terminal drought is shown to contribute to the foremost severe yield losses because it affects spikelet establishment and reduces its fertility (Bernier *et al*., 2007).

Molecular markers offer precise means to measure genetic diversity and affinity among germplasm collections than the morphological and biochemical markers due to their environmental insensitivity and abundance in genome. Characterizing germplasm collection facilitates effective use of genetic diversity in germplasm. DNA markers are best suited for genetic diversity estimates. Their use as a means to assay diversity at the locus, chromosome and whole genome level is now well established (Faseela and Salikutty, 2007).

Molecular markers offer great scope for improving the efficiency of conventional plant breeding by selecting markers linked to the trait instead of the trait itself. In case of drought resistance, availability of markers tightly linked to the resistance genes will help in identifying plants carrying these genes simultaneously. The first application of molecular markers in pearl millet was creation of genetic linkage map using Restriction Fragment Length Polymorphism (RFLP) markers (Liu *et al.,* 1994). Thereafter, sequence independent PCR based markers like RAPD, ISSR, AFLP and microsatellite probes were used for genetic diversity studies in *Pennisetum glaucum* (vom Brocke *et al.,* 2003; Govindaraj *et al.,* 2009). Meanwhile, SSR markers were also developed (Allouis *et al*., 2001). Of the several DNA markers available, microsatellites or SSRs are considered to be ideal molecular genetic markers. Microsatellites consist of simple tandemly repeated di to penta-nucleotide sequence motifs. SSRs typically provide single-locus markers, which are often co-dominantly inherited and characterized by hyper variability, abundance and reproducibility and are employing for genetic diversity and gene mapping studies in pearl millet (Mariac *et al*., 2006; Shekhra *et al.,* 2007).

Microsatellite markers are adjudged as most effective and reliable DNA markers for such studies (Kapila *et al.,* 2007) due to their abundance in genome, multi allelism, genome specificity, even distribution, high polymorphism, easy

detection, high-throughput, highly reproducible and co-dominantly inherited behaviour. Therefore, use of SSR markers is a valuable tool for the diversity analysis among pearl millet genotypes. Understanding the genetic diversity of these traits and characterizing genotypes with advent of conventional means as well as by molecular markers will help in better understanding and can be useful to identify contrasting parental materials to enhance heterozygosity or to optimize the genetic heterogeneity in a hybrid population and hence enhance yield stability in variable and changing climates (Haussmann *et al.,* 2007; 2012).The exceptionally high levels of polymorphism detected by SSRs, mentioned as Simple Sequence Length Polymorphism (SSLP) are because of the variability within the number of tandem repeats at a specific locus. They are somatically stable and inherited in a co-dominant Mendelian manner. Microsatellite sequences are abundantly present in eukaryotic genomes and have, therefore, been used for genome analysis of many crop plants including rice (Gupta *et al.,* 1994). Many microsatellite loci have been isolated and sequences flanking these loci have been used in PCR to amplify different genotypes. Presence of a high level of allelic diversity at these loci makes them informative and valuable markers, with many applications in agriculture.

An understanding of morpho-physiological, biochemical and molecular techniques or mechanisms for drought tolerance is very important to develop the breeding material as per the future need. Further, it is important to do these studies in pearl millet which is a hardy crop having drought tolerance mechanism. It is expected that the information can help to identify or transfer such mechanism through biotechnological tools in future. Keeping this in sight the current investigation entitled, “Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene linked SSR markers.” was carried out with the following objectives of accomplishment:

Objectives:

The present study was undertaken with efforts to achieve following objectives:

1. Morpho-physiological characterization of pearl millet germplasm line(s) against drought.
2. Screening of pearl millet genotypes, tolerant against drought using different biochemical parameters.
3. Validation of gene based SSR markers against drought.
4. Screening of pearl millet genotypes using highly polymorphic gene based SSR marker(s) against drought.

Chapter-II

REVIEW OF LITERATURE

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the most important food crop in the world. Pearl millet comes thi[rd] after wheat and rice in India and majorly grown in Rajasthan, Tamil Nadu, Gujarat, Maharashtra, Uttar Pradesh, Haryana, Andhra Pradesh and Karnataka. Pearl millet grown for dual purpose as grain and fodder and it is cultivated widely from well irrigated areas to the most arid regions of the world in Asian and African countries.

Pearl millet is more tolerant to drought and low soil fertility than sorghum and it is an important food crop for the drier parts of Africa and India. It exists in a number of distinct varieties or races exhibiting much variation and would appear to be amenable to considerable improvement. Water stress is one of the variables affecting plant growth and development. Conversely, plants are exposed to periods of mild or severe drought during the crop growth.

The study entitled “Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene linked SSR markers” was conducted during 2019-20. The literature cited on various aspects of the present investigation has been summarizied as under:

Factor affecting pearl millet production

There are many factors are affecting the pearl millet production including varioous biotic and abiotic stresses. Pearl millet often confronts abiotic stresses such as drought, salinity and high/low temperature *etc*. during their life cycle. They have a series of mechanisms at molecular, cellular and physiological level to respond and adapt to these stresses, thus enabling them to survive (Yamaguchi-Shinozaki and Shinozaki, 2006). It has been observed that during response and adaptation to these stresses, many stress-related genes are induced and a variety of stress-related functional proteins are accumulated in plants (Kotak *et al.*, 2007).

Abiotic stresses such as extreme temperatures, low water availability, flooding and high salt levels are the major limiting factors for plant growth and productivity. In arid and semi-arid regions, drought, as an abiotic stress, is one of the major factors limiting plant growth at various stages of its life. The plant growth

and development are restricted under water stress conditions so that under prolonged drought stress conditions, many plants will dehydrate and die. Water stress in plants reduces the water potential and turgor and increases concentration of solutes in cytosol. Consequently, cell enlargement, gas exchange, transpiration, plant nutrients uptake and transport are decreased (Lisar *et al*., 2012).

2.1 Drought and Drought Tolerance

Linsley et al. (1958) defined drought as a sustained period of time without significant rainfall. The term 'drought' describes a condition in which available soil moisture is reduced to the point where the plant cannot absorb it rapidly enough to compensate for transpiration.

Osmanzai *et al.* (1987) defined drought tolerance in plants as the ability to obtain and retain water, as well as continue its metabolic functions during a period of low water potential in its tissue.

Arnon (1972) defined drought resistance as the ability to survive drought conditions without injury.

Drought can be considered as the single most important factor adversely affecting the sustainable agricultural productivity in the developing world. The universal nature of drought throughout the rainfed cropping system of the world has increased the urgency for crop breeders to consider drought resistance as a priority in most crop breeding programmes. The major challenge in dryland agriculture is to establish ways and means by which reduction in agricultural production can be minimized during a period of water deficit.

May and Milthrope (1962) identified three primary types of drought resistance:

(a) Drought escape: The ability of a plant to complete its life cycle before a serious plant water deficit develops. Plants can achieve this by rapidd phenological development and developmental plasticity.

(b) Drought tolerance with high tissue water potential: The ability of a plant to endure periods of rainfall deficit, while maintaining high tissue water potential.

(c) Drought tolerance with low tissue water potential: The ability of a crop plant to endure rainfalls deficits at low tissue water potential achieved by maintenance of turgor and desiccation tolerance.

2.3 MECHANISMS OF DROUGHT TOLERANCE

In agriculture, the ability of a crop plant to produce its economic product with limited availability of water can be referred to as drought resistance, whereas in evolutionary context, it is the ability of a plant to survive and eventually reproduce under limited moisture.

Jones *et al.* (1985) listed several mechanisms that enable plants to resist drought which are listed below:

Mechanisms of drought tolerance and drought escape

(a) Rapid phenological development

(b) Developmental plasticity

Drought tolerance with high tissue water potential

(a) Maintenance of water uptake

(i) Increased rooting

(ii) Increased hydraulic conductance

(b) Reduction of water loss

(i) Reduction in epidermal conductance

(ii) Reduction in absorbed radiation

(iii) Reduction in evaporative surface

Drought tolerance with low tissue water potential

(a) Maintenance of turgor

(i) Solute accumulation

(ii) Increase in elasticity

(b) Desiccation tolerance

(I) Protoplasmic resistance

The traits of drought tolerance effects relevant for yield are stomatal conductance, photosynthetic capacity, timing of phenological phases, starch availability during ovary/embryo development, stem reserve mobilization, stay green, single plant leaf area, rooting depth and density, cuticular resistance

and surface roughness, osmotic adjustment, membrane composition, antioxidative defense, and accumulation of stress-related proteins (Cattivelli *et al*., 2008). More open stomata allow greater conductance and lower leaf temperature, a proxy for higher potential photosynthetic and transpiration rates. However, genotypes with higher stomatal conductance are relatively sensitive to drought stress as they lose water rapidly than those close their stomata and reduce their photosynthetic CO_2 assimilation (Serba and Yadav, 2016).

At cellular level, the abiotic stresses, especially drought, cause a decrease in pressure potential. Cell solutes concentrate due to water loss and they are actively accumulated to keep the cytoplasm osmotically balanced. Osmotic adjustment is therefore a major component of abiotic stress tolerance in plants and contributes to pressure potential. The common solutes employed in osmotic adjustment include various amines, amino acids and/or sugar alcohols. Enhanced accumulation of these osmolytes facilitates the retention of water in the cytoplasm and protection of membranes, protein complexes and cellular structure. Furthermore, plant cells contain antioxidant enzyme system, such as peroxidases and superoxide dismutases, which scavenge the reactive oxygen intermediates and provide protection against oxidative stress (Munns and Tester, 2008).

At molecular level, an assortment of genes with diverse functions are induced or repressed by these stresses. The identification and characterization of genes involved in osmotic and ionic stress tolerance have opened the possibility of engineering crop plants with increased drought/salt tolerance. The expression of genes involved in the biosynthesis of compatible solutes such as proline (Kishor *et al.,* 1995) could increase the hyper-osmotic tolerance of the plant cell. Under more prolonged water stress, dehydration of plant tissue can result in an increase in oxidative stress which increases the activity of antioxidants, production of stress proteins and accumulation of compatible solutes (Zhu, 2002).

The deficiency of water leads to severe decline in yield traits of crop plants probably by disrupting leaf gas exchange properties which not only limit the size of the source and sink tissues but the phloem loading, assimilate translocation and dry matter portioning (Farooq *et al.,* 2009). Much research on drought tolerance has focused on the characterization of plants during water deficit (Blum, 2005). In

pearl millet, stomata play an important role in minimizing crop water use in pre-anthesis water deficit (Winkel *et al*., 2001). However, controlling leaf water losses when water is non-limiting for plant development may also be a suitable adaptation strategy. It was recently shown that pearl millet genotypes carrying a terminal drought tolerance quantitative trait locus (QTL) have a lower rate of water loss per unit leaf area under well-watered (WW) conditions (Kholova *et al.,* 2008; 2010).

2.2.1 Global drought Stress, food calamity and drought situations

The world population is linearly increasing whereas the land is shrinking due to its utilization for residential and industrial purposes. The climate change scenarios again creating hurdle in our on-going agricultural production. Therefore, no option remains open except to produce more food and other commodities under conditions of diminished per capita arable land and available water resources (Swaminathan, 2006).

Drought is a foremost stress which decreases the production of crops worldwide (Yang *et al*., 2009). The problem is particularly serious in arid and semi-arid regions (Ashraf *et al.,* 1995). In these regions water potential in rhizosphere becomes more negative during water stress period which reduces water availability for plant growth and development (Ashraf, 1994).

The frequency of drought tends to increase with variability in rainfall. In India highest drought (once in 3 years) occurs in arid areas like eastern Rajasthan and the least (once in six years) in Madhya Pradesh. For semi-arid India as a whole, the chance of occurrence of drought is once in four years. Though arid areas tend to be more prone to drought, they are of less importance for food-crop production. Tropical semi-arid regions are characterised by high- evapotranspiration demand and low water holding capacity of soils making them more drought susceptible than semi-arid areas of temperate regions (Swindale and Bidinger, 1981).

The type of drought situations, in which a crop is grown, greatly determines the type of mechanisms that should be developed and decide the appropriate breeding methodology that should be used. In general, three types of environments can be associated with drought stress in plants; however, numerous combinations of these environments occur.

2.2.2 Effect of drought stress on yield and its attributing traits in pearl millet

It has been well established that drought at the initial stage is an important yield limiting factor. It affects both elongation and expansion phases of a plant (Anjum *et al.,* 2003; Bhatt and Srinivasa Rao, 2005; Kusaka *et al*., 2005; Shao *et al.,* 2008). Drought is one of major abiotic stresses constraining crop productivity worldwide, it reduces plant productivity by inhibiting growth (Singh *et al.*, 2014) and slows growth, induces stomatal closure, and therefore reduces photosynthesis (Nemeth *et al*., 2002). The relative amount of chlorophyll is directly connected to the photosynthetic capacity of the major plants (Fotovat *et al.,* 2007). Besides chlorophyll content, drought stress plays a major role in affecting the enzymes involved in the Calvin cycle (Monakhova and Chernyadev, 2002). It is reported that the production of plants also affected by showing to reactive oxygen species (Horling *et al*., 2003). Polyethylene glycol (PEG-6000) generates osmotic stress which reduces photosynthetic rate, that later effects chlorophyll-a and chlorophyll-b contents, any stress to the plant effects mechanism of photosynthesis at cellular level which includes pigments, photosystems, the electron transport system and CO_2 reduction pathways and reduce photosynthesis. PEG is mainly used for the determination of the drought stress related information's from the plants (Turkan *et al.,* 2005; Landjeva *et al*., 2008).

Drought is the major constraint to pearl millet as it is grown in the drier semi-arid and arid regions. However, adaptive evolution and natural selection made pearl millet relatively the most drought and heat tolerant among other cereals. Traditional landraces from drier regions are good sources for breeding drought tolerance (Kusaka *et al.,* 2005; Yadav, 2010). Though, the yield potential of many of the landraces is lower as compared to improved cultivars, genes for drought tolerance in the landraces can be utilized to combine with high yield potential (Yadav *et al.,* 2011). Never the less, drought tolerance is a complex polygenic trait and influenced significantly by environments. The various morphological and physiological responses to drought are controlled by 100s of genes (Hu and Xiong, 2014). Plant stress responses involve adjustment of metabolism for physiological and morphological adaptation occurring as a result of dynamic and complex cross-talk between different regulatory networks (Saito and Matsuda, 2010). Several technical limitations prohibit the study of plant responses to environmental stress that appear to be complex and pose difficulty on the classical plant-

breeding programme. Apparently, the elucidation of the components of the complex mechanisms underlying drought tolerance in crops will accelerate breeding for drought tolerant cultivars.

2.2.4 Genetic diversity for morpho-physiological characteristics in pearl millet under drought stress

Addisie and Yemane (2011) reported that the pearl millet genotype Dadda showed the maximum relative water content (45.70±1.13%) than Shella with the RWC of (32.00±1.06%) under severe water stress.

Burman *et al*. (2011) found that among various pearl millet genotypes, Pusa-266 and CZP-9802 showed lower reduction in RWC, chlorophyll content and yield under water stress as compared to genotypes ICTP-8203, Barmer population and Western Rajasthan population.

Govindaraj *et al*. (2011) conducted a study and evaluated 61 local pearl millet accessions. Observations were recorded for eight morphological traits. The phenotypic co-efficient of variation (PCV) being greater than genotypic co-efficient of variation (GCV) for all the characters studied, showed the environmental influence factors on these characters. The magnitudes of phenotypic and genotypic variances were low for the 1000-grain weight. All the traits under study showed high heritability except seed weight which had moderate heritability. They concluded that the selection based on these characters scan bring about desired improvement in yield as well as nutritional quality of pearl millet cultivars.

Amiribehzadi *et al*. (2012) analysed cytoplasmic male sterile source of pearl millet and found that the analysis of the variance for 19 parents (14 diverse cytoplasmic male sterile lines and 5 testers of A_1) and 26 F_1 crosses for the 15 characters associated with grain yield and physiological aspects showed significant differences and revealed the presence of higher amount of variability for the characters *viz.,* plant height, days to 50% flowering, days to maturity, numbers of productive tillers, grain yield per plant, spike girth, spike length and1000-grain weight.

Choudhary *et al.* (2012) evaluated fifty genotypes of pearl millet (including 3 checks *viz.* Raj-171, ICTP 3616 and ICTP8203. The analysis of variance indicated presence of significant variability among genotypes for all the characters

studied. The association analysis revealed that grain yield per plot had significant positive correlation with plant height, productive tillers per plant, ear girth, dry fodder yield per plant, test weight, harvest index and grain yield per plant and negative association with days to heading. They suggested that in breeding programme, major emphasis should be given to plant height, productive tillers per plant, ear girth, dry fodder yield per plant, test weight and harvest index (%) as they had positive correlation coefficients with grain yield with high direct effect and they also had high genetic variability.

Irfan *et al.* (2012) evaluated and characterize 27 pearl millet accessions for various morphological and fodder yield parameters. The germplasm displayed considerable variability for days to 50 % flowering, leaf area, flag leaf area, plant height and green fodder yield. Different genotypes displayed potential for selection of the desired traits.Sel.-2 (8802) was the earliest accession in terms of 50% flowering while maximum leaf area was recorded for Sel.-1(No.8802) and Sel.-2(No.8802). Sel.-4(No.8781) had the maximum plant height while Sel-3(No.8781) produced the highest green fodder yield. Cluster analysis for quantitative traits depicted five clusters at a dissimilarity level of 4.8. The first cluster consisted of four genotypes while the second cluster contained nine genotypes. Ten genotypes comprised the third cluster while the fourth cluster constituted only four genotypes. The genetic potential of Sel.-2(8802), Sel.-1(No.8802), Sel.-2(No.8802), Sel.-3(No.8781) and Sel.-4(No.8781) can be exploited in future pearl millet breeding programmes.

Shah *et al*. (2012) evaluated and characterized 27 pearl millet accessions for various morphological and fodder yield parameters. The germplasm displayed considerable variability for days to 50% flowering, leaf area, flag leaf area, plant height and green fodder yield. The genotypes, however, didn’t exhibit any variation for ligule presence, auricle absence and leaf midrib colour.

Kumari *et al.* (2013) carried out estimation of genetic variability and association among 30 genotypes of pearl millet for 11 different characters. Analysis of variance indicated presence of considerable amount of variability for all the 11 characters. The result from character association indicated that grain yield per plant had significant and positive correlation with ear head length (cm), numbers of effective tillers per plant and biological yield per plant (g) at phenotypic level. Numbers of effective tillers per plant and biological yield per plant had higher

estimate of GCV and PCV. It also had higher heritability associated with high genetic advances per cent of mean. They suggested that due emphasis should be given to these traits while making a direct selection through them. Harvest index and seed yield/plant had moderate estimates of GCV, PCV, heritability and genetic advance, thus concluded that selection for these characters would also be effective.

Subi and Idris (2013) examined 15 pearl millet genotypes [*Pennisetum glaucum* (L.) R. Br.] to assess the magnitude of genetic variability for some growth as well as grain yield characters. For days to 50% flowering and days to maturity, for plant height, leaf area, numbers of grains/plants, 1000-grain weight and grain yield (tha^{-1}) and for panicle length, highly significant differences ($P \leq 0.01$) were observed. In general, phenotypic coefficients of variation (PCV) estimates were higher than genotypic coefficients of variation (GCV) estimate for all the studied characters in all genotypes displaying the influence of environment. The combined results for heritability showed that the higher estimates of heritability and genetic advance were scored for days to 50% flowering and days to maturity signifying that these characters were under the control of additive genetic effects and can be used for any further improvement.

Vinodhana *et al.* (2013) tried to conduct an experiment using 50 genotypes of pearl millet and the observations were recorded for 10 morpho-economic characters. Higher magnitude of variation was found in the experimental material. The PCV and GCV estimates were high for seed yield potential tracked by green fodder yield potential, panicle length, spikelet density, thousand-seed weight and total numbers of tillers which suggested that there is enough scope for selection based on these characters. The higher heritability combined with high genetic advance was observed for plant height, total numbers of tillers, panicle thickness, panicle length,1000-seed weight, spikelet density, green fodder yield and seed yield which indicated that these characters were controlled by additive gene effects and phenotypic selection was likely to be effective for these characters. A study of the results obtained in correlation analysis showed that panicle length; panicle thickness, spikelet density, thousand seed weight and green fodder yield potential had positive correlation with seed yield potential. Hence, selection of these components will lead to development of dual-purpose pearl millet varieties/hybrids.

Vijaylaxmi (2013) found that water stress caused a sharp decline in RWC, Ψl and Ψs leading to lower plant water status in pearl millet genotypes. Under water deficit conditions, the hybrid maintained higher RWC (86.3%) suggesting its relatively higher ability to avoid tissue dehydration. The decrease in Ψl was concomitant to the decrease in Ψs. The decrease in Ψs (80%) was more compared to the Ψl (41%) indicating the ability of the leaves to maintain turgor through osmotic adjustment.

Singh *et al.* (2014) were performed genetic variability and correlation for yield and its contributing traits in different 55 pearl millet genotypes. Among all the characters, significant differences were observed. Genetic variability was also observed for biological yield per plant, plant height, dry fodder yield per plant and days to maturity. They observed that grain yield per plant showed significant positive genotypic as well as phenotypic correlation with productive tillers per plant, plant height, panicle length, panicle girth, biological yield per plant, dry fodder yield per plant, harvest index and test weight. Grain yield per plant had highest positive direct effect on harvest index chased by biological yield per plant.

Bind *et al.* (2015) reported wide spectrum of variation for different characters. Out of 11 characters they had studied, green fodder yield per plant had wide difference between GCV and PCV. Grain yield per plant, panicle length, dry matter yield per plant had less difference between GCV and PCV. They observed higher heritability coupled with high genetic advance as per cent of mean for these characters and thus made a conclusion that they might be under the control of additive gene effects.

Verma *et al.* (2015) studied genetic divergence in 97 pearl millet germplasm lines and grouped them into six clusters based on their relative magnitude of the D^2values. Plant height, ear diameter and grain yield were found the best discriminatory characters for better selection of diverse genotypes.

Djanaguiraman *et al*. (2018) studied pearl millet response to high temperature stress and reported that genetic variability showed temperature stress impacts at different growth stages understand genetic variations for numbers of seeds, individual seed weight and seed yield. Two periods (10 -12 days and 2 -0 days

before anthesis) were identified as most sensitive causing maximum decreases in pollen germination percentage and also decreased number of seeds and seed yield.

Kaushik *et al.* (2018) reported that days to 50% flowering, spike length, numbers of productive tillers per plant had high significant direct contribution towards grain yield per plant for which indirect selection can be made in future breeding programme to enhance grain yield.

Kumari *et al.* (2018) conducted an experiment with 150 R-lines of pearl millet and found that direct and indirect effects of component characters like biological yield per plant, leaves per plant, flag leaf length, flag leaf width, panicle length, panicle diameter, plant height and tillers per plant were found positive on seed yield. Therefore, selection based on these component characters would results in improvement of seed yield.

Sharma *et al.* (2018) evaluated 34 germplasm lines of pearl millet for genetic variability as per cent of mean along with the association for grain yield with its component traits. The ANOVA showed presence of highly significant differences among the genotypes for all the characters studied, indicating the presence of sufficient variability in the experimental material. The PCV was slightly higher than GCV indicating little influence of environment on the expression of characters.

Thomas *et al.* (2018) studied 22 pearl millet genotypes during kharif, 2017. The genotypes were analysed for genetic variability and correlation. Analysis of variance revealed highly significant differences among the genotypes for all the 12 characters viz., plant height, number of tillers per plant, number of leaves per tiller, leaf length, leaf breadth, leaf-stem ratio, dry matter yield per plant, green fodder yield per plant, crude protein, crude fibre, fat content and ash content, indicating the presence of sufficient variability in the experimental materials of pearl millet genotypes.

Kumawat *et al.* (2019) conducted an experiment for study variability parameters and character association in 50 pearl millet single cross hybrids. The material was evaluated in randomized block design with three replications. The analysis of variance indicated the presence of significant genetic variability among the single crosses for all the characters studied.

Priyanka *et al.* (2019) studied genetic variability for twenty-two physiological, nutritional and yield related traits in 42 genotypes of pearl millet. The analysis of variance revealed significant variability for all the traits. The high genetic advance as percent of mean of GCV and PCV estimates for grain yield, green fodder yield, iron content, leaf area duration, green fodder yield and zinc content indicating that these traits are governed by high additive gene effects and selection for these traits may be effective for further breeding programme.

Yadav *et al*. (2020) conducted a study comprised of three deficit irrigation regimes *viz*., 100, 80 and 60% of crop evapotranspiration (ETc) (I_1, I_2 and I_3). The trend of plant height, and physiological traits was similar under different treatments at both stages, but differed significantly only at reproductive stage. Water deficit caused significant reduction in pearl millet (5.1%) grain yields. The plant height, RWC and osmotic stress significantly reduced by water stress in pearl millet.

2.2.4 Genetic diversity for biochemical characteristics in pearl millet under drought stress

Buerkert *et al*. (2001) carried out variation in grain quality of pearl millet from Sahelian West Africa. They reported results from 22 land race populations, 22 improved varieties, six inbred x variety hybrids (IVHs, fertile inbred x open-pollinated varieties) and four topcross hybrids (TCHs, male-sterile line x open-pollinated varieties), whose grains were analyzed for protein concentration. At similar yield levels, landraces showed a 2.9 and 3.5% higher protein concentration as compared with improved varieties and hybrids without a detrimental effect on protein quality as determined by the relative amount of lysine and threonine. However, *in vitro* digestibility and ME were (79.8% and 12.2 MJ kg^{-1}, respectively) larger for both groups of hybrids.

Ouattara *et al.* (2006) studied nutritional and technological qualities of fourteen cultivars of *Pennisetum glaucum.* Proteins contents ranged from 8.66% to 17.11% for all the cultivars. Water-soluble proteins ranged from 1.81% to 3.18%. The carbohydrates were found to be the major components of these cultivars, values ranged between 71.82% to 81.02% and samples IKMP1, IKMP2, TK, B1, B2 contained more carbohydrates than all others cultivars. The energy values of cultivars flours ranged from 426.21 $Kcal100g^{-1}$ to 446.53 $Kcal100g^{-1}$.

Ashraf and Foolad (2007) reported the accumulation of free proline under stress conditions was correlated with stress tolerance in many plant species, and concentrations are generally higher in stress-tolerant as opposed to stress-sensitive plants.

Mohammadkhani and Heidari (2008) analysed drought-induced accumulation of soluble sugars and proline in two maize varieties and found a higher amount of soluble sugars and a lower amount of starch under stress coonditions. Soluble sugars concen tration increased (from 1.18 to 131.90 times) in roots and shoots of both varieties when the studied varieties were subjected to drought stress, but starch content were significantly decreased (from 16 to 84%) in both varieties. This suggests that sugars play an important role in Osmotic Adjustment (OA) in maize. The free proline level also increased (from 1.56 to 3.13 times) in response to drought stress and the increase in 704 var. was higher than 301 var. It seems to proline may play a role in minimizing the damage caused by dehydration. Increase of proline content in shoots was higher than roots, but increase of soluble sugar content and decrease of starch content in roots was higher than shoots (Ref).

Farooq *et al.* (2009) stated that photosynthetic pigments are important to plants mainly for harvesting light and production of reducing power. Both the chlorophyll a and b are prone to dehydration.

Gur *et al*. (2010) reported that there was a decline in the level of proline content in the leaves of plants subjected to 38^0C and 45^0C temperature as compared to the control plants.

Goyal et al. (2009) suggested that high temperature stress increased the proline accumulation in shoots of wheat genotypes. Highest accumulation of proline was achieved in shoots of C-306 and PBW-343. In root tissue maximum decline in proline concentration was observed in C-273 and pursueed by PBW-534.

Sarang *et al.* (2014) conducted sugars estimation on five resistant genotypes *viz.,* J-2480, J-2511, J-2290, J-2405 and J-2340 and two susceptible genotypes namely :7042S and J-2372 to downy mildew from Gujarat had the range of reducing sugars was recorded in range of.14-1.93% and non-reducing sugars 0.35-1.04%.

Vujcic and Brkanac (2014) while working on *Fibigia triquetra,* a plant that adapted to a hot and dry habitat found that dry weight and proline content in shoots increased in response to osmotic stress.

Mamta and Kawatra (2015) reported the total sugars were found to be 2.5% in HHB -67 improved, and 2.41% in HHB –223. Reducing sugars content was 0.48% in HHB-223 and 0.59% in HHB-67 improved pearl millet varieties. The non-reducing sugars content in HHB-223 and HHB-67 improved was 1.82 and 1.93%, respectively and significant between the two varieties.

Sidhu *et al.* (2016) reported crude protein in the cultivar PCB-164 had 10.74 to 10.87% milling speed, 10.66 to 11.15% storage time and 10.78 to 10.81% packaging material.

Obadina *et al.* (2017) reported that the crude protein and carbohydrate content levels of pearl millet in flour samples of Ex-Borno variety increased upon malting from 7.52% (control) to 9.19% and 74.14% (control) to 71.92% respectively n 96 h.

Bansal (2018) studied two bio-fortified varieties of pearl millet HHB-299 and Dhanshakti and reported that the crude protein content was 10.61 $g100g^{-1}$ and 11.76$g100g^{-1}$ in HHB-299 and Dhanshakti, respectively.

Iwuala *et al.* (2020) were taken and assessed two drought genotypes of pearl millet for photosynthetic performance of PS-II electron transport rate (ETR) and maximum yield under a simulated drought stress condition. Drought stress caused a gradual down regulation in the chlorophyll content in both genotypes. The elevated content of β-carotene was recorded on the 12^{th} day of the experiment. The photo inactivation of PS-II was also assessed in both the genotypes. On the basis of an overall comparison, it is evident that the genotype IP14599 has better adapted to drought than the genotype IP14222.

Yadav *et al.* (2020) applied three deficit irrigation regimes *viz*., 100, 80 and 60% of crop evapotranspiration (ETc) (I_1, I_2 and I_3). The trend of biochemical traits was similar under different treatments at both stages, but differed significantly only at reproductive stage. Water deficit caused significant reduction in pearl millet (5.1%) grain yields. The proline significantly reduced by water stress.

2.2.4 Genetic diversity for molecular characteristics using ssr markers

Shekara *et al.* (2007) studied genetic diversity of elite pearl millet inbred lines using 20 RAPD and 21 SSR markers. They found that six RAPD primers *viz*., OPD12, OPA16, OPB6, OPA19, OPB5 and OPB1 and three SSR markers *namely* Xpsmp2208, Xpsmp2223 and Xpsmp2220 were found to be highly discriminative. The PIC value ranged from 0.28 to 0.48 for RAPD and from 0.24 to 0.60 for the SSR markers. Cluster analysis and principal component analysis of combined data set of RAPDs and SSR markers indicated moderate genetic divergence in elite pearl millet germplasm lines.

Kapila *et al.* (2008) investigated genetic variability among 70 millet cultivars by using SSR molecular markers. At molecular level, 34 SSR primers were used to identify 70 cultivars of pearl millet, indicating the genetic diversity among millet genotypes with 65% polymorphism.

Yadav *et al.* (2008) studied genetic diversity among 70 maintainers and two pollinators of sub-Saharan and Indian origin for simple sequence repeat (SSR) loci using 34 primer pairs. A total of 213 alleles were detected with an average of 6.26 alleles per locus. Polymorphic information content (PIC) ranged from 0.05 to 0.96 with a mean of 0.58 for the SSR loci. Mean PIC across the linkage groups and numbers of alleles in dinucleotide motifs varied significantly.

Govindaraj *et al.* (2009) studied genetic diversity of pearl millet using RAPD analysis in order to assess the degree of polymorphisms within and among genotypes and to investigate the genetic relationship among 20 pearl millet genotypes. Twenty genotypes were evaluated using 30 different 10-mer primers of arbitrary sequence. Most of the primers did not reveal any polymorphism; however, 12 primers revealed scorable polymorphism between genotypes of pearl millet and these can be evaluated in genetic mapping.

Chakraborty *et al.* (2011) compared the level of information provided by RAPD and SSR marker systems for estimating genetic similarities in pearl millet and wheat genomes. For this investigation, sixteen pearl millet and fourteen wheat genotypes were used. The RAPD analysis of pearl millet and wheat genotypes produced 894 and 564 scorable bands respectively. RAPD primer OPN-7 revealed the highest PIC values of 0.96 and 0.97 for pearl millet and wheat respectively. As in

the case of SSR primers CTM25, CTM27 and Xpsmp 2273 gave the highest PIC value of 0.84 for pearl millet and 0.88 for wheat genotypes by Xgwm153. In both the cases *i. e.*, RAPD and SSR, the respective dendrograms revealed that, the local varieties tend to group together, showing the efficiency of these marker systems in diversity analysis. However, during the investigation, it was observed that RAPD markers were more efficient than SSR for revealing the latent diversity underlying these crop genotypes.

Somasundaram and Kalaiselvam (2011) proved that variety of different genetic markers have been proposed to assess genetic variability as a complementary strategy to more traditional approaches used for genetic resources management. Molecular tools provide valuable data on diversity through their ability to detect variation at the DNA level.

Sehgal *et al.* (2012) exploited available pearl millet Expressed Sequence Tag (EST) sequences to generate mapped resource of seventy-five new gene-based markers for pearl millet and demonstrated its use in identifying candidate genes underlying a major (Drought Tolerance-QTL) DT-QTL in this species.

Rajaram *et al.* (2013) were used Expressed Sequence Tag-SSR markers (EST-SSR markers) (99), along with previously mapped EST-SSR (17), genomic SSR (53) and Sequence Tagged Site (STS) (2) markers to construct linkage maps of four F_7 Recombinant Inbred Populations (RIP) based on crosses ICMB 841-P3 × 863B-P2 (RIP A), H 77/833-2 × PRLT 2/89-33 (RIP B), 81B-P6 × ICMP 451-P8 (RIP C) and PT 732B-P2 × P1449-2-P1 (RIP D). Mapped loci numbers were greatest for RIP A (104), tracked by RIP B (78), RIP C (64) and RIP D (59). Total map lengths (Haldane) were 615 cM, 690 cM, 428 cM and 276 cM, between the 4 RIPs, respectively. A total of 176 loci detected by 171 primer pairs were mapped among the four crosses. A consensus map of 174 loci (899 cM) detected by 169 primer pairs was constructed using Merge Map to integrate the individual linkage maps. Locus order in the consensus map was well conserved for nearly all linkage groups. Eighty-nine EST-SSR marker loci from this consensus map had significant Basic Local Alignment Search Tool (BLAST) hits (top hits with e-value ≤ 1E-10) on the genome sequences of rice, foxtail millet, sorghum, maize and Brachy podium with 35, 88, 58, 48 and 38 loci, respectively. The consensus map developed in the present study contains the largest set of mapped SSRs reported to date for pearl

millet, and represents a major consolidation of existing pearl millet genetic mapping information. This study increased numbers of mapped pearl millet SSR markers by >50%, filling important gaps in previously published SSR-based linkage maps for this species and will greatly facilitate SSR-based QTL mapping and applied marker-assisted selection programmes.

Sathya *et al.* (2013) assessed the extent of genetic diversity with relation to morpho-physiological traits of 47 pearl millet accessions (44 inbred lines and 3 checks). The hierarchical cluster analysis done using NTSYS software resulted in the formation of eight clusters indicating the presence of high level of genetic diversity among all the genotypes. Among eight clusters, cluster IV had highest number of inbreds (18) trailed by cluster I and cluster VI (9). Similarity co-efficient value ranges from 1.00 to 9.96. The results indicated that the inbreds TNBI 9 and TNBI 21 and TNBI 15 and TNBI 22 were closely related. The dissimilarity was highest between the inbreds TNBI 30 and TNBI 39 (9.96) pursueed by TNBI 44 and TNBI 9 (9.95) and TNBI 29 and TNBI 1 (9.93) for yield and yield component traits which showed that they are highly divergent among the forty-seven genotypes. It was found that the inbred TNBI 43 in cluster V recorded early flowering and highest mean value for ear head length, number of tillers, ear head breadth, root length, root-shoot ratio and grain yield among the 47 genotypes which can be used as a parent in hybridization programme to enhance yield potential of the pearl millet genotypes under moisture stress condition.

Singh *et al.* (2013) reported genetic diversity among 20 commercially released pearl millet cultivars comprising of hybrids and open pollinated varieties. Twenty-one polymorphic SSR primer pairs, out of 60 screened were used to study the diversity and amplified 64 alleles. UPGMA cluster analysis differentiated all the cultivars. Interestingly, all the cultivars developed at IARI, New Delhi, were present in a subgroup within group I. Similarly, majority of hybrids developed at HAU, Hisar were grouped in another subgroup within the group1. BAIF Bajra 1, an exclusive forage purpose variety, was genetically most diverged. Besides, a set of five polymorphic primers were found to differentiate all the cultivars. The results demonstrated presence of moderate level of genetic diversity among the pearl millet cultivars.

Sammour (2014) concluded that markers are based on the DNA sequences showing increasing variations are very useful and can be employed in crop for

phylogenetic studies, construction of genetic maps and marker assisted selection. Different biochemical and DNA based markers are employed to study the hybrid confirmation in pearl millet as well as other crops. Cultivar identification is useful for describing new cultivars, testing genotype purity and speeding up distinctness uniformity- stability (DUS) test for candidate cultivar.

Gupta *et al.* (2015) investigated genetic diversity pattern of 379 hybrid parents (current 166 parents and 213 previously developed hybrid parents) using a set of highly polymorphic 28 SSRs and detected 12.7 alleles per locus. An average of 8.5 and 8.7 SSR alleles per locus were found in previously developed and current parents, respectively.

Bashir *et al.* (2015) assessed genetic diversity for a collection of 214 pearl millet accessions from different geographical regions of Sudan and 11 accessions from West Africa using 30 SSRs. They also evaluated the accessions for 15 agro-morphological traits. A large number of alleles were detected (400). The average polymorphic information content (PIC), gene diversity and observed heterozygosity of the 30 SSRs were 0.77, 0.82 and 0.72 respectively. Low correlation was observed between the agro-morphological matrix and the genetic matrix (r= 0.20).

Animassaun *et al.* (2016) assessed fixe genetic diversity and phylogenetic relationship among Nigerian and Indian accession of pearl millet and Napier grass using microsatellite markers. They extracted genomic DNA from each accession and carried out Polymerase Chain Reaction using Inter-Simple Sequence Repeat markers. Data obtained were analysed for genetic diversity using MEGA 4.0 software. A total of 48 loci consisting of 410 bands were generated with 56.25% polymorphism. Principal Coordinates analysis revealed three principal axes contributed significantly (70.20%) to the observed variations.

Benedict *et al.* (2016) worked to understand the genetic diversity of the crop in Namibia by simple sequence repeats (SSRs) and morphological analysis. A total of 1441 genotypes were collected from the National Gene Bank representing all the Namibian landraces. A sample of 96 genotypes was further analysed by SSR using Shannon-Wiener diversity index and revealed a value of 0.45 indicating low genetic diversity.

Ramakrishnan *et al.* (2016) evaluated the genetic variation and population structure in Indian and non-Indian genotypes of finger millet using 87 genomic SSR primers. The 128 finger millet genotypes were collected and genomic DNA was isolated and further PCR was performed, these analyses confirmed that all the genotypes were genetically diverse and had been grouped based on their geographic regions.

Sudeshna *et al.* (2016) used sixteen SSR primers to check genetic diversity in 16 pearl millet genotypes. They recorded PIC value in range of 0.12 to 0.84. Genotypes CTM-25, CTM-27 and Xpsmp 2273 showed highest PIC value (0.84).

Adeoti *et al*. (2017) screened 114 accessions from different agro -ecological zones in Benin by using 14 polymorphic SSR markers to assess the level of genetic diversity. A total of 57 alleles with an average of 4.071 alleles per locus were reported. The number of alleles per locus varied from 2 to 8. Range of genetic diversity was observed from 0.633 to 0.099 with an average of 0.405. In this study they observed moderate genetic diversity among pearl millet accessions.

Chelpuri *et al*. (2017) investigated genotypic data for a total of 88 marker loci (39 Genomic SSRs and 49 EST SSRs) to construct a linkage map of the pearl millet mapping population of 188 F_8 RIL progenies based on the cross ICMB 89111-P6 x ICMB 90111-P6. A skeleton linkage map of seven linkage groups with a total map length of 725.5 cM (Haldane units) was constructed using data from 74 marker loci for 188 RILs using Join Map at LOD threshold value of 5.0 and Map Maker /Exp version 3.0b and map was drawn using Map Chart 2.2. The map length of individual linkage groups ranged from a minimum of 32.1cM (LG3) to a maximum of 140.2 cM (LG1). The average inter marker distance was 9.8 cM, with an average density of 0.102 markers/cM. The total number of mapped loci per linkage group (LG) ranged from 5 on LG3 to 23 on LG_1.

Jika *et al.* (2017) investigated genetic diversity among 69 pearl millet landraces belonging to eight ethno-linguistic farmer groups from Lake Chad basin using 17 SSR markers. Allelic richness (Ar) and expected heterozygosity (He) ranged from 2.6 to 4.0 (mean of 3.4) and from 0.4 to 0.6 (mean of 0.5), respectively. The results shown the existence of a genetic structure of pearl millet accessions mainly associated with ethno-linguistic diversity in the western side of the Lake

Chad. AMOVA results revealed that the higher genetic diversity was found within populations rather than among landraces or ethno-linguistic groups.

Nehra *et al.* (2017) reported genetic diversity among 49 stay green inbreds of pearl millet using simple sequence repeats (SSRs). Twenty-nine polymorphic SSR primers, identified after initial screening of 70, were used to study diversity among these lines. A total of 108 alleles were amplified, collectively yielding unique SSR profiles for all the 49 inbreds. The average number of SSR alleles per locus was 3.72, with a range from 2 to 13. Polymorphic information content (PIC) values of various SSR loci across all the 49 inbreds ranged from 0.14 to 0.87 with an average of 0.51 per locus. This indicated sufficient diversity among the 49-pearl millet inbreds and total 5 out of 29 polymorphic SSR loci, *namely* Xpsmp2070, Xpsmp2001, Xpsmp2008, Xpsmp2066 and Xpsmp2072 revealed PIC values above 0.70, can be considered highly useful for differentiation of pearl millet inbred lines. The lowest PIC value (0.47) for linkage group 7 showed comparatively conserved nature of this linkage group. A dendrogram obtained using WARD's minimum variance method further delineates 49 inbreds into 8 major clusters, and the clustering pattern corroborated with their pedigree and characteristics traits.

Ambawat *et al.* (2020) evaluated the diversity among 30 different released hybrids and varieties of pearl millet using 125 Simple Sequence Repeat (SSR) markers. Out of these, 61 polymorphic SSRs were reported giving 191 alleles with an average of 3.13 alleles per primer. Polymorphic Information Content (PIC) varied from 0.33 to 0.76 with an average of 0.55 PIC value. The cluster analysis based on these SSR markers categorized the genotypes into four major clusters *viz*., I, II, III, IV with similarity coefficient ranging from 0.58 to 0.73.

Jangra *et al.* (2020) were undertaken a study to improve terminal drought tolerance in pearl millet hybrid HHB 226 by introgression, drought tolerance QTLs from 863 B to the male parent HBL 11 of the hybrids. Marker-assisted foreground selection was undertaken using four polymorphic SSR primers present on linkage group 2 and 5 to identify plants possessing alleles for resistance in the segregating population (BC_4F_2) along with stringent phenotypic selection for faster recovery of the recurrent parent genome. Background selection using 32 polymorphic SSR markers spanning on seven linkage groups were used to estimate the recovery of

recurrent parent genome in improved lines. A maximum of 82% of recurrent parent genome was recovered in selected plants.

Kumar *et al.* (2020) evaluate the diversity of 17 important Indian pearl millet inbred genotypes and one popular hybrid 9444 using fluorescent labelled SSR markers. A total of 342 polymorphic alleles with an average of 4.62 alleles per primer were produced from 74 SSR markers. Polymorphic information content (PIC) ranged from 0.10 to 0.89 with an average of 0.55. A very low level of heterozygosity was detected in genotypes. The average genetic dissimilarity detected between pairs of inbred lines was 0.66. Genetic dissimilarity estimates calculated among the inbred lines varied from 0.108 (AIMP-03 and AIMP-08) to a maximum of 0.851(AIMP-03/AIMP-08 and 81B).

Tanwar *et al.* (2020) were evaluated thirty-four pearl millet restorer lines on the basis of nine seed vigour traits and molecular analysis was done with 55 SSR molecular markers to study the genetic divergence among them. Cluster analysis based on seed vigour parameters revealed the considerable amount of variability and all genotypes were divided into six clusters. Furthermore, a set of 39 SSRs, selected after initial screening of 55, amplified 226 alleles with a mean of 5.84 alleles per locus. The highest polymorphic information content (PIC) value obtained was for PSMP 2084 (0.88) with a range of 0 to 0.88 and average PIC of 0.558.

CHAPTER- III

MATERIAL AND METHODS

The field experiment was conducted at the experimental field, Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Gwalior (M.P.) during Kharif 2019-20 and the laboratory work was carried out at the Plant Molecular Biology & Biochemical Laboratories, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madhya Pradesh, India during July 2019 to March 2020 of the present study entitled **"Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene-linked SSR markers".** Present chapter reveals various materials used and methods applied to evaluate diversity assessment against drought tolerance in pearl millet using morpho-physiological, biochemical and molecular techniques.

3.1 TECHINCAL PROGRAMME OF WORK

3.1.1 Location

Experiment was conducted at the Research Farm of College of Agriculture, Gwalior located in the Gird region (Agro climatic zone No. VI, wheat-pearl millet crop zone). The Gwalior is situated at an altitude of 211.52 mean sea levels, 260 13´ N Latitude and 780 14´ E Longitude. The soil is sandy loam which is low in available nitrogen, medium in phosphorus and high in potash with pH of 8.5.

3.1.2 Climate

Hot and dry, June is the hottest months and minimum and maximum temperature varies from 14.7ºC to 42.7ºC, respectively. Hot weather about 48°C in summer and minimum temperature is 4.1°C in the winter season. The annual rainfall ranges between 750 to 800 mm, most of which received from end of June to end of September, with few showers in winter months. The weather conditions were normal during the crop season with an average maximum and minimum temperature during growing period remained as 35.2°C and 24.5°C respectively. The total rainfall received during the crop growing period from July to October 2019 was 907.7 mm. Rainfall was observed scanty and unevenly distributed during crop growing period.

The relevant meteorological data during crop season are presented in Table 3.1and Fig.1.

3.2 EXPERIMENTAL MATERIAL

The present study was consisted of 96 genotypes (Table 3.1) of (*Pennisetum glaucum* (L.) R. Br.) with divergent reactions to drought *viz*: susceptible, tolerant and resistant. The seeds were obtained from College of Agriculture, Gwalior, RVSKVV, Gwalior, M.P., India.

3.3 FIELD TRIAL

3.3.1 Pre-sowing operations

The field was prepared by tilling the land with tractor driven cultivator tracked by two harrowing with disc harrow to develop fine tilth. The field was then finally levelled by using tractor operated leveller. After this, experiment was laid out.

3.3.2 Sowing

The Seed sowing was carried out in all treatments as per the field preparation based on different treatments by hand dibbling.

3.3.3 Thinning

Thinning was done by manual laborers after 25-30 days of germination to maintain the uniform and desired plant population stand.

3.3.4 Irrigation management

Rainfall supplemented mostly the irrigation requirements. However, extra irrigation was given as and when required. Fortunately, no rainfall was recorded during period of 50-70 days, which was proved supportive for recording drought parameters.

3.3.5 Weed management

Hand weeding was done at 25 and 45 days after sowing (DAS) to keep the experimental plots free from weeds.

Table 3.1 Weekly metrological data during crop growing period

Meteoro-logical week	Date	Temperature (ºC)		Humidity (%)		Rainfall (mm)	Evaporation (mm)
		Max.	Min.	Morning	Evening		
23	June 4-10	45.8	29.1	40.6	19.7	0	19.7
24	June 11 - 17	42.7	29.4	45.6	39.4	10.8	12.4
25	June 18 - 24	37.3	25.3	79.3	51.4	72.8	8.2
26	June July 25 - 1	40.4	27.9	66.3	36.4	10.2	7.7
27	July 2 - 8	35.3	24.8	39	63	128.6	3.6
28	July 9 - 15	34.5	26.4	79.4	59.3	48.2	5.5
29	July 16 - 22	37.7	26.4	75.4	45	2.4	7.5
30	July 23 - 29	35.1	25.7	85.1	65	38.6	3.3
31	July Aug. 30 - 5	33.2	25.9	91.3	64.3	9.2	4.8
32	Aug 6 - 12	32.9	24.9	89.1	66.1	22.4	3.8
33	Aug. 13 - 19	31.1	24.1	91.1	84	59.8	2.8
34	Aug. 20 - 26	32.3	24.4	89.9	72.1	117.8	3.6
35	Aug. Sep. 27 - 2	33.5	25.2	90.1	66.7	21.8	3.5
36	Sep. 3 - 9	33.7	25.1	90.3	67.6	68.4	3.6
37	Sep. 10 - 16	32	23	94.3	78.3	67.9	2.97
38	Sep. 17 - 23	29	22.2	94.1	77.1	123.8	3.1
39	Sep. 24 - 30	31.6	21.5	95.7	80	73.6	1.1
40	Oct. 1 - 7	33.2	18	90.8	60	31.4	3.4
41	Oct. 8 - 14	32.6	17.5	32.2	40.7	0	5.2
42	Oct. 15 - 21	31.5	14.1	90.5	44.4	0	5
43	Oct. 22 - 28	33.2	18	89.4	31.7	0	4
44	Oct. Nov. 29 - 4	32	16.4	89	42.28	0	4

Source: Meteorological Observatory, College of Agriculture, Gwalior (M.P.)

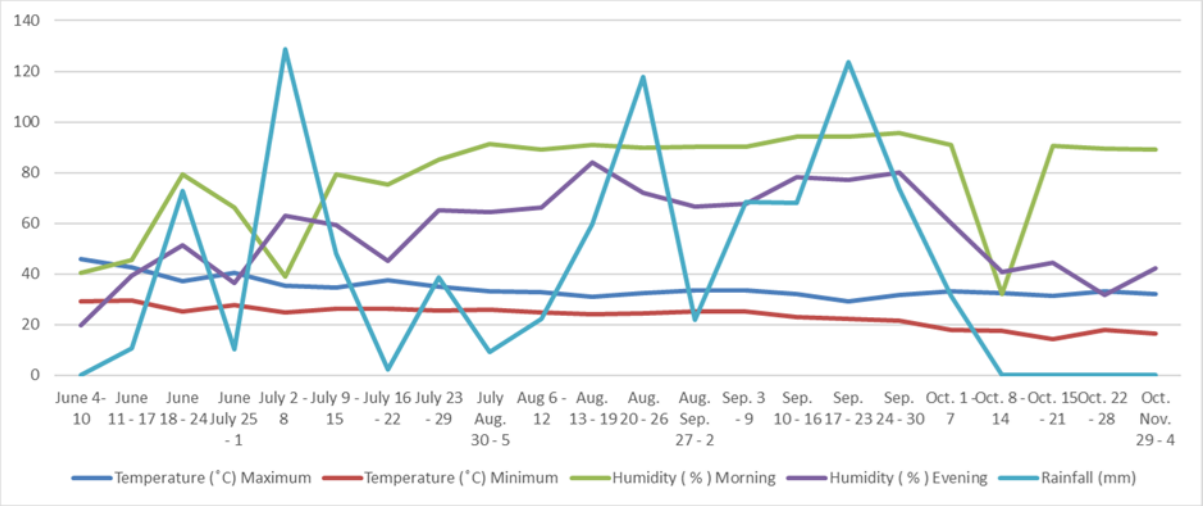

Fig 3.1 Weekly metrological data during crop growing period

3.3.6 Fertilizer and manure management

Nitrogen, phosphorus, potassium and sulphur were applied @60:30:20 kgha^{-1}, NPK respectively in field.

3.3.7 Sampling

The sampling was done at 70 days after sowing (DAS) till maturity. Five plants were randomly selected from each treatment per replication for growth analysis, dry matter produced and biochemical estimations.

3.3.8 Harvesting and threshing

The final harvesting was carried out at maturity and the plants were sun dried, threshed and analysed for various yield and its attributing components and biochemical constituents.

3.3.9 Plot description

Design	:	Randomized Design (RBD)
Number of treatments	:	96
Number of replications	:	2
Number (s) of rows	:	1
Row length	:	4 meters
Planting geometry (R x P)	:	50 cm x 15 cm
Seed rate	:	4 kg ha^{-1}
Date of sowing	:	14-07-2019
Recommended dose of fertilizers (kgha^{-1})	:	60+ 30 + 20 (N +P2O5 +K2O)

Table: 3.2 List of genotypes used in present investigation

Sr. No.	Name of germplasm	Sr. No.	Name of germplasm	Sr. No.	Name of germplasm	Sr. No.	Name of germplasm
1.	IP 132	26.	IP 136	51.	IP 187	76.	IP 162
2.	IP 118	27.	IP 171	52.	IP 159	77.	IP 115
3.	IP 152	28.	IP 130	53.	IP 139	78.	IP 170
4.	IP 175	29.	IP 166	54.	IP 146	79.	IP 109
5.	IP 133	30.	IP 128	55.	IP 196	80.	IP 154
6.	IP 173	31.	IP 183	56.	IP 186	81.	IP 174
7.	IP 199	32.	IP 165	57.	IP 158	82.	IP 108
8.	IP 127	33.	IP 192	58.	IP 151	83.	IP 189
9.	IP 198	34.	IP 122	59.	IP 193	84.	IP 110
10.	IP 177	35.	IP 143	60.	IP 105	85.	IP 117
11.	IP 182	36.	IP 167	61.	IP 123	86.	IP 169
12.	IP 147	37.	IP 172	62.	IP 131	87.	IP 114
13.	IP 107	38.	IP 106	63.	IP 178	88.	IP 163
14.	IP 140	39.	IP 137	64.	IP 121	89.	IP 274
15.	IP 164	40.	IP 116	65.	IP 104	90.	IP 283
16.	IP 142	41.	IP 194	66.	IP 134	91.	IP 236
17.	IP 180	42.	IP 195	67.	IP 112	92.	IP 291
18.	IP 188	43.	IP 126	68.	IP 141	93.	IP 230
19.	IP 181	44.	IP 155	69.	IP 145	94.	IP 262
20.	IP 129	45.	IP 149	70.	IP 144	95.	IP 231
21.	IP 119	46.	IP 185	71.	IP 138	96.	THAK 1827
22.	IP 150	47.	IP 161	72.	IP 179		
23.	IP 120	48.	IP 168	73.	IP 153		
24.	IP 111	49.	IP 190	74.	IP 101		
25.	IP 160	50.	IP 156	75.	IP 135		

Plate 1. Field view of pearl millet germplasms during Kharif 2019-20 at Research farm, College of Agriculture, Gwalior.

3.4 Screening of pearl millet genotypes against drought stress

3.4.1 Source of biological materials

A total of ninety-six pearl millet genotypes registered in India were grown in field (Table 3.2). The seeds were obtained from All India Coordinated Research Project on pearl millet, College of Agriculture, Gwalior, RVSKVV, Gwalior, M.P., India. For drought treatment, the 50-day-old plants were non-irrigated for 10 days and data were recorded for various morpho-physiological and biochemical parameters after 60 days of sowing to efficiently screen drought tolerant and susceptible genotypes. The experimental material was monitored in randomized block design (RBD) with two replications.

3.4.2 Molecular markers

In the present study, total of 35 Simple Sequence Repeats Markers (SSR) were emplyoed for screening of pearl millet genotypes against drought stress as reported by Sehgal *et al.* (2012) and described in Table 3.3.

3.4.3 Morpho-physiological parameters to screen pearl millet genotypes

Observations were recorded on five plants. Following different morpho- physiological parameters were taken under considerations:

3.4.3.1 Plant height (cm)

Plant height of five randomly selected plants from each row and replication was recorded by measuring from the base of the plant to top of the panicle of main tiller at the maturity stage**.**

3.4.3.2 Root length (cm)

Average root length of five randomly selected pearl millet genotypes was recorded with the help of 1 m scale after 60^{th} days of sowing.

3.4.3.3 Shoot length (cm)

Shoot length of randomly selected five pearl millet genotypes was measured using 1 m scale on the after 60^{th} day of sowing. Average length was used for statistical analysis.

Table 3.3 List of SSR markers were used for screening of pearl millet genotypes against drought

SSR marker	Forward	Reverse
Xibmsp01	GCAGACTGAGAAGGCTTTCC	TGCTCTTCCAGAAGCGGTTG
Xibmsp02	GGAGTACAGAGTCCGCACATT	CTTCTCAACTTTGCGACAGGT
Xibmsp03	CGCAACAGAATTTTGTCGG	TTACGCTGGTTGTCAAGTTG
Xibmsp04	AGTGAGTCAAGATCTTCATTTTTCC	AAGGGAATGGCTTGAAGATT
Xibmsp05	TCTCCTTCTCCTTGCTGATGA	GCTGAAGTTGCAGCACAGAC
Xibmsp06	CGGTGCTCATGTACACATTC	TGATAGCCTGCTGCATGAAG
Xibmsp07	GTCCCTTGCGTGGAACAAAT	AGCTAAAGCCAGTTCCAGTG
Xibmsp08	ACTTGACTCCAACCTCCAAC	TGGGGATACAGATGCTGTAG
Xibmsp09	ATACGCCGAAGAGCTGTCAG	AGCGTAATGGCAGTCATGTC
Xibmsp10	GCTGGAGCTTGACTCGTG	CAAAGAGAAACGAAATTTCCACA
Xibmsp11	CGTCAATGGCATATCTACAC	CCATACCAATGTCATTGAGC
Xibmsp12	TTTTGTTATCCACAGTCCAACTC	TGCCTTAGAAGCATCTGCAA
Xibmsp13	GGAAGTCGTAGCAGAAGTTG	CAAGGTCTCCATCAACTGGC
Xibmsp14	TCTTCAGGGATGTTCCCTACT	GAGGAAGTTTATGATGGAAGGAAA
Xibmsp15	TGCTACGCCAATTTCTAATGC	CCACCATCGTCAAGTACTGC
Xibmsp16	GAGCTCCAGATGATGAACAC	CTTGCCATAGCACCAAATGG
Xibmsp17	*CATGGCACCACTAGACATAG*	*GAAACTGACTTCATGATGGAG*
Xibmsp18	ATAGATAAAACAGGTGCAGTTTCAGA	ATGACCACAGATCAGCCTTG
Xibmsp19	GTGTTGGTTCCATCTCAGG	CTGCCTCATGGTTATGATGG
Xibmsp20	GCTGAGCTTGACCTTGTTGTC	CCTGGCATGATTCCAATTTT
Xibmsp21	GAACCTCATCCAACAATTCC	GCTGCTGATGTTGCTATTGC
Xibmsp22	CGAATCCTCTTGGTACCAAC	GATCGCTCTTCATGTGGTTC
Xibmsp23	AAAGGACCAGTCACGTGAAG	ATAGCCTGGCCATTTCCTC
Xibmsp24	CATCATTGGCCCACACAAT	GAACAACTTAAGCTGGTAGATGC
Xibmsp25	GTGAAAAAGGGTCCAAAGGG	GAAGCCCCAGTAAGTCTTC
Xibmsp26	GAGGTTCGTCAAGAGGTTCG	TCCTCGGCCTCAATAAGCTA
Xibmsp27	CATTGCTCTTCATGGTGGAG	TGGAGCACTGAAGCCAGTAA
Xibmsp28	CGGCCGAGGTACTAACAGTC	GAGAAGCTAGGGGCAACCTT
Xibmsp29	GATGCAAATTTGTGGGAACC	GCCGAGACTCGAAAACAATC
Xibmsp30	AGACAGACAGCACGCACAAC	GAGCTCGACGACATGATGG
Xibmsp31	ATCGATCTTGTGTGCAGTGG	GACCCGACATGAGGACATTC
Xibmsp32	CTGGTGACCATGTCCTTCCT	TTGGTGGTTTGGCAACATTA
Xibmsp33	GAAGGAGAAGCACCACAAGC	CCGAGGATATCCAGATCGAA
Xibmsp34	GCTCGAAACACGAAACCCTA	CTGGCAGGTGACTTCTCCA
Xibmsp35	ACGAGATGTTCCTCGTCCTG	CCTCCTTGTTCGAGATGGTG

Source: Sehgal *et al.* (2012)

3.4.3.4 Root-Shoot Ratio

After measuring the length of shoot and root, ratio of root and shoot was calculated by using following formulae.

R/S ratio = Length of Root /Length of Shoot

3.4.3.5. Spike length

It is measure from base of panicle to tip of panicle in centimetre.

3.4.3.6 Spike Girth (cm)

The girth of the panicle was measured in centimetre in all selected spike for the measurement of the spike girth. The diameter was measured using vernier calipers at the time of maturity. It was taken at bottom, middle and top region of ear head. The average was then obtained to represent spike girth.

3.4.3.7 Numbers of tillers per plant

Total numbers of panicles bearing tillers of 5 randomly selected plants were counted and averaged.

3.4.3.8 Days of 50% Heading Initiation

The number of days was taken from date of sowing to the date of spike emerge on the peduncle of 50 per cent plant in each row.

3.4.3.9 Days to 50% flowering Initiation

The number of days was taken from date of sowing to the date of stigma emergence on the main panicle of 50 per cent plant in each row.

3.4.3.10 Canopy temperature

The canopy temperature of five randomly selected plants was recorded from Infra-Red Thermometer.

3.4.3.11 Fresh weight (g)

Five plants were randomly selected and measured fresh weight (FW) after directly weighing without drying or other actions.

3.4.3.12 Dry weight (g)

Dry weight (DW) was recorded after oven-drying of five randomly preferred plants for 48 h at 70°C till stable weight.

3.4.3.13 Turgid Weight (g)

Turgid weight (TW) was determined after floating of plants on water overnight at room temperature for 24 hours.

3.4.3.14 Relative Water Content (%)

Relative water content (RWC) is considered one of the important physiological parameters to assess the water content in plants during stressed and control condition and calculated by the following formula:

$$RWC = \frac{FW - DW}{TW - DW}$$

Where, **FW**= Fresh weight of plant (g), **DW** = Dry weight of plant (g) and **TW** = Turgid weight of plant (g).

3.4.3.15 Saturation water deficit (%)

It was calculated after subtracting the value of RWC from 100.

SWD= 100—RWC

3.4.3.16 Days to physiological maturity

Numbers of days were counted from the date of sowing to physiological maturity of the plant.

3.4.3.17 Days of maturity

Days to maturity was recorded as number of days taken from the date of sowing to the date when crop ready for harvested.

3.4.3.18 Leaf area (cm sq /plant)

Leaf area of 3 leaves of each observational plant was measured in mm 2 at a 30 days interval from 30 DAS and its mean for per leaf was calculated. Further, the mean was multiplied by a mean number of leaves per plant and the leaf area was

converted into cm^2. Leaf area was measured with the help of Automatic Leaf Area Meter.

3.4.3.19 Plant Population at Harvesting

Numbers of plants was counted in every row.

3.4.3.20 Seed density of spike (cm^2)

Seed density of spike was measured with the help of 1cm square shape.

3.4.3.21 Test weight (g)

Thousand-seed were counted from the production of per plant seeds and weighted on electronic balance (g).

3.4.3.22 Yield ($qtha^{-1}$)

Grain yield of five plants were recorded and computed as yield ($qtha^{-1}$)

3.4.3.23 Biological Yield ($qtha^{-1}$)

The weight of all harvested plant parts of 5 observational plants was recorded before threshing including the dry weight of leaves, stems and spike. Then the average biomass per hectare was calculated.

3.4.3.24 Harvest index (%):

Harvest index indicates the yield efficiency of the crop to produced grain yield per unit of total biological yield. Harvest index was worked out by the formula given by Donald and Hamblin (1976).

3.4.5. Biochemical parameters

3.4.5.1 Photosynthetic pigments

Photosynthetic pigments were estimated by Arnon's method (1949). Leaf samples of pearl millet genotypes were collected after 30 and 60 days of sowing. Samples were crushed with mortar and pestles in liquid nitrogen to avoid the chemical degradation of the chlorophyll. Resultant 100 mg leaf powder was added to 10 ml of acetone (80%) in 15 ml centrifuge tubes and cooled at 4°C for 15 min tracked by centrifugation for 10 min at 10000 rpm before transferring the supernatant to fresh 15 ml centrifuge tubes.

Quantification of photosynthetic pigment was performed using UV-VIS spectrophotometer for recording absorbance at 470, 645 and 663 nm. The amount of chlorophyll-a, chlorophyll-b and total chlorophyll were calculated according to Arnon's equation (1949) as described below:

Chl_{-a} = (12.7 x Abs663) - (2.6 **x** Abs645) x 10 ml of acetone/100 mg leaf tissue

Chl_{-b} = (22.9 Abs645) -- (4.68 Abs663) x 10 ml of acetone/100 mg leaf tissue

Chl_{a+b} = (22.9 Abs645) - (4.68 Abs663) x 10 ml of acetone/100 mg leaf tissue

3.4.5.2 Estimation of proline content

Principle

Free proline content in leaves was determined according to the method proposed by Bates *et al*. (1973) based on the formation of red colored formazine by proline with ninhydrin in acidic medium, which is soluble in organic solvents like toluene.

Procedure

Leaf samples (0.5 g) were homogenized separately in 5 ml of sulphosalicylic acid (3%) using mortar and pestle. The homogenate was filtered through filter paper Whatman No.1 and filtrate was collected for further used in the estimation of proline content. Chromophore containing toluene was separated and its absorbance at 520nm was recorded in UV-VIS spectrophotometer using toluene as reference. Concentration of proline was estimated by comparing standard curve made from known concentration of proline.

3.4.5.3 Estimation of sugar content (mgg^{-1}fresh weight)

Principle

The total sugar was estimated as per protocol of Dubois et al. (1956).

Procedure

Sample (100 mg) was homogenized with 5 ml ethanol (80%). The extract of the sample was centrifuged at 10000rpm for 15 minutes. After separating of supernatant, the remaining residue was again extracted twice with 5 ml of 80% ethanol. The tubes were placed for 10 minutes in a boiling water bath tracked by cooling immediately in running tap water. Similar procedure was applied to prepare a reference having 1.0

ml distilled water to measure absorbance at 620 nm. Sugar amount in the samples was calculated by referring glucose standard curve.

3.4.5.4 Extraction and estimation of total protein

Principle

The protein content was calculated as per method given by Oliver Howe Lowry (1951) [Lowry method/Protein Extraction Buffer (PEB)] (1951). The total protein concentration is exhibited by a colour change of the sample solution in proportion to protein concentration, which can then be measured using colorimetric techniques.

Procedure

Sample (100 mg) was homogenized with 1.0 ml D/W and 100ul NaOH (0.5N). Then vertex tubes and incubates for 1 hour at room temp. The extract of the sample was centrifuged at 15000 rpm for 10 minutes. Then supernatant was taken and 50 ml HCL was added and incubated for 30 min at RT. It was centrifuged and discarded supernatant after its pellet washed with D/W.

3.4.6 Molecular markers analysis

3.4.6.1 List of molecular markers

In the present study, total of 35 SSR markers for drought were used for genetic diversity analysis (Table 3.3).

3.4.6.2 Materials required for molecular work

3.4.6.2.1 Equipment's & Consumables

Bio-Rad Thermocycler, Agilent Thermocycler, Autoclave, Refrigerator, Electronicl Analytical balance, Water bath, Cooling centrifuge, Horizontal gel electrophoresis unit, Gel documentation unit, Vortex mixture, Micropipettes, Mortar and pestle, Aluminium foil, PCR sealing film (Optical sealing film), Conical flask, Eppendorf tubes (2ml & 1.5ml), PCR Plate, Gel casting tray, Incubator, Micro pipette (100-1000µl,20-200µl,2-20µl), Microwave Oven, Scissor, Tissue paper, UV trans-illuminator *etc*.

3.4.6.2.2 Chemicals used for molecular work

Agarose, Chilled ethanol, DNA extraction buffer (Table 3.3), Ethidium Bromide, Ladder (50 &100bp), Loading dye (6x), Master mix, PCI (Phenol: Chloroform: Isoamyl alcohol, 25:24:1), Sodium acetate, TAE buffer (50X), TBE buffer (10X), TE buffer (5x).

3.4.6.3 Molecular Techniques

3.4.6.3.1 Reagents for DNA isolation

- CTAB Extraction buffer
- 2 % β-mercaptoethanol
- Chloroform: Isoamyl alcohol (24:1) (v/v)
- Phenol: Chloroform: Isoamyl alcohol (25:24:1) (v/v/v)
- 3M sodium acetate (pH 5.2)
- TE Buffer (pH 8.0)
- RNase A
- stock (10 mg/ml solution)
- Other reagents: 70 % Ethanol, 100 % chilled Isopropanol and Liquid Nitrogen

3.4.6.3.2 Reagents for Agarose Gel electrophoresis

- 1 X TAE Buffer
- 6X gel loading dye
- 10 mg/ml Ethidium bromide

3.4.6.3.2 Reagents for SSR Analysis

- 10 mM dNTPs
- 50 mM MgC12
- 1U *Taq* polymerase
- 10X PCR buffer
- Primers
- Nuclease Free Water

3.4.6.4 Sample preparation for DNA isolation

The seeds were germinated in plastic plots containing sterilized sand, soil and compost manure with appropriate ratio (1:1:2) in green house and 8-10 days-old leaves were used for DNA extraction.

3.4.6.5 DNA Extraction from Pearl Millet

High quality genomic DNA was isolated from young and fresh leaves (8-10 days old) of Pearl Millet (*Pennisetum glaucum* L.) by using Cetyl trimethyl ammonium bromide (CTAB) as per Doyle and Doyle (1987) method with some modifications.

3.4.6.5.1 Preparation of stock solutions of reagents and buffers for DNA extraction

The details of composition and procedure for preparation of various stock solutions and buffers are given in Table 3.4 and 3.5.

3.4.6.5.2 Sterilization

The chemical reagents, tips (20-200 µl and 100 µl), eppendorf tubes (1.5 ml and 0.5 ml), PCR tubes, reagents stocks, buffer, mortar and pestle and all other plastic wares were autoclaved at 121°C for 15 min, under 15 psi pressure. The glass wares were also autoclaved after washing and rinsing with double distilled water.

3.4.6.5.3 Isolation of Genomic DNA and purification

The protocol of Cetyl Trimethyl Ammonium Bromide (CTAB) DNA extraction method given by Doyle and Doyle (1987) was used for DNA isolation from pearl millet genotypes with some modifications.

1) Around 2-3 g young leaf tissue from each genotype were ground to a fine powder using liquid nitrogen (-196°C).

2) 2% CTAB extraction buffer (at 60°C) was added to tissue powder (5 ml buffer per gram of tissue works well). 1% of β-mercaptoethanol was added freshly to the mortar containing the tissue in buffer.

3) The mixture was transferred to file micro centrifuge tube, mixed well and incubated at 60°C for 30 minutes in water bath.

4) After heating, solution was allowed to cool down at room temperature and then centrifuged at 10,000 rpm for 10 minutes at 4^{0}°C.

5) The upper aqueous phase was collected in fresh microcentrifuge tube, equal volume of chloroform: Isoamyl alcohol was added and again centrifuged at 10,000 rpm for 10 minutes. (This step was repeated till no white interface has been seen).

6) To the collected supernatant, and equal volume of ice-cold isopropanol was mixed well and DNA was allowed it to precipitate overnight at -20°C.

7) The DNA was pelleted by centrifugation at 10,000 rpm for 10 minutes at 4ºC.

8) The supernatant was discarded and the pellet was washed with 70 % ethanol, then air-dried and dissolved in required quantity of TE buffer.

9) After proper dissolving, DNase free RNase was added to remove RNA by incubating at 37°C for at least 30 minutes in water bath.

10) DNA was precipitated in the aqueous phase by adding 0.1 volume of 7.5M ammonium acetate and 2 volume of absolute ethanol. After gently mixing, kept at -20°C for at least 30 minutes and DNA was pelleted at 10,000 rpm and the supernatant was removed.

11) The DNA pellet was washed twice with 70 % ethanol, air-dried and dissolved in required quantity of TE buffer. The isolated DNA sample was kept at -20°C for long term preservation.

Table 3.4. Reagents for plant total genomic DNA isolation

Reagent	Method of Preparation
0.5 M EDTA (pH 8.0)	186.1 g of sodium salt of EDTA was dissolved in 800 ml of MQ water; pH was adjusted to 8.0 with NaOH pellets. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.
4 M NaCl	233.8 g of NaCl was dissolved in 800 ml of MQ water. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.
1M Tris-Cl (pH 8.0)	121.1 g of Tris-Cl salt was dissolved in 800 ml of sterile MQ water. pH was adjusted to 8.0 with concentrated 1N HCl. The final volume was adjusted to one liter with MQ water and sterilized by autoclaving.
10% CTAB	100 gm of CTAB powder was dissolved in sterile MQ water and the volume was adjusted to one liter.
Phenol Chloroform Isoamyl alcohol (PCI)	Buffer saturated phenol, chloroform and isoamyl alcohol were mixed in the ratio of 25: 24: 1. The equilibrated mixture was stored under a layer of 0.01 M Tris-HCl (Ph 7.6) at 40C in dark glass bottle.

Table 3.5 Composition of DNA extraction buffer

Chemicals	Concentration	Quantity (for 100 ml)
Tris HCl (1M, pH 8.0)	100 mM	10 ml
EDTA (0.5M, pH 8.0)	20 mM	4.0 ml
NaCl (5M)	1.4 M	28 ml
CTAB	2 %	2.0 g
β-mercaptoethanol	0.4%	400 µl

3.4.6.6 DNA purification

The purification of DNA was done in order to remove the impurities like RNA, proteins and polysaccharides. These were considered as one of the important inhibitors in DNA amplification during PCR.

(1) 5 µl of RNase (5mg/ml) added to extracted DNA and mixed well before incubation at 37ºC for 1 h.

(2) This was followed by the addition of an equal volumes of phenol: chloroform: isoamyl alcohol (25:24:1 v/v) and mixed vigorously.

(3) Mixture was centrifuged at 12,500 rpm for 10 min and the supernatant was transferred to a fresh centrifuge tube.

(4) Tube was kept on ice and 0.1 volume of 5 M sodium acetate (pH 5.3) was added and mixed.

(5) Equal volume of pre-chilled ethanol was added and mixed by inversion. Sample was incubated at -20ºC for 10 min.

(6) Solution was centrifuged at 12,500 rpm for 10 min. Supernatant was removed and the pellet was washed with 0.5 ml 70% (v/v) ethanol, mixed by inversion and centrifuged further at 15,000 rpm for 2 min.

(7) The pellet was dried at room temperature to completely remove ethanol and was then dissolved in 100µl of TE buffer. The DNA was stored at -20°C for further use.

3.4.6.7 Quantification of DNA

Genomic DNA was quantified using Nanodrop by measuring the absorbance at 260nm and 280nm. A 50 ng/ml concentration of double stranded DNA showed an absorbance of 1 at 260nm. Concentration of DNA samples was calculated by using following formula:

$$\text{Concentration of DNA samples} = \frac{\text{O.D}_{260} \times 50 \times \text{Dilution factor}}{1000}$$

3.4.6.8 Agarose gel electrophoresis

The qualitative analysis of genomic DNA was performed by agarose gel electrophoresis. Agarose gel (0.8%) was prepared by dissolving 0.8g of agarose in 100 ml IX TAE buffer. After cooling the solution to about 45°C, 5µl of ethidium bromide (10 mg/ml) was added. After solidification, 5 µl of DNA was mixed with 1 µl of 6X gel loading dye and loaded on 0.8% agarose gel. The isolated products were resolved on 0.8% agarose gel at 100 V for 1 hour.

3.4.6.9 Dilution of DNA sample

DNA samples were diluted with appropriate quantity of sterilized distilled water to yield a working concentration of 10ng/µl for SSR markers analysis respectively. These DNA samples were stored at 4°C for further work until PCR amplification.

3.6 Composition of TBE buffer (10x) for 1 liter (pH 8.0)

S. No.	Chemical	Quantity(gm)
1.	Tris – HCl	107.8
2.	Boric acid	55.02
3.	EDTA	9.36

3.4.6.10 DNA analysis of simple sequence repeats

3.4.6.11 Selection of markers

3.4.6.11.1 Simple Sequence Repeats (SSR) Analysis

Molecular characterization of pearl millet genotypes through DNA marker (SSR) was performed. The sequence of pearl millet specific SSR marker was obtained from primer published in research paper (Sehgal *et al.* (2012). Thirty-five SSR primers used in the present investigation are listed (Table. 3.3). The components and their concentration used in the SSR - PCR reaction was prepared as described in Table 3.4 & Table 3.5.

3.4.6.12 PCR condition for (SSR) markers

PCR conditions were standardized considering different parameters *viz*., initial denaturation, denaturation, annealing, extension and final extension using Agilent Technologies Sure Cycler 8800 PCR Machines. PCR profile was optimized for

amplification by using primers of unique sequence with higher GC ratio at high stringency. The optimized conditions are presented in the Table 3.6.

Table 3.7 List of components with their concentrations used for SSR amplification using PCR

S.No.	Components	Concentration
1.	PCR buffer	1X
2.	MgCl2	1.4 mM
3.	dNTPs	100µM
4.	Primer	5pM
5.	*Taq* Polymerase	1 U
6.	MQ	As required
7.	*DNA*	10 ng

3.4.6.13 PCR Protocol

Taq buffer A (10X Tris with $MgCl_2$) was added first followed by *Taq* DNA polymerase, dNTPs, the primer and Milipore water were added in sequence and finally template DNA. The reagents were mixed thoroughly by a short spin using micro centrifuge. The tubes were then placed on the Thermal cycler for cyclic amplification. The conditions for amplification in Thermal Cycler were kept as follows:

Table 3.8 PCR conditions for SSR Primer

Temperature(°C)	Duration	Cycles	Activity
94	4 min	1	Initial denaturation
94	30 sec	35	Denaturation
T* (Opt)	30 sec		Annealing
72	45 sec		Elongation
72	5 min	1	Final elongation
4	∞		Storage

T*(opt) - Annealing temperature was optimized for each primer.

3.4.6.14 Resolution of amplified product

The amplified products were resolved on 2.5% agarose gel at 100 V for 1.5 h. 2.5% agarose gel was prepared by dissolving 2.5 g of agarose in 100 ml 1X TAE buffer, after cooling the solution to about 45°C, 5µl ethidium bromide (10 mg/ml.) was added. After solidification 5 µl of amplified products was mixed with 1 µl of 6X gel

loading dye and loaded into the wells. After electrophoresis, the gel was carefully taken out and photograph was taken on a Gel Documentation System.

3.4.6.15 Polymorphic Information Content (PIC)

3.4.6.15.1 Data scoring and analysis of SSR markers

The amplified products generated from molecular markers with PCR reaction were resolved on agarose gel. Each amplification product was considered as RAPD bands and SSR bands were scored across all samples. Bands were scored as present (1) or absent (0). Missing and doubtful cases ware scored as (9). Molecular weight of the bands was estimated using l00bp DNA ladder for SSR primers as standards.

The Polymorphism Information Value (PIC) was calculated as, where n is the number of band positions analyzed in the set of accessions and P*I is the frequency of 1^{th} allele. Data analysis was performed using NTSYS-PC (Numerical Taxonomy System, Version 2.02, Rohlf, 1994). The SIMQUAL programme was used to calculate the DICE coefficient. Dendrogram was constructed using unweighted pair group method for arithmetic mean (UPGMA) based on DICE coefficient.

3.4.6.16 Morpho-physiological and biochemical parameters analysis

The data was analyzed as per method suggested by Snedecor and Cochran (1967).

3.4.6.17 Molecular analysis

The SSR markers were scored based on the size of fragments amplified across all soybean genotypes. The major allelic frequency, polymorphism information content and genetic distance-based clustering was performed with Unweighted Pair Group Method for Arithmetic Average (UPGMA) tree using power Marker v3.25 software.

Table 3.9 ANOVA Table for RBD

Source of variation	**d. f.**	**S.S.**	**M.S.**	**F. cal.**
Replications	(r-1)	SS (R)	MS (R)	
Genotypes	(g-1)	SS (G)	MS (G)	MS (G)/MS (E)
Error (A)	(r-1) (g-1)	SS (E)	MS (E)	
Total	(rg-1)	TSS	MSS	

Where, **g** = Number of genotypes; **r** = Number of replications

CHAPTER- IV

RESULTS

The present investigation was undertaken to study “Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene- linked SSR markers”. The Ninety-Six germplasm lines were grown in randomized block design with two replications during *Kharif*, 2019 in the field of Department of Plant Molecular Biology & Biotechnology, College of Gwalior, RVSKVV, Gwalior (MP) and the laboratory analysis was carried out Biotechnology Centre, RVSKVV Gwalior. The data pertaining to various Morpho-physiological, biochemical and molecular attributes were recorded and summarized in this chapter.

4.1 Morpho-physiological variations among pearl millet genotypes

4.1.1 Plant height (cm)

Plant height varied in range of 188.05cm to 295.55cm among 96 different pearl millet genotypes with average mean value 237.30 cm. Maximum plant height was exhibited by genotype IP199 (295.55cm), trailed by IP126 (283.40) and IP137 (282.65).

4.1.2 Root length (cm)

Significant genotypic differences were observed for root length between 15.10 cm to 24.50 cm among 96 different pearl millet genotypes with average mean 17.81 cm. Maximum root length value was exhibited by genotype IP 177 (24.50 cm) followed by IP 169 (23.00 cm), IP 190 (22.80). The lowest count was 15.10 cm in the genotype IP 139 (Plate 2-Plate 5).

4.1.3 Shoot length (cm)

Shoot length differed between 162.15 cm to 273.50 cm among 96 different pearl millet genotypes with an average mean 211.62 cm. Maximum shoot length was exhibited by genotype IP199 (273.50 cm) intimately tracked by two genotypes *namely*: IP 137 (256.90 cm) and IP 126 (248.80 cm). The lowest value was 162.15 cm for the genotype IP 236.

4.1.4 Root-Shoot Ratio (R/S Ratio)

Root/shoot ratio varied in range of 0.06 cm to 0.12cm among 96 different pearl millet genotypes with the average mean 0.08cm. Maximum root/ shoot ratio was investigated with genotype IP177 (0.12 cm) intimately followed by a group of two genotypes *namely*: IP164 (0.115 cm), IP154 (0.114). The lowest value was documented 0.06 for the genotype IP180.

4.1.5 Spike length (cm)

The panicle length value is important trait in pearl millet as yield is directly proportional to it in general. The mean value for panicle length ranged from 16.40 to 40.90 cm with the average mean 25.68. Out of the Ninety-Six pearl millet germplasms studied, IP 115 (40.90 cm) recorded the highest panicle length followed by IP 170 (38.70 cm), IP 174 (34.80 cm. The minimum grain Spike length was noted with genotype IP 159 (16.40).

4.1.6 Spike Girth (cm)

Spike girth is also an important trait in pearl millet yield. The mean value for Spike girth ranged from 2.15 to 3.75 cm with the average mean 2.71 cm. Out of the Ninety-six pearl millet germplasms studied, IP 198 (3.70) recorded the highest Spike girth value followed by IP 149 (3.60), IP 173 (3.45). The minimum Spike girth value was taken by genotypes IP 230 (2.15).

4.1.7 Fresh weight (g)

Fresh weight ranged from 112.35g to 372.45g with average mean 208.41g. Maximum fresh weight value in grams was observed in genotype IP104 (372.45g) tracked by genotypes*:* IP198 (369.50g) and IP155 (332.65g). The lowest fresh weight value was recorded for the genotype IP 153 (112.35 g).

4.1.8 Turgid weight (g)

Turgid weight varied from 146.15g to 422.65g with average mean of 257.75g. Maximum turgid weight value in grams was recorded for genotype IP104 (422.65g) tracked by a group of two genotypes*: viz.,* IP198 (409.80g) and IP119 (408.75 g). The lowest turgid weight value was observed for the genotype IP153 (146.15 g).

4.1.9 Dry weight (g)

Dry weight varied in range of 55.10g to 269.25g with average mean 129.39. Maximum dry weight value in grams was recorded for the genotype IP155 (269.25g) chaseed by genotypes*:* IP104 (234.25 g) and IP180 (224.85 g). Hoowever the lowest worth was wvidenced for the genotype IP 143(55.10g).

4.1.10 Relative water content (RWC %)

RWC is considered as a prominent physiological parameter to predict tolerance against drought stress. RWC value of pearl millet genotypes varied in range of 28.80% to 86.47%with an average mean of 61.07%. Maximum RWC value was recorded in genotype IP188 (86.47%) closely chaseed by a group of two genotypes *viz*.: IP195 (82.45%) and IP126 (80.45 %). While the lowest value was exhibited by genotype IP119 (28.80 %).

4.1.11 Saturation water deficit (SWD %)

SWD value differed in range of 13.53% to 71.20% with an average mean of 38.93%. Minimum SWD was evidenceed for the genotype IP188 (13.53 %) intimately tracked by a group of two genotypes incluing IP195 (17.55%) and IP126 (19.55%). The highest SWD value was shown by genotype IP119 (71.20%).

4.1.12 Canopy temperature (ºC)

Canopy temperature ranged between 31.40ºC to 37.15ºC with an average worth of 33.63 ˚C. Maximum canopy temperature value was witnessed for the genotype IP130 (37.15˚C) intimately followed by a group of two genotypes *viz*: IP183 (36.95˚C) and IP 170 (36.10˚C). The lowest canopy temperature was recorded for the genotype IP291 (31.40 ˚C).

4.1.13 Leaf area (cm sq/plant)

Leaf area varied in range of 142.07 cm^2 to 416.38 cm^2 with an average mean of 251.89 cm^2. Maximum leaf area value in cm sq. /plant was recorded for genotype: IP 182 (416.38 cm sq/plant) intimately followed by genotype IP192 (395.73cm sq./plant) and IP129 (382.95 cm sq./plant). The lowest Leaf area was covered by genotype IP 119 (142.07 cm sq./plant).

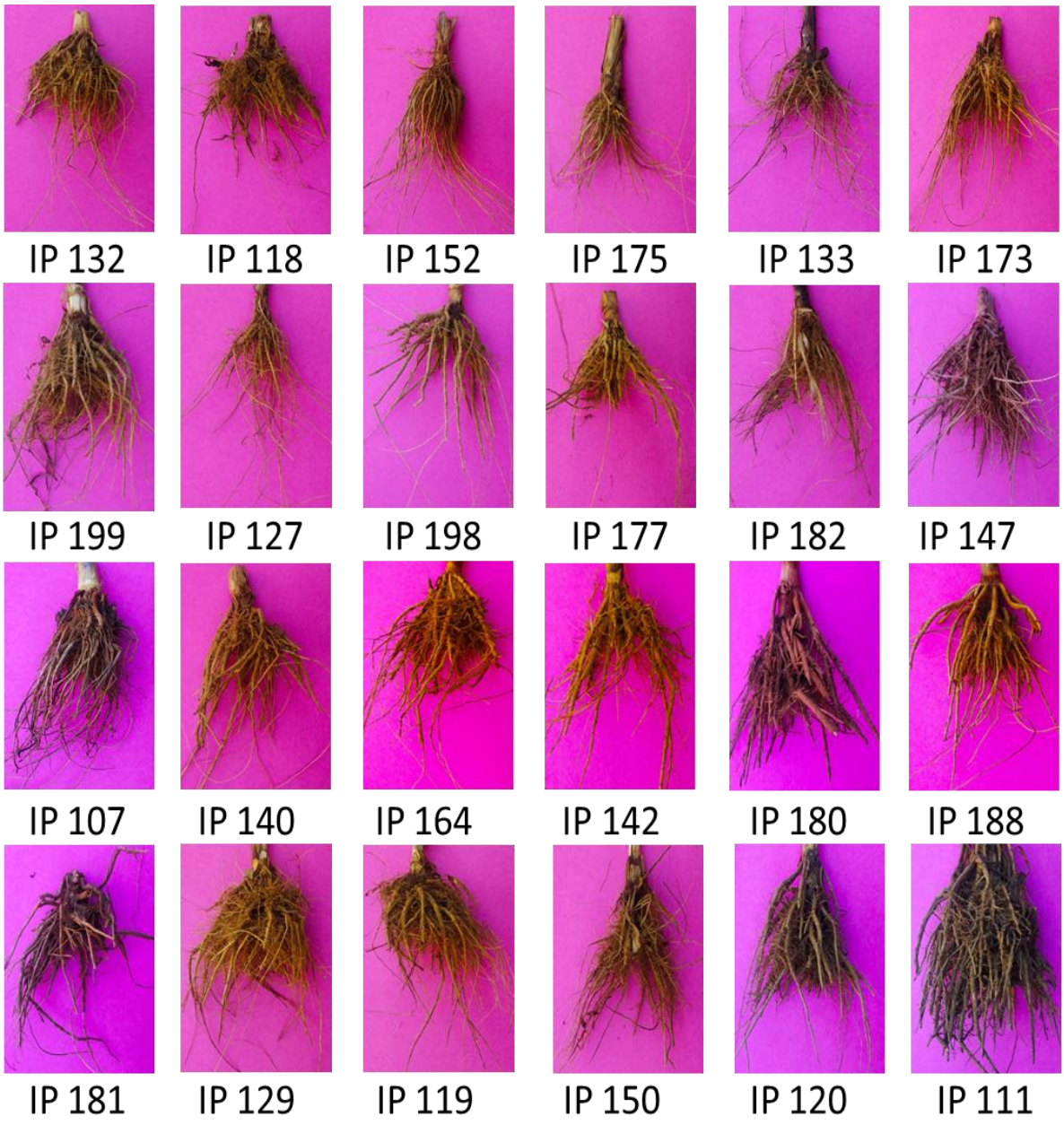

Plate 2. 1-24 Root length of pearl millet germplasm

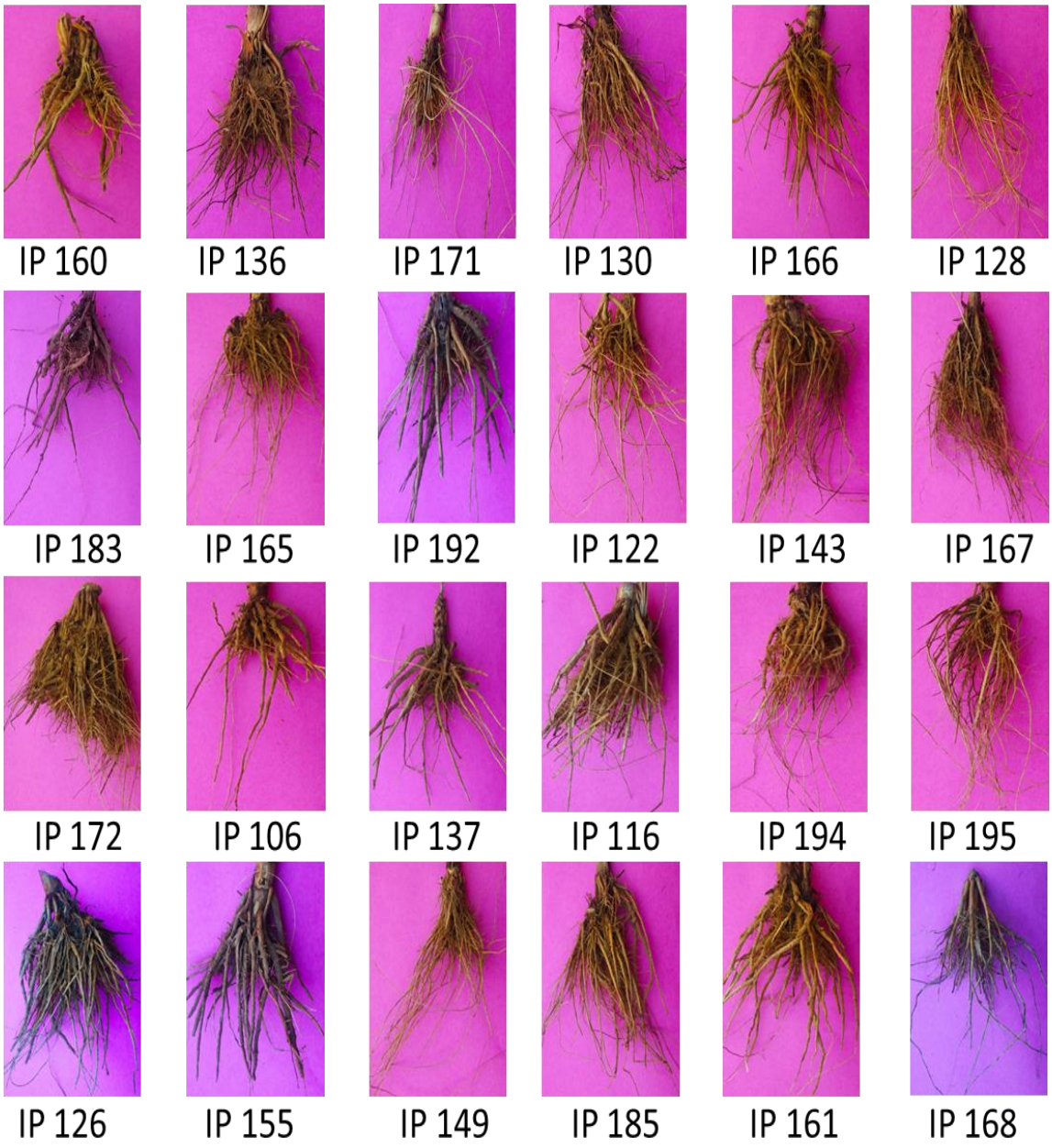

Plate 3. 25-48 Root length of pearl millet germplasm

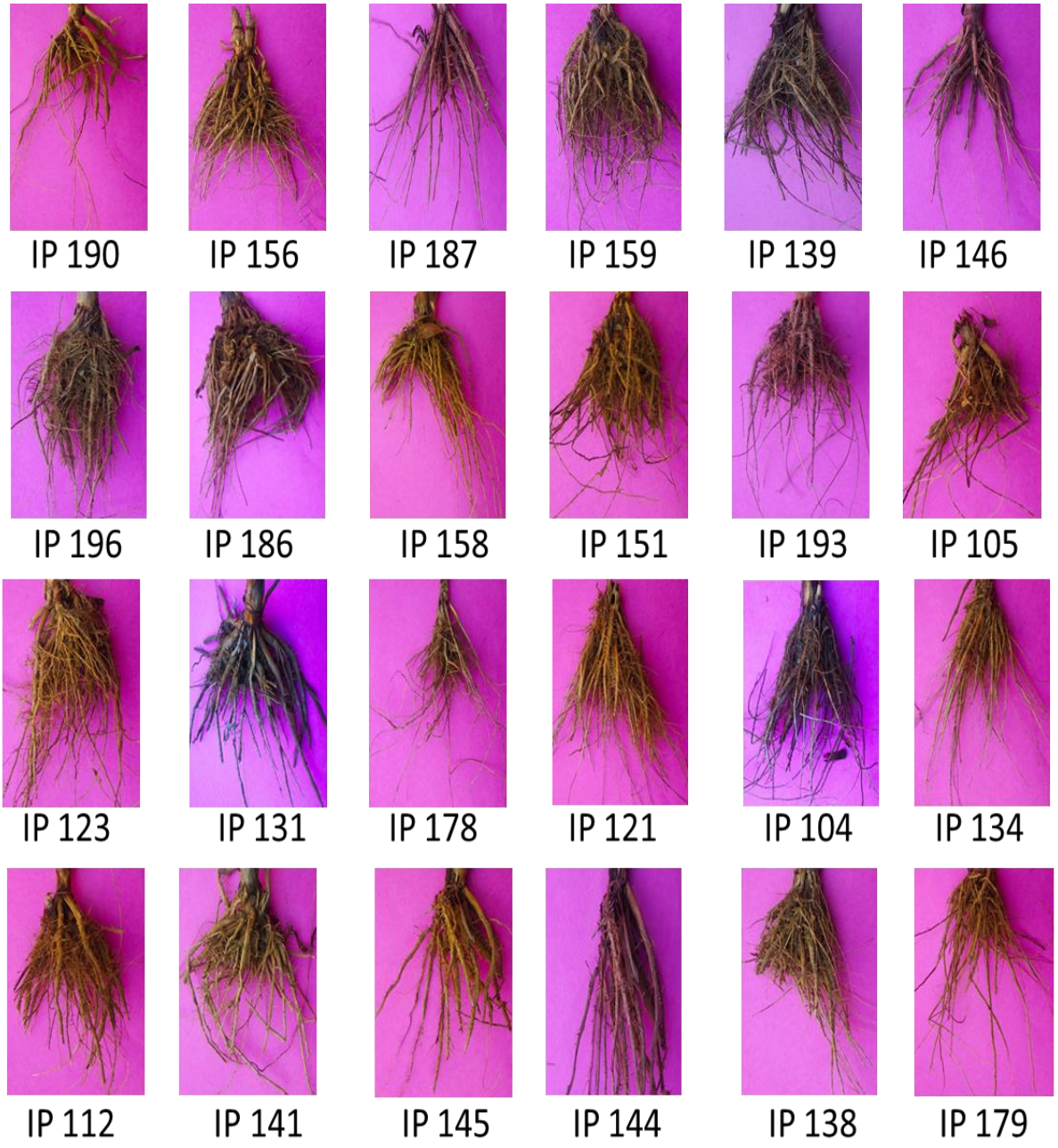

Plate 4. 49-72 Root length of pearl millet germplasm

Plate 5. 73-96 Root length of pearl millet germplasm

4.1.14 Days to 50 % flowering Initiation

Days to 50 % flowering varied significantly among 96 pearl millet genotypes in range of 47.00-56.10 days with an average mean of 50.86. Maximum numbers of days were taken to initiate 50% flowering by genotypes IP170 (56.10 days), IP 115 (54.10 days) and IP 154 (53.90 days). The minimum days to initiate 50% flowering were taken by genotypes IP 198 (47.00 days).

4.1.15 Days to 50 % Heading Initiation

Days to 50 % heading initiation differed significantly among 96 pearl millet genotypes in range of 41.1-45.5 days with an average mean of 42.97 days. Maximum numbers of days were taken to initiate 50% heading initiation by genotypes IP154 (45.5 days), IP196 (45.3 days) and IP118 (45.2 days), however the minimum days to initiate 50% heading initiation were taken by genotypes IP 159 (41.10 days).

4.1.16 Seed density of spike (cm^2)

Seed density varied significantly among ninety-six different pearl millet genotypes in range of 17.10-30.05 with an average mean of 23.66. Maximum Seed density was documented for the genotype IP120 30.05) tracked by genotypes: IP180 (29.80) and IP164 (29.70). While the minimum seed density was documented for the genotype IP 175 (17.10)

4.1.17 Numbers of tillers per plant

The mean value of numbers of tillers ranged beteen 1.20 to 2.00 with the average mean 1.56 tillers. The maximum numbers of tillers were depicted by the genotype IP154 (2.00 tiller) tracked by genotypes IP155 (2.00 tillers), IP175 (1.9), IP149 (1.90) Wheras, the tillers **in** minimum numbers were observed with genotype IP109 (1.20).

4.1.18 Days to physiological maturity

Days of Physiological Maturity of pearl millet genotypes ranged from 80.80 days to 88.40 days with average mean 83.68 days. Maximum Days of Physiological Maturity was recorded for the genotype IP 109 (88.40) intimately followed by a group of two genotypes *viz*: IP 105 (87.70) and IP 117 (87.40). The lowest Days of Physiological Maturity was recorded for the genotype IP 169 (80.80).

4.1.19 Days to maturity

Days of maturity ranged between 90.90 to 98.40 days with an average value of 95.09 days. Maximum days to maturity in days was taken by genotype IP141 (98.40) tracked by genotypes*:* IP196 (97.80) and IP 115 (97.50). However, the lowest day for maturity was recorded for the genotype IP108 (90.90g).

4.1.20 Test weight (g)

The mean value for test weight ranged 8.75g to 13.35g with the average mean 11.02g. Out of the Ninety-six pearl millet germplasms studied, THAK 1827 (13.35 g) displayed highest test weight trailed by genotypes IP156 (13.28 g) andIP 109 (13.23 g). While the minimum test weight was demnstred by genotype IP144 (8.75 g)

4.1.22 Yield (qth^{-1})

Yield is a complex character governed by large number of genes and environmental factors and their association. Grain yield value per hectare varied significantly among 96 pearl millet genotypes in range of 25.93-39.56$qtha^{-1}$ with an average value of 32.65 $qtha^{-1}$. Whereas, the maximum grain yield value per plant was documented with the genotypes THAK1827 (39.56$qtha^{-1}$) followed by IP156 (39.33$qtha^{-1}$), IP109 (39.19$qtha^{-1}$). The minimum grain yield value per plant was noted with genotype IP144 (25.93$qtha^{-1}$).

4.1.23 Biological yield (qth^{-1})

Biological yield varied significantly among ninety-six different pearl millet genotypes in range of 81.04-310.59 with an average value of 184.35 qt. Maximum biological yield value was documented with genotype IP198 (310.59$qtha^{-1}$) tracked by genotypes: IP188 (287.18 $qtha^{-1}$) and IP134 (281.85 $qtha^{-1}$).While the minimum biological yield value was observed with genotype IP143 (81.04 $qtha^{-1}$)

4.1.24 Harvest index (%)

The harvest index ranged from 10.84 to 38.87 per cent with the average mean 19.45 %. The maximum harvest index was depicted by the genotype IP143 (38.87 per cent) trailed by IP192 (36.20 per cent) and IP153 (35.78 per cent). While the minimum Harvest Index was observed for the genotype IP145 (10.84 %).

Table 4.1 Statistical analysis of morphological traits of pearl millet germplasm lines

Traits	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
DS_50	4.40	41.10	45.50	42.9677	1.07796	1.162
DAF	9.10	47.00	56.10	50.8562	1.69946	2.888
CP	5.80	31.40	37.20	33.6583	1.24703	1.555
LA	274.30	142.10	416.40	251.8792	56.66663	3211.107
PH	107.50	188.10	295.60	237.3323	20.47248	419.122
SL	24.50	16.40	40.90	25.7021	4.81369	23.172
SG	1.50	2.20	3.70	2.7344	.33113	.110
SHL	111.30	162.20	273.50	211.6427	19.94501	397.804
RL	9.40	15.10	24.50	17.8260	2.19949	4.838
RSR	.00	.10	.10	.1000	.00000	.000
SDS	13.00	17.10	30.10	23.6823	2.93003	8.585
TN	.80	1.20	2.00	1.5583	.17512	.031

DAS_50= days of 50% heading initiation, **DAF**=Days of 50% flowering initiation, **CP**= Canopy Temp., **LA**= Leaf Area, **PH**= Plant Height, **SL**=Spike length, **SG**= Spike Girth, **SHL**= Shoot Length, **RL**=Root Length, **SDS**= Seed Density, **TN**= Number of Tiller

Table 4.2 Statistical analysis of morpho-physiological traits of pearl millet germplasm lines

FW= Fresh Weight, **DW**= Dry Weight, **TW**=Turgid Weight, **RWC**= Relative Water Content, **SWD**= Saturtion Water Deficit, **DPM**= Days to physiological maturity, **DM**=Days to maturity, **TSW**= Test Weight, **YLD**=Ylyield, **BYD**= Biological Yield**, HI=**harvest Index

Paraa-meters	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
FW	260.10	112.40	372.50	208.4375	5.83303	57.15182	3266.331
DW	214.20	55.10	269.30	129.4167	4.43220	43.42649	1885.860
TW	276.50	146.20	422.70	257.7729	6.33282	62.04872	3850.044
RWC	57.70	28.80	86.50	61.0698	1.25318	12.27858	150.764
SWD	57.70	13.50	71.20	38.9302	1.25318	12.27858	150.764
DPM	7.60	80.80	88.40	83.6833	.16832	1.64922	2.720
DM	7.60	80.80	88.40	83.6833	.16832	1.64922	2.720
PPH	6.50	22.00	28.50	25.2708	.13264	1.29963	1.689
TSW	4.60	8.80	13.40	11.0302	.11128	1.09035	1.189
YLD	13.70	25.90	39.60	32.6552	.32952	3.22865	10.424
BYD	229.60	81.00	310.60	184.3510	5.44741	53.37351	2848.731
HI	28.10	10.80	38.90	19.4521	.67164	6.58071	43.306

Correlation coefficient analysis of morpho-physioolgical traits

Days to 50 % heading initiation is highly negatively and significantly correlated with days to 50 % flower initiation (r= -0.459) at 1% level of significance. Days to 50 % flower initiation negatively and significantly correlated with shoot length (r= -0.201) and had positively significant correlation with numbers of tiller (r= 0.235) at 5% significance level. Leaf area is positively and significantly correlated with spike girth (r= 0.249) at 5% level of significance. Plant height is highly, positively and significantly correlated with shoot length (r= 0.972) at 1 % significance level. While It had positively significantly correlated with spike length (r= 0.225).

Fresh weight is positively significantly correlated with dry weight (r= 0.859), turgid weight (r= 0.942) and biological yield (r= 0.806) at 1% significance level and highly and negatively correlated with saturation water deficit (r=0.410) and harvest index (r= -0.700) at 1% level oof significance. Dry weight is highly and significantly correlated with turgid weight (r= 0.856), biological yield (r=0.900) and harvest index (r= 0.835) at 1% significance level and plant population at harvesting (r= 0.232) at 5% significance level. While turgid weight is negatively and significantly correlated with harvest index (r= -0.710) and positively and significantly correlated with biological yield (r= 0.799) at 1% significance level. Whereas relative water content is highly, negatively and significantly correlated with saturation water deficit (r= -1.000) at 1% significance level. Days of physiological maturity is positively and significantly correlated with days of maturity (r= 1.000) at 1% significance level. Plant population at harvesting is negatively and ignificantly correlated with harvest index (r= -0.217) and positively and significantly correlated with biological yield (r= 0.236) at 5 % significance level. Test weight is positivelyand significantly correlated with yield (r= 1.000) and harvest index (r= 0.271) at 1% level of significance. Yield had positive and significant correlation with harvest index (r= 0.274) at 1% significance level. While biological yield is negatively and significantly correlated with harvest index (r= -0.893) at 1 % significance level.

Table 4.3 Correlation coefficient among different morpho-physiological traits of pearl millet germplasm lines

	DAS_50	DAF	CP	LA	PH	SL	SG	SHL	RL	SDS	NT
DAS_50	1	.459**	.095	-.061	-.162	-.027	-.174	-.160	.064	-.164	.110
DAF		1	.007	-.004	-.156	.170	-.176	-.201*	.004	-.003	.235*
CP			1	-.112	.076	.144	.027	.043	.063	.062	-.136
LA				1	.122	.018	.249*	.121	-.071	.088	.118
PH					1	.225*	.157	.972**	-.018	-.004	-.031
SL						1	.132	-.010	-.140	-.087	.055
SG							1	.129	.051	-.005	.014
SHL								1	.015	.017	-.045
RL									1	-.081	.127
SDS										1	-.095
NT											1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

DAS_50= days of 50% heading initiation, **DAF**=Days of 50% flowering initiation, **CP**= Canopy Temp., **LA**= Leaf Area**, PH=** Plant Height, **SL**=Spike length, **SG**= Spike Girth, **SHL**= Shoot Length, **RL**=Root Length, **SDS**= Seed Density, **NT**= Number of Tiller

Table 4.4 Correlation coefficient among morpho-physiological traits of pearl millet germplasm lines

	FW	DW	TW	RWC	SWD	DPM	DM	PPH	TSW	YLD	BYD	HI
FW	1	.859**	.942**	.410**	-.410**	.053	.053	.129	.032	.030	.806**	-.700**
DW		1	.856**	.001	-.001	.018	.018	.232*	-.073	-.075	.900**	-.835**
TW			1	.156	-.156	-.002	-.002	.134	.039	.036	.799**	-.710**
RWC				1	-1.000**	.134	.134	-.085	.127	.130	.042	.059
SWD					1	-.134	-.134	.085	-.127	-.130	-.042	-.059
DPM						1	1.000**	.013	.063	.063	.031	-.006
DM							1	.013	.063	.063	.031	-.006
PPH								1	-.012	-.015	.236*	-.217*
TSW									1	1.000**	.045	.271**
YLD										1	.045	.274**
BYD											1	-.893**
HI												1

*. Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

FW= Fresh Weight, **DW=** Dry Weight, **TW**=Turgid Weight, **RWC**= Relative Water Content, **SWD**= Saturtion Water Deficit, **DPM**= Days to Physiological Maturity, **DM**=Days to Maturity, **TSW**= Test Weight, **YLD**=Ylyield, **BYD**= Biological Yield, **HI**=harvest Index.

4.2 Cluster analysis of morpho-physiological traits

Cluster analysis of morpho-physiological traits for pearl millet 96 germplasms was done on the basis of similarity using NTSYS ver 2.0 software. Dendrogram formed two clusters one major and one one minor. Minor cluster had one genotype *i.e*., IP 104 and major cluster had 95 germplasm lines which further divided in to groups one minor cluster and one major cluster. Minor cluster consist 16 germplasm lines *namely*; THAK1827, IP107, IP231, IP140, IP291, IP139, IP283, IP236, IP274, IP196, IP146, IP230, IP163, IP198, IP147 and IP114. Major cluster had 83 germplasm lines and it further divided in to two groups one major and one minor. Minor cluster obtain 21 germplasm lines including IP193, IP188, IP105, IP101, IP119, IP167, IP154, IP 129, IP156, IP122, IP152, IP165, IP153, IP143, IP141, IP149, IP109, IP108, IP120, IP169 and IP183. Major cluster consist 61 germplasm lines which again divided into two clusters one minor and one major. Minor cluster had 22 germplasm lines *namely*; IP115, IP116, IP110, IP142, IP133, IP199, IP131, IP123, IP138, IP159, IP 186, IP130, IP189, IP126, IP126, IP162, IP132, IP134, IP145, IP262, IP177, IP168, IP133 and IP195. While major cluster had 36 germplasm lines *i.e*., IP160, IP171, IP121, IP111, IP192, IP185, IP106, IP178, IP164, IP136, IP117, IP158, IP112, IP180, IP104, IP150, IP172, IP166, IP155, IP161, IP137, IP151, IP135, IP174, IP128, IP127, IP182, IP175, IP179, IP181, IP187, IP194, IP190, IP144 and IP170.

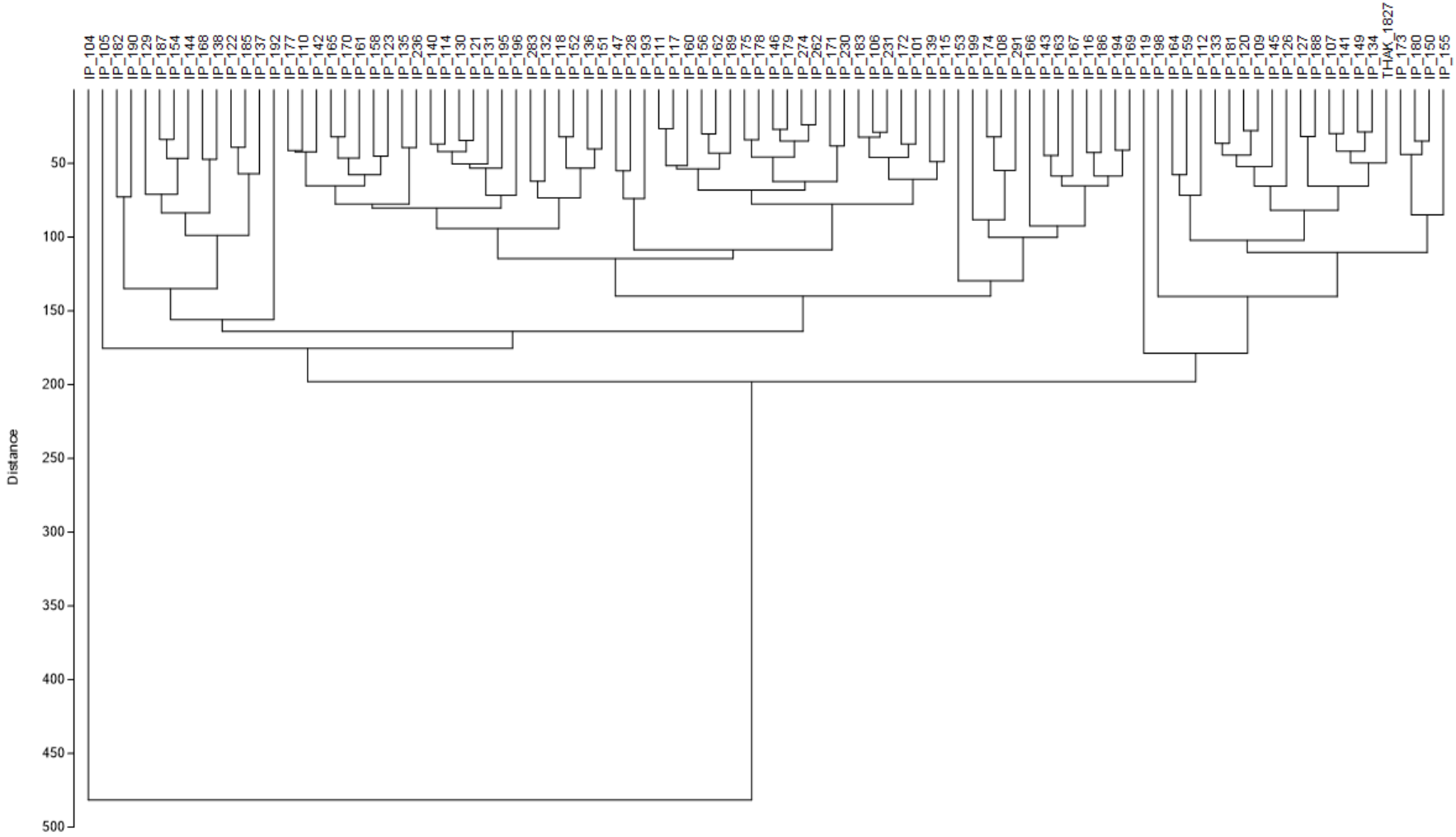

Fig 4.2 Dendrogram of pearl millet germplasm lines based on different mprpho-physiological traits

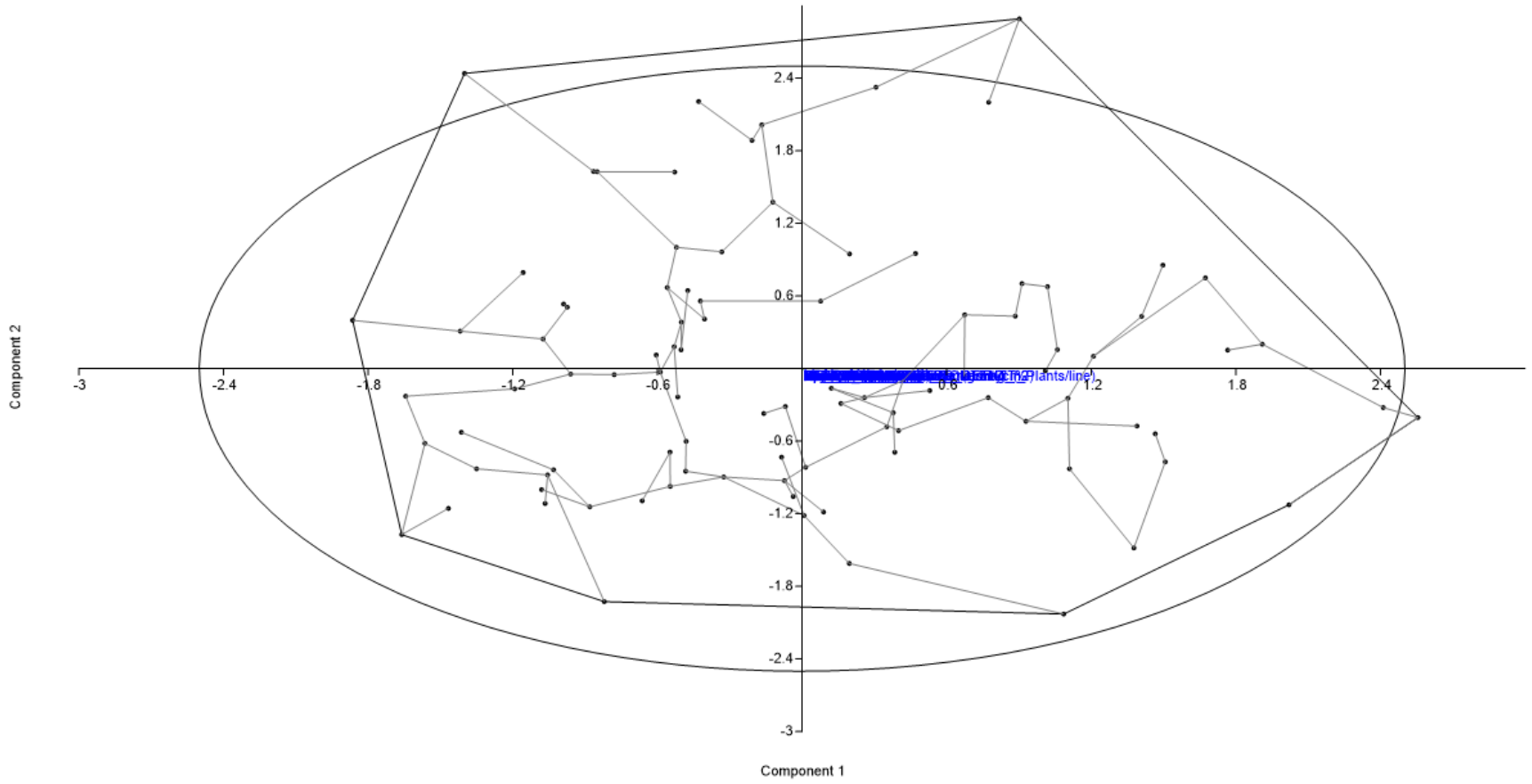

Fig 4.3 PCA diagram of pearl millet germplasm lines based on different mprpho-physiological traits

4.3 Analysis of biochemical parameters

In the current study, different biochemical parameters like total chlorophyll content, proline content, soluble sugar and protein percentage in immature seeds (under developmental phase) were measured in ninety-six pearl millet germplasm lines.

4.3.1 Chlorophyll content (mgg^{-1} fr.wt.)

Data depicted reveals that there was presence of significant number of variations in chlorophyll content among ddiffrent germplasm lines. Chlorophyll content taken at 30 days ranged from 1.31 mgg^{-1} to 4.69 mgg^{-1} with average value of 2.29 mgg^{-1}**.** Out of the ninety-six pearl millet germplasm lines studied, genootpe IP194 (4.69 mgg^{-1} fw) conained highest chlorophyll content followed by IP168 (4.35 mg g^{-1} fw), IP161 (4.13 mg g^{-1} fw) and minimum chlorophyll content depited by genotype IP127 (1.31 mg g^{-1} fw).

Chlorophyll content at 60 days varied between 1.46 mgg^{-1} to 3.84 mgg^{-1} with an average of 3.02 mgg^{-1}. Out of the ninety-six germplasm lines studied, genotypes IP161 (3.84 mg g^{-1} fw) depicted highest chlorophyll content chaseed by IP165 (3.83 mg g^{-1} fw) and IP143 (3.80 mg g^{-1} fw) while minimum chlorophyll content displayed by genotype IP127 (1.46 mg g^{-1} fw).

4.3.2 Carotenoid content (mgg^{-1} fr.wt.)

Carotenoid content at 30 days ranged from 4.5 mgg^{-1} to 11.44 mgg^{-1} with an average of 7.23 mgg^{-1}. The highest carotenoid content at 30 days was recorded for the genotype IP194 (11.44 mgg^{-1}) trailed by genotypes IP159 (10.61 mgg^{-1} fw) and, IP192 (10.57 mgg^{-1} fw) and minimum carotenoids content demonstrated by genotype IP154 (4.5 mgg^{-1} fw).

Carotenoid content at 60 days differed from 5.001 mgg^{-1} to 10.10 mgg^{-1}with mean worth of 6.66 mgg^{-1}. The highest carotenoid content at 60 days was recorded for the genotype IP164 (10.10 mg g^{-1} fw) tracked by Thak1827 (8.93 mgg^{-1} fw), IP 274(8.82 mg g^{-1} fw) and minimum carotenoids content was demostrated by genootype IP154 (5.01 mgg^{-1} fw).

4.3.3 Total soluble sugars (TSS) content (mgg^{-1} fr. Wt.)

Soluble sugars are the key osmotic adjustment substances and important indicators of drought tolerance. The mean value of this character varied from 1.0 mgg^{-1}fr.wt.10

to 2.20 mgg^{-1}fr.wt with the average mean of 1.70. On the basis of mean performance, the highest TSS content was recorded in IP133, IP144, IP181, IP136, IP196, IP262 (2.20 mg g^{-1}fr. Wt.) and minimum TSS content was recorded for the genotpe IP 173 (1.10 mg g^{-1}fr. Wt.)

4.3.4 Proline content (mg g^{-1} fr. Wt.):

Proline is believed as an imperative drought tolerance indicator and estimated in ninety-six genotypes during the present investigation. A reference to the data revealed that there was a significant variation in proline content of seven pearl millet germplasm studied. The mean value of this character ranges from 0.10 to 0.17 mg g^{-1}fr. wt. with the average mean of 0.13. Highest proline content was recorded in genootypes IP147folloowed by IP160, IP106, IP187 and IP121 and minimum in genotypes IP134 followed by IP162, IP274, IP2426 and IP291 (0.10 mg g^{-1}fr. wt).

4.3.5 Protein content (mgg^{-1} fr. wt.)

Protein content varied significantly among 96 pearl millet genotypes in range of 9.2-26.60 mgg^{-1} with an average of 13.02 mgg^{-1}. Maximum protein content was recorded foor the genotype IP130 (16.60 mgg^{-1} fr. wt.) tracked by genotypes IP126 (16.6 mgg^{-1}), IP110 (16.2 mgg^{-1}) and minimum in genotype IP188 (9.2 mgg^{-1}).

Table 4.5 Statistical analysis of different biochemical parameters of pearl millet germplasm lines

Parameters	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
CH_{30}	3.40	1.30	4.70	2.8979	.06733	.65966	.435
CA_{30}	6.90	4.50	11.40	7.2313	.14388	1.40969	1.987
CH_{60}	2.30	1.50	3.80	3.0156	.05086	.49828	.248
CA_{60}	5.10	5.00	10.10	6.6615	.11581	1.13466	1.287
Prolien	.07	.10	.17	.1273	.00230	.02255	.001
Sugar	1.10	1.10	2.20	1.6969	.03037	.29752	.089
Protein	7.40	9.20	16.60	13.0208	.17031	1.66865	2.784

CH_{30}= Chlorophyll 30 days, **CA_{30}**= Carotenoid 30days, **CH_{60}**= Chlorophyll 60 days, **CA_{60}**= Carotenoid 60days

Correlation coefficient analysis biochemical parameters

Chlorophyll at 30 days is highly, positively and significantly correlated with carotenoid 30 days (r= 0.537) and chlorophyll at 60 days (r= 0.885) at 1% level of significance and protein (r= 0.213) at 5 % significance level. Carotenoid at 30 days had positive and significant correlation with chlorophyll at 60 days (r= 0.425) and carotenoid at 60 days (r= 0.354).

4.4 Dendrogram based on different biochemical parameters

Dendrogram analysis of 96 pearl millet germplasm lines based on similarity between these germplasm lines on the basis of different biochemical parameters *i.e*., proline, sugar and protein were depicted by using NTSYS ver 2.0 software. On the basis of dendrogram pearl millet germplasm grouped into two clusters one major and one minor. Major cluster had one genotype *i.e.,* IP104 and major cluster consist 95 germplasm lines that further divided into two groups one major and one minor. Minor cluster consist only one germplasm *viz*., IP105 and major cluster had 72 germplasm lines and again divided into two groups one major and minor. Minor cluster had 12 germplasm lines *namely*; IP182, IP190, IP129, IP187, IP154, IP144, IP168, IP138, IP122, IP185, IP137 and IP192. Major cluster had 60 germplasm lines which further grouped into two clusters one major and one minor. Minor cluster consist 13 germplasm lines including IP153, IP199, IP174, IP108, IP291, IP166, IP143, IP163, IP167, IP166, IP186, IP194 and IP169 and major cluster consist 47 germplasm lines *i.e*., IP177, IP110, IP142, IP165, IP170, IP161, IP158, IP123, IP135, IP236, IP140, IP114, IP130, IP121, IP131, IP195, IP196, IP283, IP132, IP118, IP152, IP136, IP151, IP147, IP128, IP193, IP111, IP117, IP160, IP158, IP162, IP189, IP175, IP178, IP146, IP179, IP274, IP262, IP171, IP230, IP183, IP108, IP231, IP172, IP101, IP139 and IP115.

Table 4.6 Correlation coefficient among different biochemical parameters of pearl millet germplasm lines

Correlations							
Parameters	**CH_{30}**	**CA_{30}**	**CH_{60}**	**CA_{60}**	**Prolein**	**Sugar**	**Protein**
CH_{30}	1	.537**	.885**	-.097	.131	.097	.213*
CH_{30}		1	.425**	.354**	.157	-.137	-.016
CH_{30}			1	-.173	.075	.073	.197
CH_{30}				1	-.072	-.191	-.015
CH_{30}					1	-.099	.085
CH_{30}						1	.111
CH_{30}				•			1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

CH_{30}= Chlorophyll 30 days, **CA_{30}**= Carotenoid 30days, **CH_{60}**= Chlorophyll 60 days, **CA_{60}**= Carotenoid 60days

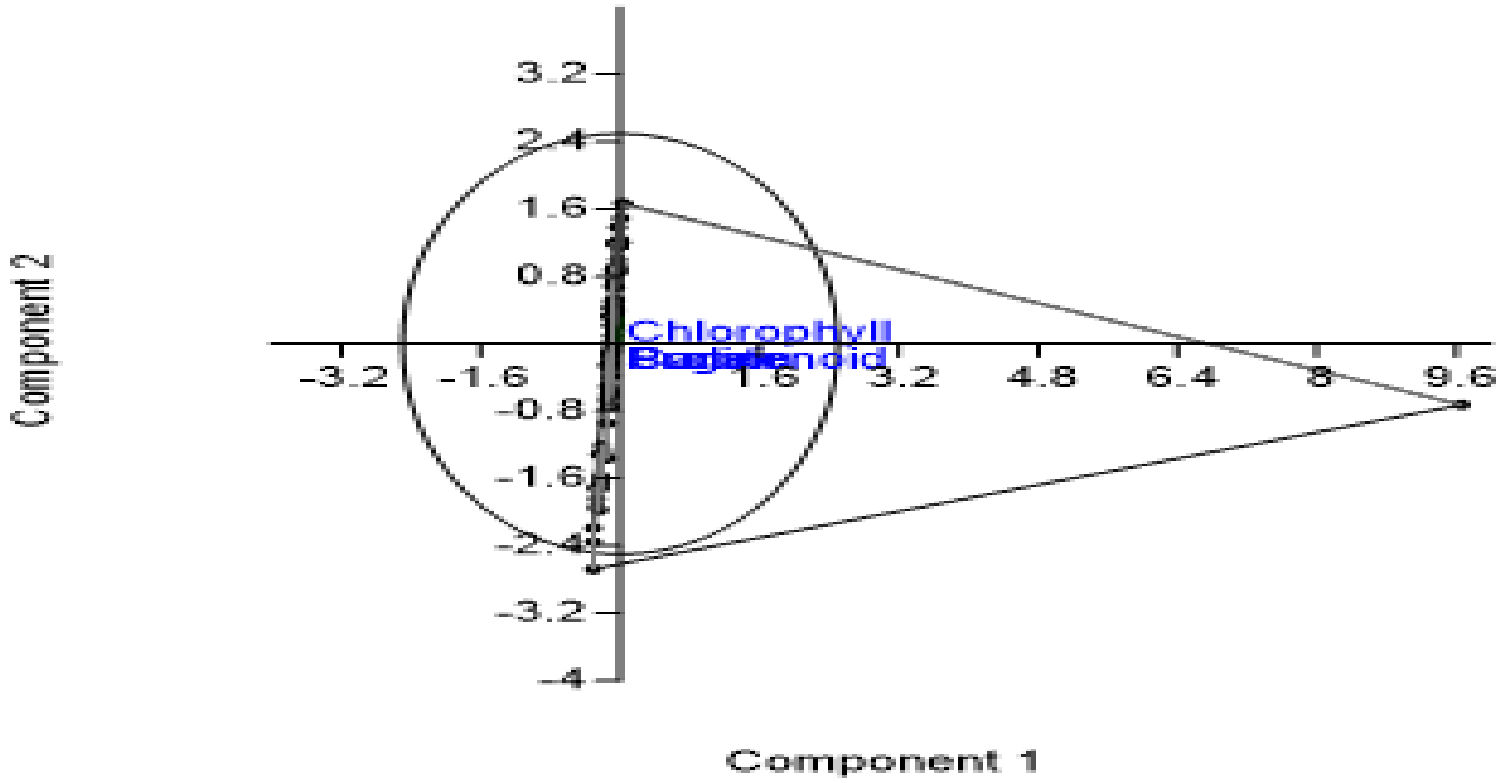

Fig 4.4 Diagram of pearl millet germplasm lines based on Chlorophyll and carotenoid attributes

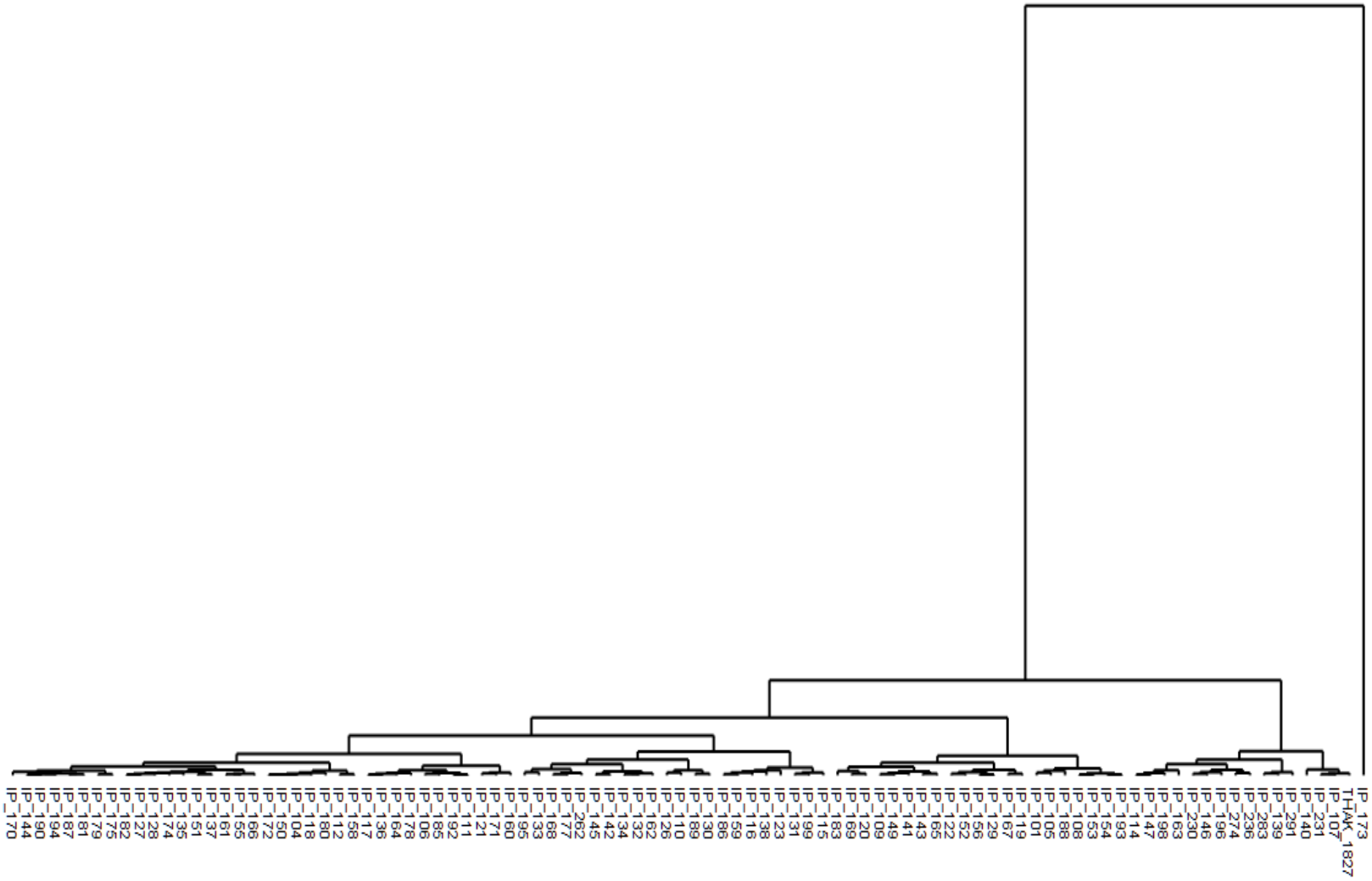

Fig 4.5 Dendogram of pearl millet germplasms based on different biochemical parametrs

4.5 Validation of gene-linked SSR markers against drought in pearl millet

In the present investigation, a total of 35 reported gene-linked SSR markers were used for validation in check variety THAK1827and 4 germplasm lines including IP132, IP199, IP135 and IP 177 of pearl millet against drought tolerance initially.

High quality gnomic DNA was used for validation. The quantification of extracted DNA was done by measuring absorbance at 260 nm wavelengths. Purity of DNA was checked by reading absorbance ratio of A260/280 for protein contamination and also the quantitative and qualitative analysis was done by resolving DNA on 0.8 % agarose gel. The concentrations of all samples were ranged between 400-3000 ngµl^{-1}. Working samples were prepared in different concentration by diluting with nuclease free sterile water.

Pearl millet genotypes DNA were amplified by SSR markers in Thermal Cyclers. The optimized PCR reaction mixture contained 1.0 U/µl *Taq* DNA polymerase, 0.25 mM dNTPs, 1.5 mM and 1.7 mM MgCl2. Among the various tried concentrations of DNA 5, 10, 15, 20, 25 and 30 ng per µl reaction mixture, 10ng found optimum. Lower quality of DNA resulted in less intense bands, whereas higher concentration added background effect. A working concentration of genomic DNA was 10ng/µl resulted in good amplification. The primer concentration of 5pM per reaction produced best amplification. Among different primer concentrations (5, 10, 15, 20, 25 and 30pM) tried, 5pM primer concentration was found to be optimum and gave complete amplification products. The different concentrations of $MgCl_2$ (1mM, 1.5mM, 1.7mM and 2mM) were tried because concentration of $MgCl_2$ effects on number and intensity of bands. Appropriate annealing temperature was 57°C. The Agarose gel electrophoresis (1.5 %) was used to separate amplified product of SSR primer

Twenty-two SSR molecular markers were successfully amplified out of thirty-five SSR markers across all germplasm lines and seven SSR molecular markers were found to be polymorphic and fifteen markers were monomorphic. So, for final experimentation seven polymorphic SSR molecular markers were used for amplification of all the 96 germplasm lines of pearl millet (Fig 4.10).

The range of major allele frequency value was 0.7604 to 0.9479 with the average 0.8363. The highest major allele frequency value (0.9479) was observed for

the markers Xibmsp01 chaseed by Xibmsp07 (0.8750), Xibmsp06 (0.8438), Xibmsp03 (0.8333) and Xibmsp09 (0.8333) while the lowest (0.7604) for the markers Xibmsp26 and Xibmsp29. The range of genetic diversity value was 0.0987 to 0.3644 with the average 0.2665. The highest genetic diversity value (0.3644) was demonstrated by markers Xibmsp26 and Xibmsp29 trailed by Xibmsp03 (0.2778), Xibmsp29 (0.2778), Xibmsp06 (0.2637) and Xibmsp07 (0.2188) while the lowest (0.0987) was in marker Xibmsp01. The range of PIC value was 0.0939 to 2980 with the average 0.2274. The highest PIC valuewas recoored for the markers Xibmsp26 and Xibmsp29 (0.2980) pursueed by Xibmsp03 (0.2392), Xibmsp29 (0.2392), Xibmsp06 (0.2289) and Xibmsp07 (0.1948) while the lowest for the markers Xibmsp01 (0.0939).

4.7 Cluster analysis molecular analysis

The pearl millet germplasm lines showing the genetic relationships are presented in UPGMA tree based on SSR markers. The clustering was based on genetic similarity between and among investigated pearl millet germplasm lines. Initially 96 pearl millet germplasm lines were divided into two clusters minor and major (Fig.16.). Minor cluster contained one germplasm *i.e*., IP 274 (Highly diverse). The major cluster contained 95 germplasm lines and further divided into two clusters one major and one minor. Minor cluster had five germplasm lines. Among these five germplasm lines genotypes IP132, IP156 and IP190 showed highly similarity and grouped together. In the same way, genotypes IP160 and IP164 also grouped together. The major cluster had 90 germplasm lines and again divided into two clusters: minor and major clusters. Minor cluster contain 4 germplasm lines including IP177, IP172, IP116 and IP182. Major cluster had 86 germplasm lines and further sub divided into two clusters major and minor. The minor cluster had only one germplasm *i.e*., IP101 and major had 85 germplasm *viz*., IP138, IP137, IP159, IP187, IP262, IP108, IP130, IP175, IP179, IP192, THAK 1827, IP118, IP139, IP114, IP152, IP171, IP231, IP165, IP189, IP110, IP183, IP104, IP140, IP111, IP112, IP131, IP141, IP158, IP198, IP115, IP122, IP134, IP146, IP127, IP128, IP129, IP133, IP135, IP142, IP143, IP145, IP147, IP149, IP153, IP154, IP155, IP161, IP162, IP166, IP168, IP169, IP173, IP174, IP178, IP180, IP181, IP185, IP186, IP188, IP194, IP195, IP196, IP291, IP108, IP109, IP123, IP126, IP167, IP107, IP119, IP120, IP150,IP170, IP230, IP 236, IP283, IP136, IP199, IP117, IP105, IP163, IP121, IP144, IP151 and IP193.

Table 4.7 Allele specific SSR markers presenting Major allele frequency, gene diversity and Polymorphic Information Content (PIC) in pearl millet.

Marker	Major Allele Frequency	Gene Diversity	PIC
Xibmsp01	0.9479	0.0987	0.0939
Xibmsp03	0.8333	0.2778	0.2392
Xibmsp06	0.8438	0.2637	0.2289
Xibmsp07	0.8750	0.2188	0.1948
Xibmsp09	0.8333	0.2778	0.2392
Xibmsp26	0.7604	0.3644	0.2980
Xibmsp29	0.7604	0.3644	0.2980
Mean	0.8363	0.2665	0.2274

Table: 4.7 Cluster of germplasm lines based on UPGMA tree

All the germplasm lines were grouped into following 10 different clusters based on UPGMA tree:

Cluster	Number of germplasms	Name of germplasm lines
1	1	IP 274
2	5	IP 132, IP 156, IP 190 IP 160, IP 164
3	4	IP177, IP 172, IP 116, IP 182.
4	1	IP 101
5	17	IP 138, IP 137, IP 159, IP 187, IP 262, IP 108, IP 130, IP 175, IP 179, IP 192, THAK 1827, IP 118, IP 139, IP 114, IP 152, IP 171, IP 231
6	12	IP 165, IP 189, IP 110, IP 183, IP 104, IP 140, IP 111, IP 112, IP 131, IP 141, IP 158, IP 198,
7	4	IP 115, IP 122, IP 134, IP 146
8	34	IP 127, IP 128, IP 129, IP 133, IP 135, IP 142, IP 143, IP 145, IP 147, IP 149, IP 153, IP 154, IP 155, IP 161, IP 162, IP 166, IP 168, IP 169, IP 173, IP 174, IP 178, IP 180, IP 181, IP 185, IP

		186, IP 188, IP 194, IP 195, IP 196, IP 291, IP 108, IP 109, IP 123, IP 126
9	9	IP 167, IP 107, IP 119, IP 120, IP 150, IP 170, IP 230, IP 236, IP 283
10	9	IP 136, IP 199, IP 117, IP 105, IP 163, IP 121, IP 144, IP 151 and IP 193.

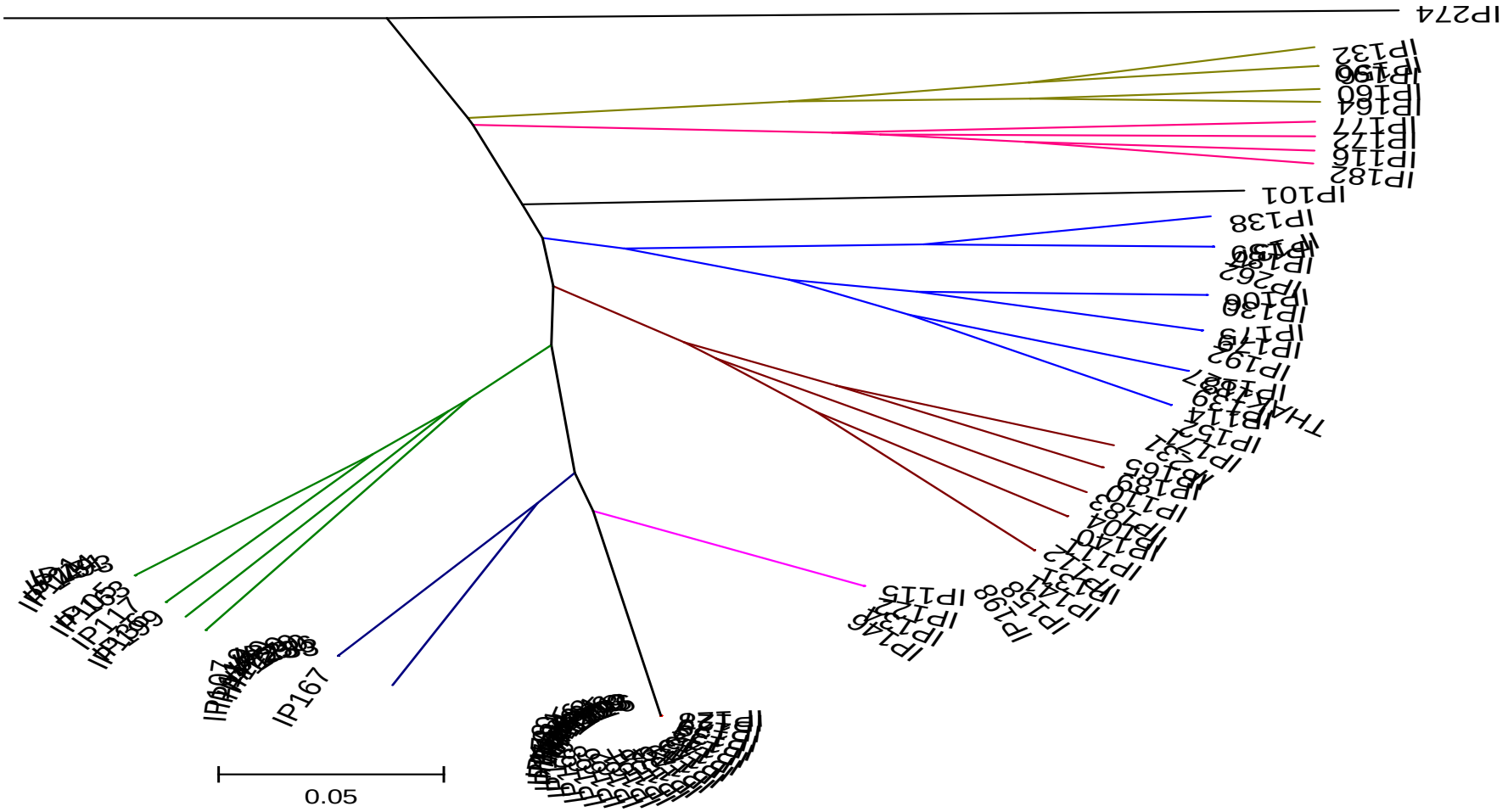

Fig. 4.6. Tree diagram of 96 pearl millet germplasm lines showing clusters-based similarity using UPGMA relationship

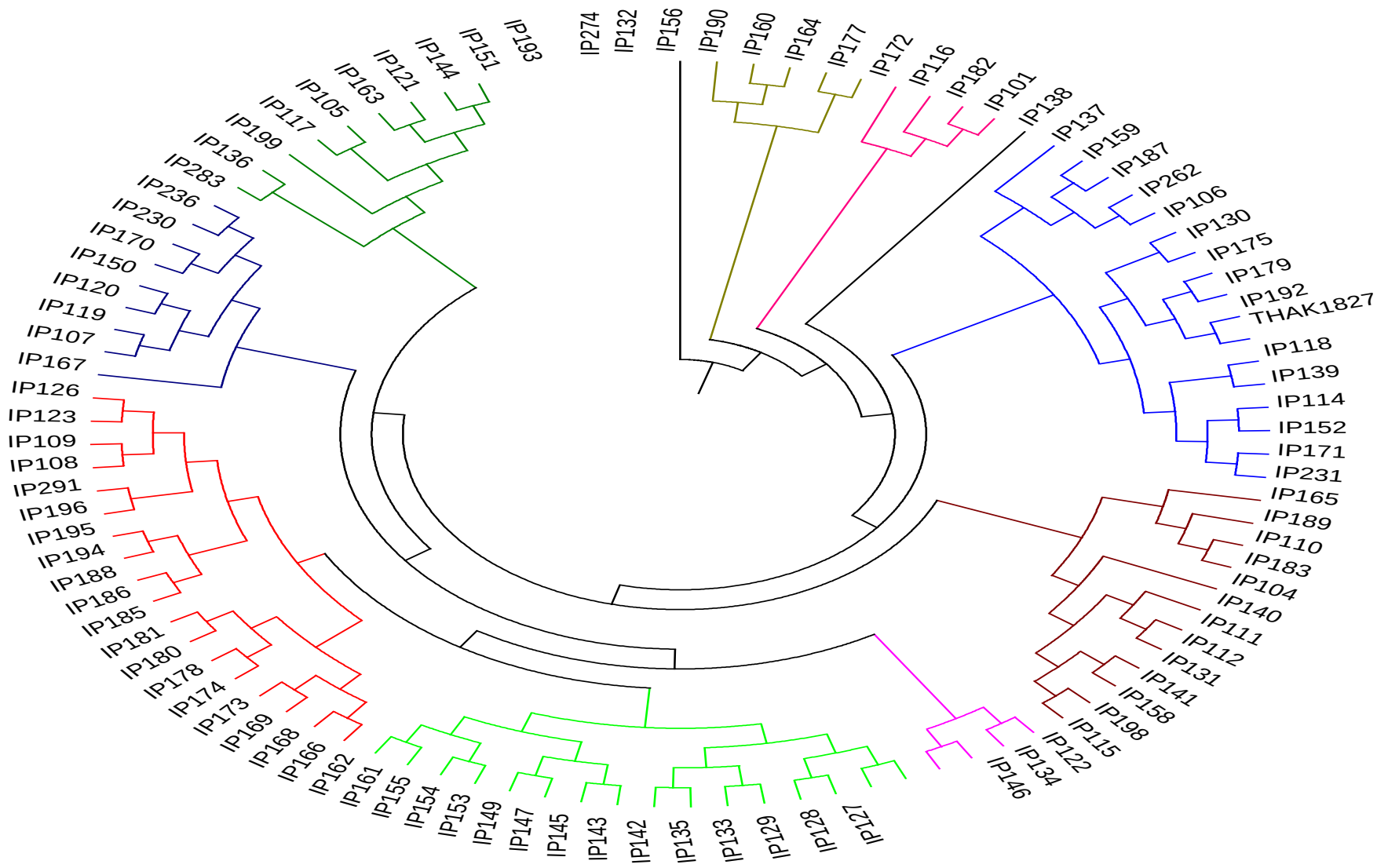

Fig. 4.7 Dendrogram of 96 pearl millet germplasm lines showing clusters based similarity using UPGMA relationship.

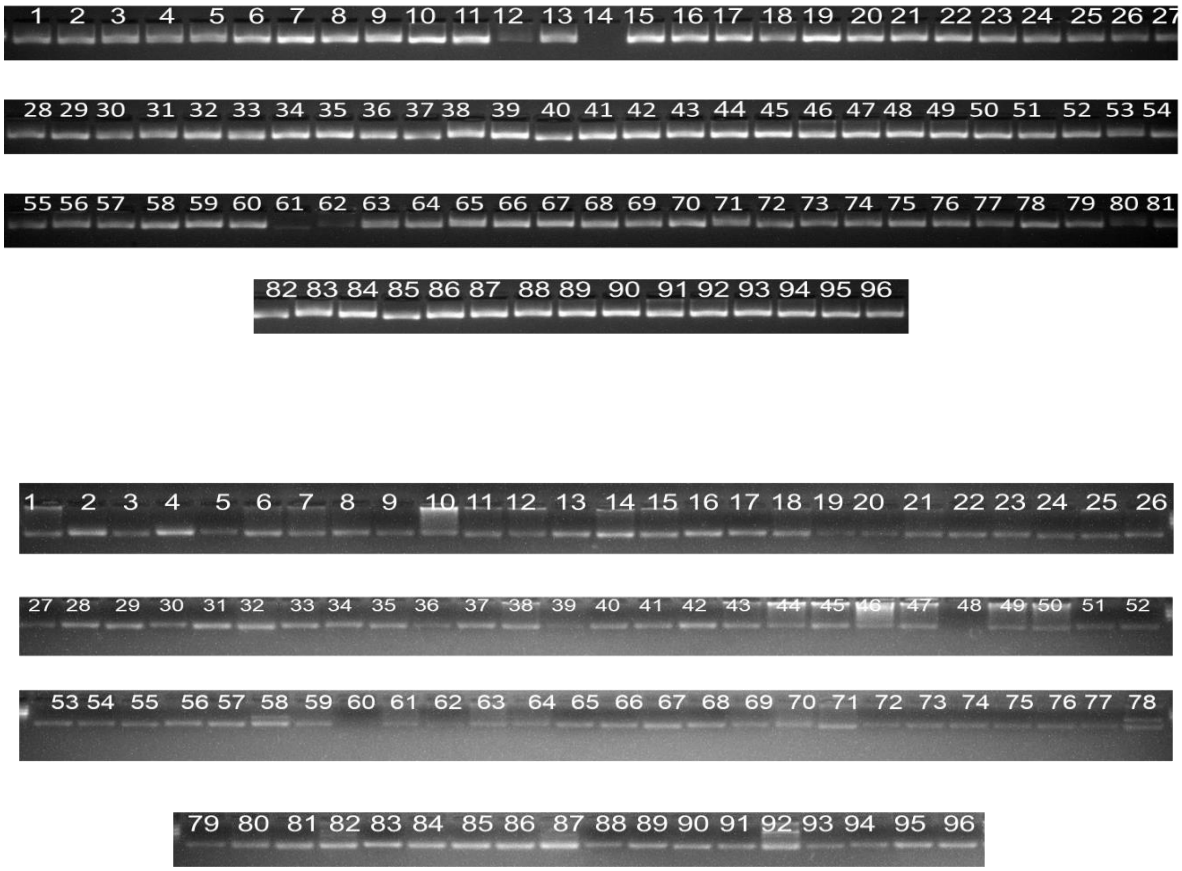

Plate 6 - Allelic variation using markers Xibmsp01 and Xibmsp29 showing polymorphism among pearl millet germplasms.

Chapter- V

DISCUSSION

Drought is the most devastating abiotic constraint affecting crop productivity, which is caused by insufficient precipitation and/or altered rainfall patterns. Drought stress causes many different physiological responses in plants. Drought, being the most important abiotic stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other abiotic factor. Pearl millet is one of cereal which has strong development of roots and tends to have effective adaptive mechanism to cope with drought. Plants respond to water deficit at cellular as well as molecular level. Therefore, the present investigation entitled **"Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene-linked SSR markers"** was undertaken in RBD during kharif season of 2019 at Research Farm and Laboratory, Department of Plant Molecular Biology & Biotechnology, college of Agriculture, Gwalior.

The current investigation results of field and laboratory experiment described in the previous chapter. In this chapter only significant differences have been discussed on various aspects of study on pearl millet [*Pennisetum glaucum* (L.) R. Br] refer to the present investigation. In the current investigation the significant variances in morpho-physiological, biochemical and molecular responses of 96 pearl millet germplasm to drought stress was evaluated and discussed as under:

5.2.1 Morpho-physiological variations among different pearl millet germplasm lines

Several morpho-physiological characters are determining processes in plants respond to drought stress. Drought effects growth, yield, membrane integrity, pigment content, osmotic adjustment water relations and photosynthetic activity (Benjamin and Nielsen, 2006; Praba *et al*., 2009). All 96 pearl millet germplasms differed significantly in plant height, days to 50% heading initiation, days to 50% flowering, leaf area, chlorophyll and carotenoid content, fresh weight, dry weight, relative water content, days to physiological maturity, plant population at harvesting, test weight, yield and biological yield.

Among the 96 different pearl millet germplasm lines studied, root-shoot ratio, relative water content, days to physiological maturity, chlorophyll content and carotenoid content are the important morpho-physiological traits to predict drought. Investigation of data presented that genotype IP177 (0.12 cm) recorded the highest root-shoot ratio chaseed by genotypes IP164 (0.115 cm) and IP154 (0.114 cm). Drought adversely affects the shoot growth and increased root development; similar result was also reported by Govindaraj *et al*. (2010).

Relative water content (RWC) and canopy temperature are important features that influence plant water relationships. RWC is considered a measure of plant water status, reflecting the metabolic activity in tissues and used as an index for dehydration tolerance. RWC is related to water uptake by the roots as well as water loss by transpiration (Anjum *et al*., 2011). RWC was recorded highest in genotypes IP188 (86.47%) tracked by IP195 (82.45%) and IP 126 (80.45). Canopy temperature was recorded highest in genotypes IP130 (37.15^{o}C) trailed by IP183 (36.95^{o}C) and IP170 (36.10^{o}C). High percentage of RWC % showed that genotypes are more tolerant against drought. Similar results also adressed by Schonfeld *et al*. (1988).

Chlorophyll is one of the most important chloroplast components responsible for photosynthesis and relative chlorophyll content has a positive relationship with photosynthetic rate. Chlorophyll content decrease in plants, showing to drought has widely been reprted. Drought stress transformed the ratio of chlorophyll ‘a’ and ‘b’ and carotenoids (Anjum *et al*., 2003; Farooq *et al*., 2009). Chlorophyll content was recorded highest in genotype IP194 (4.69 mgg^{-1} fw) chaseed by IP168 (4.35 mgg^{-1} fw), IP161 (4.13 mgg^{-1} fw). There was a general reduction in chlorophyll content on account of drought stress. Comparable results also reported by Massacci *et al*. (2008).

The observations on leaf area, days to 50% flowering and days to physiological maturity investigated that the pearl millet germplasm lines took lesser number of days to reach maturity under drought condition. The drought resulting early flowering and early maturity as well as decrease in leaf growth. Leaf area was recorded highest in genotypes IP182 (416.38 cm sq./plant). Days to 50% flowering initiation was recorded highest IP170 (56.10 days). Days of physiological maturity was recorded in range 80.80 days to 88.40 days with highest in genotype IP109 (88.40). Winkel *et al*. (1997) had reported parallel results.

Yield is a complex characteristic which governed by large number of genes, biotic and abiotic factors and their connotation. Drought stress leads to severe decline in yield. Spike length and test weight are the important qualities of yield. Droughts bring lesser spike length and low-test weight resulting decrease in yield. Investigated data were recorded as range of spike length was 16.40 to 40.90 cm, test weight was 8.75 to 13.35 g and yield were 25.93-39.56qt./ha. Similar results are in close agreements with the findings of Farooq *et al*. (2009) and Anjum et al. (2011).

5.2.2 Biochemical variations among pearl millet germplasm lines

In the present study, biochemical traits such as total soluble sugars, proline and protein were also investigated on explanation of drought stress. It was found that drought increased level of sugar, proline and protein. As recorded data of current investigation were revealed the highest TSS (2.20 mg g-1), proline (0.17 mg g-1) and protein content (16.60 mg g-1).

The accumulation of soluble sugars in plants response to drought stress is well recorded. The role of soluble sugars in plant metabolism as typical osmo protectants, stabilizing cellular membranes and maintaining turgor pressure. It was claimed that under drought conditions, sugar fluidity may even be a signal for metabolic directive. Soluble sugars are the key osmotic adjustment substances and important indicators of drought tolerance. Sugar content was recorded in range of 1.10 to 2.20 mgg^{-1} with the highest in genotypes IP133, IP144, IP181, IP136, IP196, IP262 (2.20 mgg-1fr. wt.) and minimum TSS content was recorded in fgenootype IP173 (1.10 mgg-1fr. wt.). Comparable findings have also been reported by Watanabe *et al*. (2000) and Izanloo *et al*. (2008).

Proline acts as imperious drought tolerance indicator. Interest in proline accumulation in its possible involvement in drought tolerance was stimulated when Singh et.al. (2013) indicated that proline accumulation ability of ten Barley cultivars was correlated with their grain yield. It seems to proline may play a role in minimizing the injury caused by dehydration. Proline was recorded in range 0.10 to 0.17 mg g-1fr. Similar results investigated by Mohammadkhani and Heidari (2008).

Protein synthesis responds to drought stress. Late embryogenesis abundant (LEA) proteins play an important role in the protection of plants under drought.

Protein was recorded in range of 9.2-16.60 mgg-1during present investigation. Comparable study has also done by Hadimani *et al*. (2001).

5.2.3 Molecular variations among pearl millet germplasm lines

The assessment of genetic variability on the basis of morpho-physiological and biochemical features alone might not provide an accurate classification of the genetic divergence between the genetic resources, due to the restricted number of morpho-physiological traits evaluated, environmental influence and development-specific trait appearance. Application of molecular markers having a better view for assessment of genetic variability present between the breeding materials.

Molecular markers offer great opportunity for improving the effectiveness of conventional plant breeding by selecting markers linked to the attribute rather than the trait itself. In case of drought resistance, accessibility of markers closely linked to the resistant/tolerant genes will help in detecting plants carrying these genes simultaneously. PCR based simple sequence repeats (SSRs) are often considered the most appropriate. SSRs features are single-locus markers, co-dominant, hyper variable, multi-allelic and reproducible. SSR molecular markers are considered to be model molecular genetic markers. SSR marker consists of simple tandemly repeated di to penta-nucleotide sequence motifs.

Due to high level of co-dominant inheritance, multi-allelic and reproducibility, SSR markers have been used for characteristic germplasm and analysing genetic contacts among 96 pearl millet germplas lines. SSR markers have been suitable for genetic diversity investigation among pearl millet germplasm lines by various research groups (Segal *et al*., 2012; Nehra *et al*., 2017).

In the present investigation with 96 germplasm lines to characterize the diversity at molecular level the 35 SSR molecular markers were used and presented appreciate information about genetic diversity existing in pearl millet germplasm lines. For effective genetic variability analysis, allele frequency, genetic diversity and polymorphism data content for each SSR locus were assessed. Major Allele Frequency was recorded highest with marker Xibmsp01 (0.9479) and lowest with Xibmsp01 (76.04) with a mean value of (0.8363). Genetic diversity was highest in Xibmsp01 (0.3699) and lowest in Xibmsp01 (0.987) with an average of (0.2665). The Polymorphic Information Content values were mostly decent for all the SSR loci

tested with an average 0.227 and highest for the marker Xibmsp29 (0.2980) and lowest for Xibmsp01 (0.093). Kapila *et al*. (2008) and Singh *et al*. (2013) also documented similar findings However; the lower Polymorphic Information Content (PIC) values show low allelic diversity in current investigation of pearl millet germplasm lines. The SSR allelic variability detected among pearl millet genotype in this study was low associated to earlier investigation (Nehra *et al*., 2017).

CHAPTER- VI

SUMMARY, CONCLUSION AND SUGGESTIONS

FOR FURTHER WORK

Summery

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the most important cereal crop and poor man's food. It is an important food crop for the drier parts of Africa, India and other countries of world.

The experiment entitled "Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene-linked SSR markers" was carried out at Department of Plant Molecular Biology & Biotechnology, College of Agriculture, Gwalior, RVSKVV, Gwalior during kharif 2019.Present investigation 96 pearl millet germplasm lines were screened for drought using different morphological, physiological, biochemical and molecular characterizations. Morpho-physiological characters were *viz*., days to 50 % heading initiation, days to 50 flower initiation, plant height, shoot length, root length, root shoot ratio, spike length, spike girth, seed density of spike, fresh weight, dry weight, turgid weight, relative water content, saturation water deficit, chlorophyll content, carotenoid content, leaf area, numbers of tillers, canopy temperature, days to physiological maturity, days to maturity, plant population at harvesting, test weight, yield, biological yield and harvest index.

Root/shoot ratio varied in range of 0.06 cm to 0.12with an average of 0.08. Maximum RWC value varied in range of 28.80% to 86.47% with a mean value 61.07%. Chlorophyll content at 30 days ranged from 1.31mgg^{-1} to 4.69 mgg^{-1} with mean of 2.29 mgg^{-1}, whereas chlorophyll content at 60 days varied from 1.46 mgg^{-1} to 3.84 mgg^{-1} with a mean value of 3.02 mgg^{-1}. Carotenoid content at 30 days ranged from 4.5 mgg^{-1} to 11.44 mgg^{-1} with an average of 7.23 mg. however, carotenoid content at 60 days differed from 5.001 mgg^{-1} to 10.10 mgg^{-1} with average of 6.66 mgg^{-1}.

In the present investigation, biochemical traits such as total soluble sugars, proline and protein were also investigated in the relation of drought. Total soluble

sugar varied from 1.10 to 2.20 mgg-1fr. wt. with the average value of 1.70mgg^{-1}. Proline value ranged from 0.10mgg^{-1} to 0.17 mgg-1 with the average value of 0.13. mgg-1 Protein content was varied in range of 9.2 mgg-1 -26.60 mgg-1 with an average of 13.02 mgg-1.

In the present investigation, a total of 35 reported gene-linked SSR markers were used for validation adainst drought. Twenty-two SSR molecular markers were successfully amplified out of thirty-five against drought across all germplasm lines and seven SSR markers were found to be polymorphic and fifteen markers were monomorphic. All seven polymorphic SSR markers were used for amplification of all the 96 germplasm lines of pearl millet. The range of PIC value was 0.0939 to 2980 with the average of 0.2274. The highest PIC value was recorded for the markers Xibmsp26 and Xibmsp29 (0.2980) chaseed by Xibmsp03 (0.2392), Xibmsp29 (0.2392), Xibmsp06 (0.2289) and Xibmsp07 (0.1948) while the lowest for the marker Xibmsp01 (0.0939). The range of major allele frequency value was 0.7604 to 0.9479 with the average of 0.8363. The range of genetic diversity value was 0.0987 to 0.3644 with the average of 0.2665.

Conclusion

The present investigation concluded as below:

- Genotypes *viz*: IP133, IP127, IP177, IP198, IP107, IP140, IP164, IP181, IP160, IP 166, IP194, IP195, IP126, IP190, IP196, IP158, IP178, IP121, IP110, IP236, IP230 and THAK 1827 were made their position in distinict group regarding drought by using different morpho-physiological, biochemical and gene- linked SSR molecular markers.
- The highest PIC value (0.2980) was found for the markers Xibmsp26 and Xibmsp29 among all the polymorphic markers investigated. The average PIC value was found to be 0.2274.
- The highest alleles frequency (0.9479) was found for the marker Xibmso01 ssr and average allele frequency were 0.8363. The genetic diversity average was recorded.2665.

Suggestions for further Work

Future scenarios of present study entitled “Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene- linked SSR markers” are given below:

- Genotype(s) obtained tolerance against drought may be used further in molecular breeding programmes to develop tolerant varieties. SSR molecular markers with higher PIC value may be used for screening of large numbers of germplasm line(s).
- The genotypes: IP133, IP127, IP177, IP198, IP107, IP140. IP164, IP181, IP160, IP166, IP194, IP195, IP126, IP190, IP196, IP158, IP178, IP121, IP110, IP236, IP 230 and THAK 1827 has been recommended as a donor parent for future breeding programme for development of drought tolerant genotype(s).
- The contrasting genotypes identified for drought tolerance can be used for identification of genes for drought tolerance and QTLs by development of RILs through forward genetics approaches.
- Application of some drought stress alleviating bioregulators will need further investigations.

BIBILIOGRAPHY

Abdalla A. A., Ahmed U. M., Ahmed A. R., Tinay A.H. and Ibrahim K. A. 2009. Physicochemical Characterization of Traditionally Extracted Pearl Millet Starch (Jir). Journal of Applied Sciences Research. **5**(11): 2016-2027, © 2009, insinet Publication.

Addisie, Y. and Yemane G. E. 2011. Response of pearl millet (*Pennisetum glaucum*) cultivars to post flowering drought stress. International Journal of Plant Physiology and Biochemistry 3:150-154.

Adejare F. B. and Umebese C. E. (2007). Tomatal resistance to low leaf water potential at different growth stages affects plant biomass in *glycine max* l. Unilag research repository.

Adeoti K., Africa W., Djedatin G., Ewedje E., Beule T., Rival A. and Jaligot E. (2017). Assessment of genetic diversity among cultivated Pearl millet (*Pennisetum glaucum*, Poaceae) accessions from Benin, West Africa. African Journal of Biotechnology 16, 782-790.

Alam M. K., Ogata Y., Sako Y., Al-Mamun M. and Sano H. 2010. Intermediary Metabolism of Plasma Acetic Acid, Glucose and Protein in Sheep Fed a Rice Straw-based Diet. Asian-Aust. J. Anim. Sci. Vol. 23, No. 10: 1333 - 1339.

Allouis S., Qi X., and Lindup S. (2001). Construction of a BAC library of pearl millet (*Pennisetum glaucum*). Theor Appl Genet 102, 1200–1205.

Ambawat S., Satyavathi C. T., Meena R., Khandelwal V. and Meena R. C. (2020). Molecular Characterization and Genetic Diversity Analysis of Released Hybrids and Varieties of Pearl Millet [*Pennisetum glaucum* (L.) R. Br.]. Current Journal of Applied Science and Technology. **39**(31): 92-104, 2020; Article no. CJAST.61543, ISSN: 2457-1024.

Amiribehzadi A., Satyavathi C.T., Singh S.P., Bharadwaj C. and Singh M. P. (2012). Estimation of heterosis in diverse cytoplasmic male sterile sources of Pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Annual Agricultural Research New Series **33**(4):220-227.

Animassaun A., Joseph A., Morakinyol O. T., Mustaphal and Ramar K. M. (2016). Assessment of genetic diversity in accessions of pearl millet (*Pennisetum glaucum*) and napier grass (*Pennisetum purpureum*) using micro satellite (issr) markers, Iranian journal of genetics and plant breeding, vol.4, no.1

Anju. 2005. Nutritional evaluation and product development from white and yellow pearl millet varieties. M.Sc. Thesis CCSHAU HISAR.

Anjum F., Yaseen M., Rasool E., Wahid A. and Anjum S. 2003. Water stress in barley (*Hordeum vulgare* l.). Effect on chemical composition and chlorophyll contents. Pak. J. Agri. Sci., vol. **40**(1-2).

Anjum, S.A., Xie, X., Wang, L.C., Saleem, M.F., Man, C., Lei, W., 2011. Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research **6**, 2026–2032.

Arnon I. 1972. 'Crop Production in Dry Regions.' Leonard Hill, London.

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol 24: 1–15.

Ashraf M. and Rauf H. 2001. Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts: Growth and ion transport at early growth stages. Acta Physiol Plant **23,** 407–414.

Ashraf M. 1994. Genetic variation for salinity tolerance in spring wheat. - Hereditas 120: 99-104. Lund, Sweden. ISSN 0018-0661.

Ashraf M. and Foolad M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59: 206-216.

Ashraf M., Zafar U. and Oleary J. W. 1995. Genetic variation for salt tolerance in sunflower (*Hrliunthus annuus* L.). Herrditus 123: 141- 145. Lund, Sweden. ISSN 0018-0661.

Bansal T. (2018). Development of bio-fortified pearl millet-based value-added products incorporating carrot and sesame seeds. M.Sc. Thesis, CCSHAU, Hisar, India.

Bashir E. M. A., Bali A. M., Ali A. M., Mohamed E.T.I., Melchinger A.E., Parzies H. K. and Haussmann B. I. G. 2015. Genetic diversity of Sudanese pearl millet (*Pennisetum glaucum* (L.) R. Br.) landraces as revealed by SSR markers, and relationship between genetic and agro morphological diversity. Genetic Resources and Crop Evolution 62: 579–591.

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.

Benedict B. M., Chimwamurombe P., Kwembeya E. and Maggs K. G. (2016). Genetic Diversity of Namibian *Pennisetum glaucum* (L.) R. BR. (Pearl Millet) Landraces Analyzed by SSR and Morphological Markers, Scientific World Journal 1-10.

Benjamin J. G., and Nielsen D. C. (2006). Water deficit effects on root distribution of soybean, field pea and chickpea. Field Crops Research **97**(2-3), 248–253.

Bernier J., Kumar A., Ramaiah V., Spaner D.and Altin G. 2007. A Large-Effect QTL for Grain Yield under Reproductive-Stage Drought Stress in Upland Rice. Crop Breeding & Genetic.

Bhatt R. M. and Srinivasa R. N. K. 2005. Influence of pod load on response of okra to water stress. Indian journal Plant physiol., vol. 10, no. 1, (n.s.) pp. 54-59.

Bind H., Bharti B., Kumar S., Pandey M. K., Kumar, D. and Vishwakarma D. N. (2015). Studies on genetic variability for fodder yield and its contributing characters in bajra [*Pennisetum glaucum* (L.) R. Br.]. Agricultural Science Digest 35: 78-80.

Blum A. 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive. Crop and Pasture Science. Australian Journal of Agricultural Research 56(11) 1159-1168.

Brunken J. N.1977. A systematic study of *Pennisetum sect. Pennisetum (Gramineae*). Am. J. Bot. 64, 161-176.

Buerkert A., Moser M., Kumar A. K., FuErst P. and Becker K. (2001). Variation in grain quality of pearl millet from Sahelian West Africa. Field Crops Research., 69:1-11.

Burton G. W. 1974. Factors Affecting Pollen Movement and Natural Crossing in Pearl Millet. Crop Science.

Burman U., Garg B.K., Yadav O.P. and Kathju S. (2011) Effect of terminal water stress on growth, plant water status and yield of pearl millet genotypes. Indian Journal of Plant Physiology 16:276-284.

Carnovale E., Lugaro E., and Boccia L. (1988). Phytic acid in Faba bean and pea: effect on protein availability. Cereal Chem. 65(2): 114-117.

Cattivelli L., Fulvia R., Franz-W. B., Elisabetta M., Anna M. M., Enrico F., Caterina M., Alessandro T., Stanca A.M. 2008. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics, Field Crops Research, Volume 105, Issues 1–2,2008, Pages 1-14, ISSN 0378-4290.

Chakraborty S., Thakare I., Ravikran R., Nikam V., Trivedi R., Sasidharan N. and Jadeja G C. (2011). Assessment of diversity using RAPD and ISSR markers in sorghum varieties across Gujarat, India, Elect. J. Plant Breeding, **2**(4): 488-493.

Chase A. 1921. The Linnaean Concept of Pearl Millet. American Journal of Botany, **8**(1).

Cheik A.T., Aly S., Yaya B. and Alfred T. S. 2006. A Comparative Study on Nutritional and Technological Quality of Fourteen (14) Cultivars of Pearl Millets [*Pennisetum glaucum* (L) Leeke] in Burkina Faso. Pakistan Journal of nutrition. Volume: 5 | Issue: 6 | Page No.: 512-521.

Chelpuri D., Srivastava K., Reddy N., Katiyar P., Durga K. and Kilaru. (2017). Genetic Linkage Map Construction in Pearl Millet Using SSR Markers, Int. J. Pure App. Biosci. (5): 1304-1315.

Choudhary R., Jat B. L., Anwala R., Dhikwal S. R. and Sharma K. C. (2012). Studies on estimates of genetic variability and character association of yield components and protein content in pearl millet. Forage Research 38: 80-85.

Clayton R. N., O'Neil J. R., and Mayeda T. K. (1972). Oxygen isotope exchange between quartz and water. Journal of Geophysical Research, **77**(17), 3057–3067.

De Wet, J. M. J. (1977). Pearl millet (Pennisetum glaucum) in Africa and India. In Proceedings, International Pearl Millet Workshop (J. R. Witcombe and S. R. Beckerman, eds.), pp. 3, 4. ICRISAT, Patancheru, India.

Demirevska K., Zasheva D. and Dimitrov R. (2009). Drought stress effects on Rubisco in wheat: changes in the Rubisco large subunit. Acta Physiol Plant 31, 1129.

Djanaguiraman M., Perumal R., Ciampitti I.A., Gupta S.K. and Prasad P. V. V. 2018. Quantifying pearl millet response to high temperature stress: thresholds, sensitive stages, genetic variability and relative sensitivity of pollen and pistil. Plant cell & environment, **41**(5): 993-1007.

Doyle J. J., and Doyle J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Duan X.J., Deng M., Zhao W.Z., and Wu K. (2007). The prolongation structure of the inhomogeneous equation of the reaction–diffusion type. Journal of Physics A: Mathematical and Theoretical, **40** (14), 3831–3837.

DuBois M., Gilles K. A., Hamilton J.K., Rebers P. K., and Smith F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. Analytical Chemistry 28 (3), 350-356.

Farooq M., Wahid A., Kobayashi N., Fujita D. and Basra S. M. A. (2009). Plant drought stress: Effects, mechanisms and management. Agron. Sustain. Dev.29: 185-212.

Faseela K.V. and Salikutty J. 2007. Molecular characterization of amaranthus landraces and assessment of interspecific relationships among *Amaranthus spp*. (L.) Using RAPD markers. Indian J. Genet.; 67:12-17

Fotovat R., Valizadeh M. and Toorchi M. 2007. Association between water-use efficiency components and total chlorophyll content (SPAD) in wheat (*Triticum aestivum* L.) under well-watered and drought stress conditions.

Govindaraj M. B., Selvi and Rajarathinam S. (2009). Correlation Studies for Grain Yield Components and Nutritional Quality Traits in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.) Germplasm, World J. Agri. Sci. **5**(6): 686-689.

Govindaraj, M., P. Shanmugasundaram, P. Sumathiand A.R. Muthiah. 2010. Simple, rapid and cost-effective screening method for drought resistant breeding in pearl millet. Elect. J. Plant Breed., 1: 590-599iological, biochemical and gene linked SSR molecular markers.

Govindaraj M., Selvi B. and Sudhir K.I. (2011). Genetic diversity studies in indigenous pearl millet [Pennisetum glaucam (L.) R. Br.] accessions based on biometrical and nutritional quality traits. Indian Journal of Plant Genetic Resources 24: 186-193.

Govt. of India. 2019. Agricultural Statistics at a glance.

Goyal V.K. 2009. "Multiple description coding: compression meets the network," in IEEE Signal Processing Magazine, vol. 18, no. 5, pp. 74-93.

Gupta S. C., Kessler A. C., Brown M. K., and Zvomuya F. (2015). Climate and agricultural land use change impacts on streamflow in the upper midwestern United States, Water Resour. Res., 51, 5301–5317.

Gupta S. K., Nepolean, T., Sankar S. M., Rathore A., Das R. R., Rai K. N. and Hash C. T. (2015). Patterns of molecular diversity in current and previously developed hybrid Parents of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. American Journal of Plant Sciences, 6:1697-1712.

Gupta S., Swinton J., and Anderson R. M. (1994). Theoretical Studies of the Effects of Heterogeneity in the Parasite Population on the Transmission Dynamics of Malaria. Proceedings of the Royal Society B: Biological Sciences, **256**(1347), 231–238.

Goyal V., Jain S., Bishnoi N. R. and Munjal R. 2001. Leaf water relations, diffusive resistance and proline accumulation in hybrid pearl millet under depleting soil moisture content. Dianj plant physiol., vol. 6. No. I. (n.s.) pp. 41-45.

Gur U. D., Mustafa O., Abdullah K. and Osman C. (2010). Diurnal gradual heat stress affects antioxidant enzymes, proline accumulation and some physiological components in cotton (*Gossypium hirsutum* L.). Afr. J. Biotech.9: 1008-1015.

Hadimani, N.A., Muralikrisna, G., Tharanathan, R.N., Malleshi, N.G., 2001. Nature of carbohydrates and proteins in three pearl millet varieties varying in processing characteristics and kernel texture. Journal of Cereal Science 33, 17–25.

Harland B. F., and Oberleas D. 1987. Phytate in Foods. World Review of Nutrition and Dietetics, 235–259.

Haussmann M. F., Longenecker A. S., Marchetto N. M., Juliano S. A. and Bowden R. M. 2012. Embryonic exposure to corticosterone modifies the juvenile stress response, oxidative stress and telomere lengthproc. R. Soc. B.2791447–1456.

Haussmann R., Rantner W., Cerrito S., and Zwerger W. (2007). Thermodynamics of the BCS-BEC crossover. Physical Review A, **75**(2).

Horling F., Lamkemeyer P., König J., Finkemeier I., Kandlbinder A., Baier M., Dietz K.J. 2003. Divergent Light-, Ascorbate-, and Oxidative Stress-Dependent Regulation of Expression of the Peroxiredoxin Gene Family in Arabidopsis. Plant Physiology.

Hu H. and Xiong L. 2014. Genetic Engineering and Breeding of Drought-Resistant Crops. Annual Review of Plant Biology Vol. 65:715-741.

Ihsan M. Z., Ismail S. M., El-Nakhlawy F. S., Fahad S.and Daur I. (2016). Wheat Phenological Development and Growth Studies as Affected by Drought and Late Season High Temperature Stress under Arid Environment. Front. Plant Sci ence.

Irfan A.S., Hidayat U.R., Syed M.A.S., Zahir S., Saeedur R., Ihteramullah and Muhammad N. (2012). Characterization of pearl millet germplasm various morphological and fodder yield parameters. Pakistan journal of Botany **44**(1):273-279.

Iwuala E., Odjegbab V., Sharmac V. and Alamc A. (2020). Drought stress modulates expression of aquaporin gene and photosynthetic efficiency in *Pennisetum glaucum* (L.) R. Br. Genotypes. Current Plant Biology volume 21,100:131.

Iwuala E. N., Odjegba V. J., Sharma V. and Alam, A. (2020). Highlights of strategies adapted by two *Pennisetum glaucum* (L.) R. Br. races in a simulated drought stress experiment. Plant Gene, 100238.

Izanloo, A., Condon, A.G., Langridge, P., Tester, M., Schnurbusch, T., 2008. Different mechanisms of adaptation to cyclic water stress in two South Australian bread wheat cultivars. J. Exp. Bot. **59**, 3327–3346.

Jafar S.A. and Goldsmith A. 2004. "Transmitter optimization and optimality of beamforming for multiple antenna systems," in IEEE Transactions on Wireless Communications, vol. 3, no. 4, pp. 1165-1175.

Jangra S., Rani A., Yadav R. C., Yadav N. R. and Yadav D. (2019). Introgression of terminal drought stress tolerance in advance lines of popular pearl millet hybrid through molecular breeding. Indian Society for Plant Physiology.

Jika A.N., Dussert Y., Raimond C., Garine E., Luxereau A., Takvorian N., Djermakoye R., Adam T. and Robert T. 2017. Unexpected pattern of pearl millet genetic diversity among ethno-linguistic groups in the Lake Chad Basin. Heredity. 118: 491–502.

Jones H. G., Lakso A. N. and Syvertsen J. P. 1985. Physiological control of water status in temperate and subtropical fruit trees. -Hortic. Rev. 7: 301-344.

Kapila R. K., Yadav R. S., Plaha P., Rai K. N., Yadav O. P., Hash C. T. and Howarth C. J. (2008). Analysis of genetic diversity in pearl millet inbreds using microsatellite markers. Plant Breeding, 127: 33-37.

Kaushik J., Vart D., Kumar A. and Kumar R. 2018. Phenotypic diversity in pearl millet [*Pennisetum glaucum* (L.) R.Br.] germplasm lines. International Journal of Chemical Studies, **6**(5):1169-1173.

Khairwal I.S., Yadav S.K., Rai K. N., Upadhyaya H. D., Kachhawa D. Nirwan B., Bhattacharjee R., Rajpurohit B.S., Dangaria C. J. and Srikant. (2007). Evaluation and identification of promising pearl millet germplasm for grain and fodder traits. Journal of SAT Agricultural Research, **5** (1). Pp. 1-6. ISSN 0973-3094.

Kholová J, Vadez V. and Hash CT. 2008. Mechanisms underlying drought tolerance in pearl millet (*Pennisetum americanum* L.). 5th International Crop Science Congress, Jeju, South Korea Book of abstracts.

Kholová J, Hash CT, Kakkera A, Kočová M, Vadez V. Constitutive water conserving mechanisms are correlated with the terminal drought tolerance of pearl millet [*Pennisetum glaucum* (L.) R. Br.], Journal of Experimental Botany, 2010, vol. 61 (pg. 369-377).

Kishor PBK, Hong Z., Miao G.H., Hu CAA. and Verma DPS.1995. Overexpression of [delta]-Pyrroline-5-Carboxylate Synthetase Increases Proline Production and Confers Osmotolerance in Transgenic Plants. Plant Physiology.

Knuckles E., Kuzmicky D. and Betschart A. (1985). Effect of phytate and partially hydrolyzed phytate on in vitro protein digestibility. Journal of food science, **50**(4), 1080–1082.

Kotak S., Larkindale J., Lee U., Kuskull D. P. V., Vierling E., Dieter S. K. 2007. Complexity of the heat stress response in plants. Current Opinion in Plant Biology volume 10 issue 3, page 310 – 316.

Kumari M., Garg D.K., Saini R.S. and Jat B.L. 2018. Genetic variability and correlation coefficient in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Forage Research **39**(2):83-87.

Kumari M., Garg D. K., Saini R S. and Jat B. L. (2013). Genetic variability and correlation coefficient in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Forage Research, 39: 83-87.

Kumar S., Hash C. T., Singh G., Basava R. K. and Srivastava R. K. (2020). Identification of polymorphic SSR markers in elite genotypes of pearl millet and diversity analysis. Ecological Genetics and Genomics 14 (2020) 100051.

Kumawat K., Sharma N.K. and Nemi chand. 2019.Genetic variability and character association analysis in pearl millet single cross hybrids under dry conditions of Rajasthan. Electronic Journal of Plant Breeding, 10(3):1067-1070.

Kusaka M., Lalusin A. G. and Fujimuram T. 2005. The maintenance of growth and turgor in pearl millet (*Pennisetum glaucum* L. Leeke) cultivars with different root structures and osmo-regulation under drought stress. Plant Sci. 168:1-14.

Landjeva S., Neumann K., Lohwasser U., Borner A. 2008. Molecular mapping of genomic regions associated with wheat seedling growth under osmotic stress. Biologia Plantarum 52, 259-266.

Linsley R. K., Kohler M. A., and Paulbus J.L.H.1958. Hydrology for Engineers, 340 pp., McGraw; Hill, New York.

Lisar S., Motafakkerzad R., Hossain M. and Rahman I. M. 2012. Water stress in plant: causes, effects, responses. Water stress.

Liu C. J., Witcombe J. R., Pittaway T.S., Nash M., Hash C. T., Busso C.S. and Gale M. D.1994. An RFLP.based genetic map of pearl millet (*Pennisetum glaucum*). Theor Appl Genet 89:481-487.

Lobell D. B., Schlenker W., and Costa-Roberts J. (2011). Climate Trends and Global Crop Production Since 1980. Science, 333(6042), 616–620.

Lowry O. H., Rosebrough N. J., Farr A.l. and Randall R. J. 1951. Protein measurement with the folin phenol reagent. The journal of biological chemistry.

Maciel and Tabosa. 1982. Competição de Cultivares de Capim-Elefante (*Pennisetum purpureum*, Schum.) E de Híbridos de Capim-Elefante x Milheto (*Pennisetum americanum* (L.) Leeke) sob Pastejo. Rev. Bras. Zootec., v.28, n.5, p.936-946.

Mahajan S., and Chauhan B. M. (1987). Phytic acid and extractable phosphorus of pearl millet flour as affected by natural lactic acid fermentation. Journal of the Science of Food and Agriculture, **41**(4), 381–386.

Manivannan P., Jaleel C.A., Kishore K. A., Sankar B., Somasundaram R., Sridharan R. and Panneerselvam R. (2007). Changes in antioxidant metabolism of Vigna unguiculata L. Walp. by propiconazole under water deficit stress. Colloids Surf B: Biointerf.57: 69-74.

Mamta and Kawatra A. (2015). Development and nutritional evaluation of pearl millet (*Pennisetum glaucum*) based convenience foods.

Mariac C., Luong V. and Kapran I. (2006) Diversity of wild and cultivated pearl millet accessions (*Pennisetum glaucum* [L.] R. Br.) In Niger assessed by microsatellite markers. Theor Appl Genet 114, 49–58.

Massacci A, Nabiev SM, Pietrosanti L, Nematov SK, Chernikova TN, Thor K, Leipner J (2008) Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. Plant Physiol Biochem **46**:189–195.

May L.H. and Milthorpe F.L. 1962. Drought resistance of crop Plants. Field Crop Abstracts 171-179.

Mitra J. (2001). Genetics and genetic improvement of drought resistance in crop plants. Current Science, 80(6), 758-763.

Mohammad K.N. and Heidari R. (2008). Drought-induced Accumulation of Soluble Sugars and Proline in Two Maize Varieties. World Applied Sciences Journal 3 (3): 448-453.

Monakhova O.F. and Chernyadev I.I. (2002): Protective role of kartolin-4 in wheat plants exposed to soil drought. Applied and Environmental Microbiology, 38: 373–380.

Munns R. and Tester M. 2008. Mechanisms of Salinity Tolerance. Annual Review of Plant Biology. Vol. 59:651-681.

Nehra M. K., Devvart, Kaushik J. and Sharma R. K. (2017). Molecular characterization of pearl millet [*Pennisetum glaucum* (L.) R. Br] inbreds using microsatellite markers Journal of Applied and Natural Science 9 (1):357 – 363.

Nemeth M., Janda T., Horvath E., Paldi E., Szalai G. 2002. Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. Plant Science volume 162, issue 4, page 569-574.

Obadina A. O., Ishola I. O., Adekoya O., Soares A. G., De Carvalho C. W. P. and Barboza H. T. (2017). Nutritional and physicochemical properties of flour from native and roasted whole grain pearl millet (*Pennisetum glaucum* [L.] R. Br.). Journal of Cereal Science 70:247-252.

Osmanzai, M., Rajaram S. and Knapp E.B.1987. Breeding for moisture-stress areas. In: J.P. Srivastava, E. Porceddu, E. Acevedo & S. Varma (Eds) Drought Tolerance in Winter Cereals, pp. 151-161. John Wiley & Sons, New York.

Ouattara C. A. T., Aly S. Y., Traore S. and Alfred S.T. 2006. A Comparative Study on Nutritional and Technological Quality of Fourteen (14) Cultivars of Pearl Millets [*Pennisetum glaucum* (L) Leeke] in Burkina Faso. Pakistan Journal of Nutrition,5 (6): 512-521.

Poonam.2002. Effect of acid and heat treatment on nutrient composition and shelf life of pearl millet (*Pennisetum glaucum*) flour. M.Sc. Thesis, CCSHAU, Hisar, India.

Praba M. L., Cairns J. E., Babu R. C., and Lafitte H. R. (2009). Identification of Physiological Traits Underlying Cultivar Differences in Drought Tolerance in Rice and Wheat. Journal of Agronomy and Crop Science, **195**(1), 30–46.

Priyanka V., P. Shanthi P., Reddy D.M. and Reddy B.R. 2019. Genetic Variability Studieson Yield, Physiological and Nutritional Traitsin Pearl Millet [*Pennisetum glaucum* (L.) R.Br.]. International Journal of Current Microbiology and Applied Sciences **8**(7):501-508.

Rajaram V., Nepolean T., Senthilve S., Varshney R., Vadez V., Srivastava R. K., Trushar M., Ambawat S. S. Kumar S., Ramana K. B., Amindala B., Mangamoori L.N., Oscar R. L. and Charles T. A. (2013). Pearl millet [*Pennisetum glaucum* (L.) R. Br.] consensus linkage map constructed using four RIL mapping populations and newly developed EST-SSRs J, BMC, Genomics, 14:159.

Ramakrishnan M., Ceasar S. A.., Duraipandiyan V., Al Dhabi N. A. Ignacimuthu S. (2016). Assessment of genetic diversity, population structure and relationships in Indian and non-Indian genotypes of finger millet (*Eleusine coracana* (L.) Gaertn) using genomic SSR markers, Springer Plus Journal 5:120.

Rekha. 1997. Efficacy of processing techniques in the utilization of pearl m i l l e t for value added products. M.Sc. thesis, CCS Haryana Agricultura l University, Hisar, Haryana, India. 125 pp.

Rohlf FJ (1994) Numerical taxonomy and multivariate analysis system. Exeter Software, Setauket, N.Y.

Saito K. and Matsuda F. 2010. Metabolomics for Functional Genomics, Systems Biology, and Biotechnology. Annual Review of Plant Biology Vol. 61:463-489.

Sammour H. R. (2014). Genetic diversity in lathrus sativus L. germplasm. Bioscience. 8:325-336.

Sarang S. S., Tejas C B., Avadh K S. and Talati J. G. (2014). Enzymatic study and proximate composition of pearl millet genotypes in relation to Downy Mildew. Indian J Agric Biochem. 27(1):13-19.

Sathya M., Kumari V.N. and Sumathi P. (2013). Hierarchical clustering of millet (*Pennisetum glaucum* (L.) R. Br) inbreds for morpo physiological traits, Int.J. Curr. Microbiol.App. Sci. 2(12):647-652.

Schonfeld M, Johnson R, Carver B, Mornhinweg D (1988). Water relations in winter wheat as drought resistant indicators. Crop Sci. **28**: 526-531.

Sehgal D., Rajaram V., Armstead I. P., Vadez V., Yadav Y. P., Hash C. T. and Yadav R. S. (2016). Integration of gene-based markers in a pearl millet genetic map for identification of candidate genes underlying drought tolerance quantitative trait loci, BMC Plant Biology 2012, 12:9.

Serba D.D. and Yadav R.S. 2016. Genomic Tools in Pearl Millet Breeding for Drought Tolerance: Status and Prospects. Front. Plant Science.

Serraj R., Hash C. T., Rizvi S. M. H., Sharma A., Yadav R. S. and Bidinger F. R. (2005). Recent Advances in Marker-Assisted Selection for Drought Tolerance in Pearl Millet. Plant Production Science, **8**(3), 334–337.

Shah A. I., Rahman H., Shah S.M.A., Shah Z., Rahman S., Ihteramullah and Noor M. (2012). Characterization of pearl millet germplasm for various morphological and fodder yield parameters. Pak. J. Bot., **44**(1): 273-279.

Sharma B., Chugh L.K., Sheoran R.K., Singh V.K. and Sood M. 2018. Study on genetic variability, heritability and correlation in pearl millet germplasm. Journal of Pharacognosy and Phytochemestry,**7**(6):1983-1987.

Sharma B. D., Mitter J., and Mohan M. (1978). On measures of “useful” information. Information and Control, 39(3), 323–336.

Shao HB, Chu LY, Jaleel CA and Zhao CX (2008). Water-deficit stressinduced anatomical changes in higher plants. CR. Biol. 331: 215-225. Shao HB, Li B, Wang BC, Tang K, Liang YL (2008). A study on differentially expressed gene screening of Chrysanthemum plants under sound stress. CR. Biol. 331: 329-333

Shekhra A. C., Chandra-Prasanna B. M., Bhat S. R. and Singh B. B. (2007). Genetic Diversity Analysis of Elite Pearl Millet Inbred Lines using RAPD and SSR Markers. Journal of Plant Biochemistry and Biotechnology volume 16, pages23–28.

Sidhu G. K., Sharma S. and Neha. (2016). Influence of milling speed on proximate composition of pearl millet flour during storage. Intl. J. Food. Ferment. Technol. **6**(1):49-56.

Singh A. K., Rana M. K., Singh S., Kumar S., Durgesh K. and Arya L. (2013). Assessment of genetic diversity among pearl millet [*Pennisetum glaucum* (L.) R Br.] cultivars using SSR markers. Range Management. & Agroforestry 34: 77-81.

Singh B., Upadhyay P. K. and Sharma K. C. (2014). Genetic variability, correlation and path analysis in pearl millet (*Pennisetum glaucum* (L.) R. Br.). Indian Research Journal of Genetics and biotechnology,6: 491-500.

Snedecor G. and Cochran WG. (1967). Statistical Methods, 6th ed. Iowa State University Press, Ames, Iowa, USA.

Somasundaram S. T. and Kalaiselvam M. (2011) Molecular tools for assessing genetic diversity, International Training Course on Mangroves and Biodiversity, Annamalai University, India, pp. 82 - 91.

Subi M.I.M. and Idris A.E. (2013). Genetic variability, heritability and genetic advance in pearl millet [*Pennisetum glaucum* [L.] R. Br.] Genotypes. British Biotechnology Journal,**3**(1): 54-65.

Sudeshna C., Sasidharan N., Ruchi T., Bhushan A. and Patel, D. A. (2016). Assessment of genetic diversity of *Pennisetum glaucum* (L.) R. Br. and *Triticum aestivum* L. genotypes through RAPD and SSR markers. International Journal of Agriculture Sciences, 8, 947-951.

Swaminathan.2006. The need to explore human security. Human security and international insecurity.

Swindale L. D., Bidinger F.R., 1981. Introduction: the human consequences of drought and crop research priorities for their alleviation. Agris.

Tanwar H., Yashveer S., Sangwan V. P., Sharma S., Sangwan S. and Mor V. S. (2020). Genetic diversity in pearl millet restorers based on seed vigour traits and ssr markers. Bangladesh j. Bot. **49**(2): 357-365, 2020 (june).

Terzi R. and Kadioglu A. 2006. Drought stress tolerance and the antioxidant enzyme system in ctenanthe setosa. Acta biologica cracoviensia series botanica 48/2: 89–96.

Thomas A.M., Babu C. and Iyanar K. 2018. Genetic variability and association studies in pearl millet for green fodder yield and quality traits, Electronic Journal of Plant Breeding,9(3):1263-1271.

Toker, C., Canci, H. & Yildirim, T. Evaluation of perennial wild Cicer species for drought resistance. Genet Resour Crop Evol **54,** 1781–1786.

Turkan I., Bor M., Ozdemir F., Koca H. 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant P. acutifolius Gray and drought-sensitive P. vulgaris L. subjected to polyethylene glycol mediated water stress. Plant Science volume 168, issue 1, page 223- 231.

Uppal R.K., Wani S.P., Garg K.K. and Alagarswamy G. (2015) Balanced nutrition increases yield of pearl millet under drought. Field Crop Res 177:86–97.

Vavilov NI (1950). The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica. 13:1-366.

Verma U., Kumar R., Malik V. and Vart D. (2015). Multivariate analysis for selection of diverse genotypes in pearl millet germplasm. Forage Research,**41**(2): 73-77.

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. American J. of Plant Sciences, 3, 1730-1740.

Vinodhana K., Sumathi P. and Sathya M. (2013). Genetic variability and inter-relationship among morpho-economic traits of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and their implications in selection. International Journal of Plant, Animal and Environmental Sciences, **3**(2):145-149.

Vom B. K., Christinck A., Weltzien, R. E., Presterl T., and Geiger H. H. (2003). Farmers' Seed Systems and Management Practices Determine Pearl Millet Genetic Diversity Patterns in Semiarid Regions of India. Crop Science, 43(5), 1680.

Vujcic V. and Brkanac S.R. (2014). Physiological and biochemical responses of Fibiger triquetra (DC.) Boiss. to osmotic stress. Acta Bot. Croat. 73 (2), 347–358.

Watanabe S, Kojima K, Ide Y, Sasaki S (2000). Effects of saline and osmotic stress on proline and sugar accumulation in Populus euphratica in vitro. Plant Cell Tissue Organ Cult **63**: 199–206

Winkel, T., Renno. J. F. and Payne, W. A. (1997). Effect of timing of water deficit on growth, phenology and yield of pearl millet (*Pennisetum glaucurn* (L) R BR) grown in Sahelian conditions. J. EXp. Bot. **48**, 1001 - 1009

Winkel B. 2001. Flavonoid Biosynthesis. A Colorful Model for Genetics, Biochemistry, Cell Biology, and Biotechnology. Plant Physiology.

Yadav O., Mitchell S., Zamora T., and Fulton M. (2008). Development of new simple sequence repeat markers for pearl millet. Journal of SAT Agricultural Research, ed. **3**(1): 34-37.

Yadav O. P. (2010). Drought response of pearl millet landrace-based populations and their crosses with elite composites. Field Crops Research118:51-56.

Yadav R. S., Sehgal D., Vadez V. 2011. Using genetic mapping and genomics approaches in understanding and improving drought tolerance in pearl millet. Journal of Experimental Botany, Volume 62, Issue 2, Pages 397–408.

Yadav T., Kumar A., Yadav R.K., Yadav G., Kumar R., and Kushwaha M. (2020). Salicylic acid and thiourea mitigate the salinity and drought stress on physiological traits governing yield in pearl millet- wheat. Saudi Journal of Biological Sciences.

Yamaguchi S. K. and Shinozaki K. 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annual review of plant biology vol. 57:781-803.

Yang, F., Xu, X., Xiao, X. & Li C. 2009. Responses to drought stress in two poplar species originating from different altitudes. Biologia Plantarum 53: 511–516.

Yoon J.H., Thompson L. U. and Jenkins D.J.1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response, The American Journal of Clinical Nutrition, Volume 38, Pages 835–842.

Zhu J. K. (2002). Salt and Drought Stress Signal Transduction in Plants. Annual Review of Plant Biology. Vol. 53:247-273.

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THESIS ABSTRACT

S. No			
1.	**Title of the Thesis**		**Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene linked SSR markers.**
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6.	Degree awarded		**Master of Science in Agriculture**
7.	Year of award of the degree		2019-2020
8.	Major subject		**Plant Molecular Biology and Biotechnology**
9.	Total number of pages in the thesis		98
10.	Number of words in the abstract		127

Signature	**Signature**	**Signature**
(Dr. M.K. Tripathi)	**(Dr. M. K. Tripathi)**	**(Mohan Lal Choudhary)**
Advisor	**Professor and Head**	**Student**

ABSTRACT

The current investigation entitled, “Diversity assessment of pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm lines against drought using morpho-physiological, biochemical and gene-linked SSR markers" was conducted during *Kharif* season of 2019 at Research Farm, Department of Plant Molecular Biology & Biotechnology, RVSKVV, College of Agriculture, Gwalior (M.P.). In present investigation pearl millet germplasm lines was screened against drought using morpho-physiological characteristics, biochemical parameters and gene- linked SSR markers. Ninety-six pearl millet germplasms were screened for drought tolerance. Out of 96 germplasm lines, 22 including IP133, IP127, IP177, IP198, IP107, IP140, IP164, IP181, IP160, IP166, IP194, IP195, IP126, IP190, IP196, IP158, IP178, IP121, IP110, IP236, IP 230 andTHAK 1827 were formed distinct group regarding drough information generated by validation of gene-linked SSR molecular markers, morphological data and by biochemical analysis.These germplasm lines may be used further in molecular breeding programmes to develop tolerant varieties and SSR molecular markers with higher PIC value may be used for screening of large numbers of germplasm line(s).

Table 1 Morpho-physiological data of 96 pearl millet germplasm lines

Sr. No.	Name of genotype	Days of 50% heading initiation	Days of 50% flowering initiation	Canopy temp.	Leaf area (cm2)	Plant height (cm)	Spike length (cm)	Spike girth (cm)	Shoot length (cm)
1	IP 132	44.00	48.10	35.00	153.73	229.05	23.45	3.20	205.60
2	IP 118	45.20	51.00	33.25	216.78	213.65	26.25	2.65	187.40
3	IP 152	44.40	50.90	33.75	197.51	218.05	24.85	3.35	193.20
4	IP 175	43.00	49.10	32.80	306.77	244.20	26.10	2.75	218.10
5	IP 133	41.90	47.30	35.55	235.82	253.75	24.40	3.05	229.35
6	IP 173	44.20	49.00	34.30	293.04	277.10	32.10	3.45	245.00
7	IP 199	44.20	52.00	34.40	185.34	295.55	22.05	2.65	273.50
8	IP 127	41.70	48.00	32.35	259.39	233.60	25.90	3.10	207.70
9	IP 198	41.30	47.00	34.30	234.80	228.80	21.00	3.70	207.80
10	IP 177	42.70	49.30	34.55	240.10	230.10	21.35	2.45	208.75
11	IP 182	42.10	50.20	34.40	416.38	276.35	33.40	2.85	242.95
12	IP 147	41.90	48.40	34.55	271.32	260.25	31.15	2.65	229.10
13	IP 107	41.20	47.00	33.30	284.86	238.15	25.75	3.20	212.40
14	IP 140	42.10	50.80	32.70	241.75	217.05	20.25	2.60	196.80
15	IP 164	42.80	51.00	33.50	230.45	206.10	18.90	2.50	187.20
16	IP 142	43.20	50.00	34.65	236.33	243.65	24.45	3.10	219.20
17	IP 180	42.00	50.10	34.30	248.37	263.85	24.20	3.20	239.65
18	IP 188	45.00	53.10	34.30	306.53	233.95	20.40	2.35	213.55
19	IP 181	41.70	51.00	34.70	254.15	256.50	30.40	2.50	226.10
20	IP 129	43.90	50.80	33.40	382.95	234.65	17.95	3.10	216.70
21	IP 119	41.90	49.80	33.70	142.07	250.10	30.40	2.45	219.70
22	IP 150	41.90	48.20	35.90	255.55	280.25	27.60	2.85	252.65
23	IP 120	42.40	51.10	35.15	245.81	268.20	24.90	3.05	243.30
24	IP 111	42.10	49.00	35.10	205.99	244.55	16.80	2.60	227.75
25	IP 160	43.30	52.00	36.45	237.85	232.55	20.20	2.45	212.35
26	IP 136	44.90	51.20	35.35	202.22	233.60	25.95	2.50	207.65
27	IP 171	42.85	52.90	34.95	278.73	199.55	24.75	2.70	174.80
28	IP 130	43.00	51.30	37.15	205.04	226.10	30.40	2.60	195.70
29	IP 166	44.70	52.00	36.00	153.68	229.70	36.50	2.30	193.20

30	IP 128	41.70	50.10	36.05	282.26	265.60	22.90	2.45	242.70
31	IP 183	44.50	50.90	36.95	266.63	236.20	30.20	2.55	206.00
32	IP 165	42.00	53.10	35.30	229.15	229.45	23.40	2.55	206.05
33	IP 192	43.70	51.10	33.70	395.73	248.55	25.10	2.65	223.45
34	IP 122	44.20	51.10	34.55	333.11	261.55	25.60	2.70	235.95
35	IP 143	42.10	53.10	32.95	210.47	237.05	29.65	2.35	207.40
36	IP 167	45.00	52.00	33.10	190.12	217.50	23.70	3.30	193.80
37	IP 172	42.20	50.00	34.50	257.47	238.75	29.00	3.05	209.75
38	IP 106	42.70	53.10	33.00	278.32	244.35	23.80	3.25	220.55
39	IP 137	42.30	52.00	32.15	341.59	282.65	25.75	3.10	256.90
40	IP 116	44.30	50.30	32.65	224.40	241.45	26.20	2.60	215.25
41	IP 194	44.40	51.00	35.85	180.33	242.30	30.40	2.50	211.90
42	IP 195	42.90	49.10	32.35	228.30	223.55	22.80	2.70	200.75
43	IP 126	41.50	49.50	31.90	211.80	283.40	34.60	2.55	248.80
44	IP 155	43.00	54.10	33.10	222.62	268.60	40.90	3.25	227.70
45	IP 149	43.00	53.10	33.15	260.87	229.05	31.70	3.60	197.35
46	IP 185	41.70	49.80	32.15	349.12	238.45	27.00	3.25	211.45
47	IP 161	42.90	50.80	31.95	236.28	234.10	19.90	2.60	214.20
48	IP 168	44.10	51.00	31.95	325.45	251.60	27.80	2.35	223.80
49	IP 190	43.00	50.20	31.95	371.93	266.40	27.45	2.85	238.95
50	IP 156	43.30	53.10	32.05	266.25	222.50	21.05	2.45	201.45
51	IP 187	41.90	49.90	32.65	377.77	223.25	27.25	3.10	196.00
52	IP 159	41.10	48.00	32.15	217.63	210.00	16.40	2.60	193.60
53	IP 139	42.60	50.90	32.45	303.90	215.25	27.45	2.55	187.80
54	IP 146	42.80	51.20	32.55	290.48	238.45	27.45	2.55	211.00
55	IP 196	45.30	53.20	33.30	232.25	206.10	18.70	2.70	187.40
56	IP 186	42.30	50.80	32.70	241.01	232.00	23.05	2.50	208.95
57	IP 158	45.20	53.30	33.50	220.81	265.45	24.75	2.35	240.70
58	IP 151	42.70	50.20	34.20	182.75	228.95	30.25	2.45	198.70
59	IP 193	43.10	51.00	33.25	299.78	258.65	19.10	2.60	239.55
60	IP 105	43.10	52.80	31.70	190.16	207.55	27.85	2.60	179.70
61	IP 123	42.00	50.30	31.85	232.94	243.80	19.25	3.20	224.55
62	IP 131	43.10	50.80	33.40	184.01	232.25	24.00	2.60	208.25
63	IP 178	41.90	50.80	33.70	301.23	246.25	25.90	2.75	220.35

64	IP 121	43.40	52.10	33.40	189.06	214.05	22.85	2.70	191.20
65	IP 104	42.90	53.10	33.15	261.40	233.55	28.65	2.50	204.90
66	IP 134	44.60	50.70	33.50	243.74	225.35	26.40	2.50	198.95
67	IP 112	42.60	50.90	33.00	160.83	237.75	27.30	2.30	210.45
68	IP 141	44.30	49.40	33.55	283.54	221.00	23.30	2.20	197.70
69	IP 145	41.90	48.90	31.65	192.92	246.65	19.05	2.35	227.60
70	IP 144	42.10	51.10	33.60	373.71	213.60	31.75	2.65	181.85
71	IP 138	41.60	49.80	33.55	303.53	233.25	27.35	2.60	205.90
72	IP 179	43.40	50.40	32.80	271.07	247.25	31.40	2.70	215.85
73	IP 153	42.20	49.70	34.05	290.74	213.55	25.75	2.55	187.80
74	IP 101	41.90	48.80	32.45	241.13	220.55	27.10	3.05	193.45
75	IP 135	42.60	51.50	33.45	230.61	202.25	25.40	2.55	176.85
76	IP 162	41.40	49.80	34.05	250.38	238.85	24.60	2.55	214.25
77	IP 115	43.90	53.00	34.65	279.53	207.30	26.95	2.40	180.35
78	IP 170	42.50	56.10	36.10	240.57	223.80	38.70	3.30	185.10
79	IP 109	43.00	53.10	33.50	220.15	258.35	18.55	2.45	239.80
80	IP 154	45.50	53.90	33.00	361.75	212.25	25.75	3.10	186.50
81	IP 174	43.30	51.00	32.15	181.59	248.55	34.80	2.35	213.75
82	IP 108	41.40	48.20	32.45	198.51	237.05	25.05	2.65	212.00
83	IP 189	42.70	50.70	32.40	261.49	260.60	27.35	2.45	233.25
84	IP 110	43.00	48.10	33.75	287.12	231.40	24.25	3.05	207.15
85	IP 117	44.00	51.00	32.90	204.54	230.25	17.10	2.20	213.15
86	IP 169	41.40	50.00	34.15	186.08	244.55	23.05	2.55	221.50
87	IP 114	43.80	52.00	33.05	209.13	229.95	24.25	2.40	205.70
88	IP 163	43.80	52.80	33.35	206.97	245.75	34.55	2.35	211.20
89	IP 274	42.70	51.20	34.50	271.18	232.75	31.35	2.80	201.40
90	IP 283	43.00	50.90	34.40	200.01	238.55	21.20	2.55	217.35
91	IP 236	42.90	50.30	33.60	209.64	188.05	25.90	2.25	162.15
92	IP 291	43.00	52.20	31.40	209.71	248.45	27.80	2.55	220.65
93	IP 230	44.20	53.10	32.50	262.46	204.55	19.25	2.15	185.30
94	IP 262	42.50	50.00	33.45	273.10	236.70	22.55	2.60	214.15
95	IP 231	43.50	51.90	33.45	289.82	235.80	18.90	2.75	216.90
96	THAK 1827	42.70	53.20	33.30	274.83	228.60	24.95	2.80	203.65

Table2 Morpho-physiological data of 96 pearl millet germplasm lines

Sr.No.	Name of genotype	Root length (cm)	Root shoot ratio	Seed density of spike (grain/cm2)	No of tillers	Fresh weight (gm)	Dry weight (gm)	Turgit weight (gm)	Relative water content %
1	IP 132	20.95	0.10	18.75	1.40	193.45	144.25	288.60	33.96
2	IP 118	19.60	0.10	21.95	1.50	169.30	114.90	225.25	49.31
3	IP 152	20.90	0.11	22.60	1.70	166.85	129.15	222.70	40.28
4	IP 175	16.50	0.08	17.10	1.90	195.50	111.05	231.95	70.29
5	IP 133	17.50	0.08	20.30	1.30	262.50	183.55	318.35	58.59
6	IP 173	16.50	0.07	17.90	1.50	290.60	218.55	372.05	47.26
7	IP 199	17.55	0.06	23.40	1.50	139.45	96.60	183.30	49.10
8	IP 127	18.75	0.09	28.40	1.30	292.50	201.25	322.00	75.58
9	IP 198	20.80	0.10	19.55	1.30	369.50	209.65	409.80	79.86
10	IP 177	24.50	0.12	29.35	1.50	240.60	134.40	288.70	68.76
11	IP 182	16.35	0.07	27.10	1.40	247.50	172.60	313.00	53.22
12	IP 147	16.05	0.07	27.75	1.30	188.05	140.75	274.40	35.68
13	IP 107	18.73	0.09	26.00	1.80	251.90	183.65	303.25	57.03
14	IP 140	22.14	0.11	23.05	1.90	218.55	96.20	259.65	75.45
15	IP 164	21.50	0.11	29.70	1.70	307.95	141.25	365.05	75.16
16	IP 142	16.35	0.07	27.95	1.70	233.25	127.10	305.25	59.59
17	IP 180	15.55	0.06	29.80	1.60	278.35	224.85	364.60	38.94
18	IP 188	17.10	0.08	27.25	1.30	310.10	193.85	331.55	86.47
19	IP 181	19.50	0.09	29.25	1.50	280.65	190.05	311.05	75.39
20	IP 129	16.45	0.08	26.10	1.80	210.60	139.00	284.65	49.26
21	IP 119	17.35	0.08	19.10	1.60	210.95	131.90	408.75	28.80
22	IP 150	17.80	0.07	23.05	1.40	288.10	209.35	369.10	49.45
23	IP 120	16.80	0.07	30.05	1.70	250.65	159.05	293.35	68.88
24	IP 111	15.76	0.07	27.75	1.60	189.65	98.70	228.15	70.91
25	IP 160	18.85	0.09	22.05	1.90	180.85	113.65	214.50	68.07
26	IP 136	16.50	0.08	26.75	1.50	190.30	125.75	229.00	61.69
27	IP 171	17.95	0.10	21.30	1.70	176.55	119.40	228.85	52.48
28	IP 130	18.45	0.09	22.45	1.30	209.45	108.30	260.85	66.60
29	IP 166	16.70	0.09	23.60	1.40	179.35	72.05	214.85	75.55

30	IP 128	16.35	0.07	23.30	1.50	183.65	108.60	249.60	57.09
31	IP 183	20.85	0.10	22.90	1.70	149.45	96.05	188.55	58.91
32	IP 165	17.20	0.08	22.75	1.40	220.50	159.80	258.60	63.93
33	IP 192	15.65	0.07	27.25	1.50	142.45	69.70	178.00	67.01
34	IP 122	16.55	0.07	22.45	1.80	158.30	99.30	202.90	56.95
35	IP 143	18.85	0.09	22.80	1.80	149.60	55.10	184.90	73.32
36	IP 167	17.45	0.09	22.15	1.40	147.40	85.35	203.45	53.77
37	IP 172	15.85	0.08	25.30	1.50	152.80	90.55	199.60	60.26
38	IP 106	18.60	0.08	25.30	1.50	149.30	90.65	201.90	52.89
39	IP 137	16.80	0.07	23.30	1.50	178.60	103.10	225.30	63.89
40	IP 116	15.25	0.07	27.00	1.50	124.75	65.15	169.90	57.78
41	IP 194	21.90	0.10	21.05	1.60	121.75	62.25	159.85	61.18
42	IP 195	20.55	0.10	25.95	1.50	267.40	107.40	301.30	82.45
43	IP 126	21.80	0.09	21.05	1.80	297.45	145.85	334.60	80.45
44	IP 155	15.20	0.07	24.80	2.00	332.65	269.25	377.30	59.19
45	IP 149	20.15	0.10	22.45	1.70	257.05	189.95	316.05	53.25
46	IP 185	16.80	0.08	25.85	1.50	159.50	93.85	210.15	56.43
47	IP 161	17.55	0.08	21.50	1.60	228.55	167.60	283.20	52.74
48	IP 168	19.45	0.09	20.05	1.70	211.50	109.25	248.05	73.85
49	IP 190	22.80	0.10	22.10	1.70	268.85	136.20	304.10	79.02
50	IP 156	16.90	0.08	19.00	1.60	173.80	95.85	207.15	70.20
51	IP 187	15.70	0.08	20.35	1.30	200.15	121.85	252.15	59.70
52	IP 159	15.30	0.08	28.25	1.50	300.05	187.95	343.30	72.56
53	IP 139	15.10	0.08	23.60	1.40	142.15	73.15	199.00	54.79
54	IP 146	15.80	0.07	27.15	1.70	173.10	120.55	215.60	55.96
55	IP 196	16.80	0.09	19.90	1.80	212.45	93.50	245.75	78.19
56	IP 186	19.10	0.09	23.80	1.80	113.60	61.50	165.10	49.91
57	IP 158	22.20	0.09	23.10	1.40	232.60	142.00	275.25	68.28
58	IP 151	15.90	0.08	23.90	1.40	203.75	144.65	253.40	54.97
59	IP 193	17.20	0.07	22.00	1.50	221.55	159.10	289.65	47.35
60	IP 105	16.65	0.09	23.60	1.50	170.25	107.75	217.70	57.11
61	IP 123	17.65	0.08	24.05	1.50	214.35	139.65	267.25	59.31
62	IP 131	15.80	0.08	24.75	1.30	220.70	124.10	259.60	75.60
63	IP 178	21.90	0.10	24.30	1.70	181.75	118.35	209.90	69.12

64	IP 121	19.70	0.10	21.85	1.30	209.55	87.90	279.80	63.85
65	IP 104	15.90	0.08	20.50	1.90	372.45	234.25	422.65	73.35
66	IP 134	17.60	0.09	20.00	1.60	261.55	190.25	300.75	64.54
67	IP 112	15.65	0.07	23.65	1.50	301.25	160.55	338.25	78.99
68	IP 141	15.30	0.08	25.10	1.40	259.55	188.30	306.75	60.53
69	IP 145	15.15	0.07	21.00	1.50	257.10	178.05	326.85	53.76
70	IP 144	18.05	0.10	19.80	1.50	176.10	118.75	233.50	50.07
71	IP 138	16.10	0.08	22.75	1.40	234.65	123.20	266.00	78.11
72	IP 179	18.15	0.08	18.95	1.50	182.90	126.80	224.45	57.71
73	IP 153	16.50	0.09	23.80	1.70	112.35	68.15	146.15	56.57
74	IP 101	15.70	0.08	25.65	1.30	139.70	93.95	177.70	55.36
75	IP 135	16.25	0.09	27.90	1.70	228.75	150.90	276.00	62.24
76	IP 162	17.50	0.08	24.60	1.50	183.50	96.05	222.00	69.48
77	IP 115	17.05	0.09	22.75	1.40	131.25	85.65	169.20	54.30
78	IP 170	15.25	0.08	24.85	1.60	214.75	148.60	253.80	62.85
79	IP 109	21.90	0.09	24.55	1.20	260.85	165.75	307.35	67.16
80	IP 154	21.25	0.11	24.95	2.00	200.65	123.10	239.95	66.36
81	IP 174	15.25	0.07	23.10	1.60	151.95	114.05	190.95	49.78
82	IP 108	18.20	0.09	19.85	1.70	138.70	107.60	186.25	39.54
83	IP 189	15.95	0.07	24.25	1.50	177.40	98.25	223.15	63.58
84	IP 110	17.00	0.08	23.50	1.50	253.45	144.00	297.30	71.42
85	IP 117	16.30	0.08	20.65	1.60	191.35	92.50	230.50	71.61
86	IP 169	23.00	0.10	23.65	1.50	127.65	73.05	165.20	60.59
87	IP 114	18.20	0.09	19.75	1.90	228.50	100.55	263.65	77.93
88	IP 163	15.70	0.07	23.25	1.50	158.10	76.75	199.05	66.49
89	IP 274	16.60	0.08	24.20	1.60	180.50	106.20	226.65	61.95
90	IP 283	17.00	0.08	21.05	1.40	180.45	149.40	251.85	30.59
91	IP 236	16.85	0.10	25.00	1.50	234.65	158.70	263.75	72.79
92	IP 291	15.85	0.07	25.85	1.50	113.65	90.85	166.00	32.42
93	IP 230	18.60	0.10	28.25	1.60	183.50	108.80	227.05	64.21
94	IP 262	17.20	0.08	24.10	1.50	179.65	101.65	223.65	65.47
95	IP 231	16.65	0.08	20.90	1.60	146.85	110.30	191.05	45.18
96	THAK 1827	20.95	0.10	24.70	1.50	249.15	162.65	325.85	54.53

Table 3 Morpho-physiological data of 96 pearl millet germplasm lines

Sr. No.	Name of genotype	Saturation water deficit	Days of physiological maturity	Days of maturity	Plant population at harvesting (plants/line)	Test weight (gm)	Yield (qt./hac)	Biological yield (qt./hac)	Harvest index
1	IP 132	66.04	83.50	96.20	26.00	11.35	33.63	208.70	16.11
2	IP 118	50.69	81.50	95.40	26.00	9.40	27.85	170.22	16.41
3	IP 152	59.72	82.50	95.55	24.50	12.50	37.04	191.33	19.41
4	IP 175	29.71	83.80	94.20	25.50	10.89	32.25	164.52	19.69
5	IP 133	41.41	82.10	95.60	27.00	10.63	31.48	271.93	11.59
6	IP 173	52.74	84.40	93.70	26.00	10.68	31.63	229.28	13.80
7	IP 199	50.90	82.30	94.30	25.00	11.21	33.21	143.11	23.21
8	IP 127	24.42	81.30	95.80	27.50	9.03	26.74	218.15	12.28
9	IP 198	20.14	83.00	95.30	25.50	12.20	36.15	310.59	11.66
10	IP 177	31.24	84.70	95.20	25.50	10.65	31.56	199.11	15.87
11	IP 182	46.78	82.80	96.00	24.50	11.83	35.04	255.70	13.72
12	IP 147	64.32	82.60	93.20	27.00	10.83	32.07	208.52	15.39
13	IP 107	42.97	81.50	93.80	27.00	11.10	32.89	272.07	12.09
14	IP 140	24.55	82.60	97.20	23.50	11.00	32.59	119.93	27.26
15	IP 164	24.84	84.00	94.70	26.00	11.38	33.70	209.26	16.13
16	IP 142	40.41	82.50	95.30	25.00	12.13	35.93	188.30	19.12
17	IP 180	61.06	82.40	93.80	26.50	10.35	30.67	233.11	13.17
18	IP 188	13.53	83.40	95.20	26.00	11.23	33.26	287.18	11.59
19	IP 181	24.61	83.30	96.30	27.00	11.53	34.15	281.56	12.13
20	IP 129	50.74	83.20	95.30	23.50	9.93	29.41	205.93	14.28
21	IP 119	71.20	80.90	93.20	24.50	11.66	34.53	195.41	17.70
22	IP 150	50.55	83.30	94.90	26.00	10.10	29.93	222.65	13.47
23	IP 120	31.12	83.60	95.60	25.50	11.72	34.73	250.44	14.02
24	IP 111	29.09	82.50	95.70	26.00	11.40	33.78	126.22	26.76
25	IP 160	31.93	82.50	94.70	24.50	9.15	27.11	168.37	16.16
26	IP 136	38.31	80.80	95.00	25.00	11.46	33.94	186.30	18.27
27	IP 171	47.52	82.00	93.40	27.50	11.42	33.82	176.89	19.14
28	IP 130	33.40	84.00	93.30	25.50	10.65	31.56	160.44	19.67

29	IP 166	24.45	83.50	95.80	25.50	12.57	37.23	106.56	35.03
30	IP 128	42.91	82.50	95.70	26.00	10.98	32.52	160.89	20.21
31	IP 183	41.09	84.70	94.20	25.50	12.28	36.39	142.30	25.73
32	IP 165	36.07	82.80	96.00	25.00	12.95	38.37	236.74	16.25
33	IP 192	32.99	81.50	96.90	25.00	12.58	37.26	103.26	36.20
34	IP 122	43.05	81.50	95.70	24.50	10.75	31.85	147.11	21.65
35	IP 143	26.68	84.50	96.30	23.00	10.52	31.17	81.04	38.87
36	IP 167	46.23	84.40	94.40	24.00	12.42	36.79	126.44	29.15
37	IP 172	39.74	82.40	97.00	25.50	11.70	34.67	134.15	25.85
38	IP 106	47.11	84.10	92.30	23.00	11.49	34.03	134.30	25.40
39	IP 137	36.11	82.80	93.20	25.50	9.55	28.30	152.74	18.52
40	IP 116	42.22	82.20	96.10	27.50	10.93	32.37	96.52	33.56
41	IP 194	38.82	83.40	91.80	25.00	10.78	31.93	92.22	34.73
42	IP 195	17.55	83.50	93.70	23.50	12.43	36.81	153.93	23.93
43	IP 126	19.55	82.70	95.80	25.00	10.90	32.30	253.11	13.15
44	IP 155	40.81	83.00	95.60	25.50	9.85	29.19	218.89	13.36
45	IP 149	46.75	83.40	95.50	25.00	10.60	31.41	281.41	11.16
46	IP 185	43.57	86.00	91.70	23.50	12.33	36.52	139.04	26.28
47	IP 161	47.26	84.50	94.80	26.50	12.00	35.56	248.30	14.33
48	IP 168	26.15	86.00	94.50	25.00	10.29	30.47	161.85	18.91
49	IP 190	20.98	83.70	94.60	23.50	11.63	34.44	201.78	17.23
50	IP 156	29.80	84.40	93.20	27.00	13.28	39.33	142.00	27.94
51	IP 187	40.30	84.60	96.20	25.00	11.68	34.59	180.52	19.19
52	IP 159	27.44	85.90	95.50	28.50	12.25	36.30	278.44	13.13
53	IP 139	45.21	81.90	96.40	24.00	12.17	36.06	108.37	33.34
54	IP 146	44.04	83.60	94.80	27.00	12.30	36.44	178.59	20.41
55	IP 196	21.81	82.10	97.80	25.50	10.44	30.92	138.52	22.65
56	IP 186	50.09	83.40	93.30	23.00	9.93	29.41	91.11	32.41
57	IP 158	31.72	83.00	94.60	25.00	12.03	35.63	210.37	16.96
58	IP 151	45.03	82.00	94.60	24.00	11.40	33.78	214.30	15.78
59	IP 193	52.65	85.80	97.00	22.00	11.28	33.41	235.70	14.23
60	IP 105	42.89	87.70	95.20	26.50	10.30	30.52	159.63	19.13
61	IP 123	40.69	82.50	95.70	25.50	12.40	36.74	206.89	17.97
62	IP 131	24.40	83.00	94.80	26.50	12.58	37.26	183.85	20.34

63	IP 178	30.88	83.40	94.00	24.50	11.00	32.59	175.33	18.60
64	IP 121	36.15	83.50	95.00	25.00	10.45	30.95	145.04	22.32
65	IP 104	26.65	85.50	94.70	24.00	10.20	30.22	247.04	12.25
66	IP 134	35.46	84.50	94.80	26.50	10.53	31.19	281.85	11.08
67	IP 112	21.01	83.50	91.30	24.00	11.15	33.04	237.85	13.92
68	IP 141	39.47	86.50	98.40	25.00	8.98	26.59	223.96	11.87
69	IP 145	46.24	82.00	97.10	27.00	9.65	28.59	263.78	10.84
70	IP 144	49.93	85.40	94.40	26.50	8.75	25.93	175.93	14.73
71	IP 138	21.89	83.40	93.80	24.50	10.59	31.38	182.52	17.33
72	IP 179	42.29	82.10	96.70	23.00	12.03	35.63	187.85	18.97
73	IP 153	43.43	87.40	95.80	26.50	12.18	36.07	100.96	35.78
74	IP 101	44.64	82.90	94.20	24.00	9.88	29.26	139.19	21.02
75	IP 135	37.76	85.90	92.90	24.00	10.45	30.96	223.56	13.88
76	IP 162	30.52	84.50	95.20	27.50	11.15	33.04	142.30	23.36
77	IP 115	45.70	85.50	97.50	24.50	11.58	34.30	126.89	27.08
78	IP 170	37.15	85.40	96.60	24.50	9.40	27.85	220.15	12.66
79	IP 109	32.84	88.40	93.80	25.50	13.23	39.19	245.56	15.98
80	IP 154	33.64	83.40	94.10	24.00	9.98	29.56	182.37	16.21
81	IP 174	50.22	82.00	95.50	24.50	9.95	29.48	168.96	17.48
82	IP 108	60.46	83.50	90.90	27.00	8.93	26.44	159.41	16.59
83	IP 189	36.42	85.10	93.90	27.50	10.35	30.67	145.56	21.12
84	IP 110	28.58	82.50	96.60	25.00	11.65	34.52	213.33	16.20
85	IP 117	28.39	87.40	95.70	24.00	11.77	34.86	137.04	25.45
86	IP 169	39.41	80.80	97.00	22.50	9.30	27.56	108.22	25.49
87	IP 114	22.07	83.50	97.30	24.00	9.98	29.56	128.96	22.97
88	IP 163	33.51	85.40	95.50	25.50	10.23	30.30	113.70	27.08
89	IP 274	38.05	85.60	95.80	26.00	10.49	31.08	157.33	19.77
90	IP 283	69.41	84.40	95.00	27.00	10.60	31.41	221.33	14.19
91	IP 236	27.21	86.90	96.30	24.00	9.30	27.56	235.11	11.72
92	IP 291	67.58	82.30	95.50	26.00	8.98	26.59	134.59	19.81
93	IP 230	35.79	83.10	97.00	25.00	11.80	34.96	161.19	21.72
94	IP 262	34.53	86.70	94.10	26.50	9.83	29.11	150.59	19.36
95	IP 231	54.82	84.30	95.80	24.50	11.50	34.07	163.41	20.94
96	THAK 1827	45.47	86.90	97.50	25.50	13.35	39.56	240.96	16.43

Table: 4 Complete Biochemical data of 96 pearl millet germplasm lines.

Sr. No.	Name of Genotype	Chlorophyll content (30 days) (mg/g)	Chlorophyll content (60 days) (mg/g)	Carotenoid content (30 days) (mg/g)	Carotenoid content (60 days) (mg/g)	Proline (mg/g)	Sugar (mg/g)	Protein (mg/g)
1	IP 132	3.57	3.46	8.29	7.50	0.10	1.60	12.40
2	IP 118	3.63	3.47	8.13	7.56	0.12	2.10	11.20
3	IP 152	4.06	3.64	9.35	8.12	0.16	1.20	11.80
4	IP 175	2.75	3.09	5.91	6.35	0.10	1.30	12.80
5	IP 133	2.43	2.69	4.54	5.94	0.11	2.20	14.20
6	IP 173	3.43	3.44	6.00	5.02	0.14	1.10	12.70
7	IP 199	1.76	2.06	6.22	7.46	0.16	1.40	11.90
8	IP 127	1.31	1.46	6.03	7.00	0.11	1.70	13.60
9	IP 198	1.98	2.04	6.74	7.92	0.12	1.80	11.40
10	IP 177	3.47	3.26	7.96	5.51	0.14	1.90	14.20
11	IP 182	2.46	2.70	5.92	7.34	0.10	1.70	13.90
12	IP 147	2.84	3.59	7.12	5.46	0.17	1.40	11.20
13	IP 107	3.19	3.44	7.81	7.28	0.12	1.80	15.90
14	IP 140	3.00	3.50	5.83	5.38	0.10	2.20	16.20
15	IP 164	3.33	3.21	8.50	10.10	0.12	1.40	15.40
16	IP 142	3.40	3.10	6.68	6.58	0.16	1.50	14.20
17	IP 180	3.11	3.47	6.31	6.60	0.10	1.40	11.00
18	IP 188	2.90	3.10	6.35	6.42	0.11	1.80	9.20
19	IP 181	1.71	2.58	4.85	5.41	0.14	2.20	12.70
20	IP 129	1.51	1.81	5.77	5.68	0.16	1.40	11.90
21	IP 119	2.36	2.23	6.13	6.78	0.11	1.50	13.60
22	IP 150	2.31	2.63	5.62	5.42	0.12	1.60	11.40
23	IP 120	2.50	3.04	6.36	6.71	0.14	2.10	14.20
24	IP 111	1.61	2.18	6.14	6.38	0.10	1.20	13.90
25	IP 160	2.73	3.28	5.56	5.78	0.17	1.30	16.10
26	IP 136	1.87	2.36	5.86	7.75	0.12	2.20	15.20
27	IP 171	2.63	3.02	7.26	5.95	0.10	1.10	15.50
28	IP 130	3.16	3.38	7.71	5.38	0.12	1.40	16.60

29	IP 166	3.50	3.34	8.67	7.86	0.16	1.70	12.70
30	IP 128	3.58	3.66	8.12	5.29	0.10	1.80	13.50
31	IP 183	3.44	3.46	7.85	6.85	0.11	1.90	14.70
32	IP 165	3.63	3.83	9.96	6.68	0.14	1.70	12.80
33	IP 192	3.24	3.54	10.57	6.59	0.16	1.20	14.20
34	IP 122	3.99	3.54	7.23	5.68	0.11	1.30	12.70
35	IP 143	4.00	3.80	9.45	5.30	0.12	1.50	11.90
36	IP 167	3.65	3.38	8.78	6.21	0.14	1.40	13.60
37	IP 172	2.91	3.34	6.14	7.89	0.10	1.80	11.40
38	IP 106	3.27	3.22	8.20	6.03	0.17	1.90	14.20
39	IP 137	3.39	3.63	7.58	5.82	0.12	2.00	13.90
40	IP 116	3.51	3.59	7.20	6.34	0.10	2.10	11.20
41	IP 194	4.69	3.77	11.44	5.01	0.12	2.20	12.60
42	IP 195	3.76	3.70	9.02	7.99	0.16	1.90	15.50
43	IP 126	3.08	3.09	4.73	5.06	0.10	2.10	16.60
44	IP 155	2.64	2.77	6.35	6.02	0.12	1.60	12.70
45	IP 149	3.32	3.22	5.90	5.85	0.14	1.70	13.50
46	IP 185	3.64	3.57	7.84	5.16	0.16	1.80	14.70
47	IP 161	4.13	3.84	7.55	5.08	0.14	1.90	12.80
48	IP 168	4.35	3.80	5.66	5.13	0.12	1.90	14.20
49	IP 190	3.64	3.40	7.85	5.31	0.14	1.70	12.70
50	IP 156	1.66	2.01	7.86	7.36	0.10	1.80	11.90
51	IP 187	4.10	3.46	8.63	8.09	0.17	1.90	12.40
52	IP 159	3.12	3.00	10.61	8.00	0.12	1.70	11.50
53	IP 139	2.81	2.96	7.81	5.51	0.10	1.80	11.00
54	IP 146	3.12	3.47	6.38	5.78	0.12	1.60	13.10
55	IP 196	2.86	2.83	9.49	8.28	0.16	1.40	13.20
56	IP 186	2.01	2.52	7.43	8.59	0.10	1.20	11.40
57	IP 158	2.58	2.75	7.55	6.64	0.11	2.10	12.30
58	IP 151	2.92	3.29	4.61	5.78	0.14	1.90	13.40
59	IP 193	2.79	3.02	9.36	8.17	0.16	1.50	11.60
60	IP 105	2.71	3.15	6.23	6.39	0.13	2.00	10.60
61	IP 123	2.84	3.13	8.63	7.99	0.12	1.20	9.90
62	IP 131	3.11	3.01	7.86	7.26	0.14	1.40	11.20

63	IP 178	3.15	3.13	6.68	5.86	0.12	2.10	14.80
64	IP 121	2.75	2.78	5.90	5.68	0.17	1.60	15.20
65	IP 104	2.65	2.98	6.87	5.97	0.12	1.70	11.60
66	IP 134	2.66	3.05	7.46	5.49	0.10	1.80	12.90
67	IP 112	2.38	2.62	5.44	5.45	0.12	1.90	11.80
68	IP 141	2.52	2.62	7.07	8.26	0.13	1.90	12.80
69	IP 145	1.98	2.34	4.87	7.09	0.11	1.70	14.20
70	IP 144	2.07	2.12	5.69	8.30	0.11	1.80	12.70
71	IP 138	2.23	2.60	7.53	5.77	0.14	1.90	11.90
72	IP 179	2.38	2.65	7.70	6.05	0.16	1.70	12.40
73	IP 153	2.69	2.83	6.84	6.03	0.13	1.80	11.50
74	IP 101	2.50	2.24	5.94	6.14	0.12	1.60	11.00
75	IP 135	2.52	2.87	6.30	6.12	0.14	1.40	13.10
76	IP 162	2.72	3.15	7.90	8.14	0.10	1.20	13.20
77	IP 115	2.46	2.77	6.21	5.96	0.13	2.10	11.40
78	IP 170	3.10	3.23	7.73	5.99	0.12	1.90	12.30
79	IP 109	2.72	2.71	7.45	8.16	0.10	1.50	13.40
80	IP 154	2.19	2.78	4.50	5.57	0.12	2.00	11.60
81	IP 174	2.90	2.84	7.54	8.20	0.16	1.20	13.90
82	IP 108	2.68	2.88	7.84	6.60	0.12	1.40	11.20
83	IP 189	3.59	3.35	9.55	8.23	0.11	1.70	15.90
84	IP 110	3.09	3.01	7.63	8.27	0.14	1.80	16.20
85	IP 117	3.26	2.99	7.82	7.26	0.16	1.90	15.40
86	IP 169	2.65	3.04	7.52	6.83	0.11	1.70	14.20
87	IP 114	2.25	2.35	8.61	8.44	0.12	1.40	11.00
88	IP 163	2.04	2.41	5.55	5.80	0.14	1.80	9.40
89	IP 274	2.99	3.32	9.11	8.82	0.10	2.20	11.60
90	IP 283	2.10	2.76	6.70	6.47	0.17	1.40	11.90
91	IP 236	2.76	2.95	6.36	6.89	0.10	1.50	12.40
92	IP 291	2.52	2.66	7.62	7.15	0.10	1.40	11.40
93	IP 230	2.69	2.78	7.39	7.69	0.12	1.80	13.10
94	IP 262	3.18	2.68	7.73	5.72	0.16	2.20	13.40
95	IP 231	2.98	2.92	7.65	6.06	0.14	2.00	16.10
96	THAK 1827	3.62	3.79	8.17	8.93	0.11	1.80	15.20

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