

**GENETIC ASSOCIATION MAPPING TO IDENTIFY
QTLs FOR DROUGHT TOLERANCE AND
EXPRESSION ANALYSIS OF SELECTED GENES
UNDER DROUGHT ENVIRONMENT IN FINGER
MILLET [*Eleusine coracana* (L.) Gaertn]**

**ANTRE SURESH HARIBHAU
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**DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE
2022**

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**ANTRE SURESH HARIBHAU
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Thesis submitted to the
UNIVERSITY OF AGRICULTURAL SCIENCES, BANGALORE
*In partial fulfillment of the requirements
for the award of the degree of*

DOCTOR OF PHILOSOPHY
in
PLANT BIOTECHNOLOGY

BENGALURU

AUGUST, 2022



*Affectionately
dedicated to
My beloved Family,
Friends
&
Teachers*

DEPARTMENT OF PLANT BIOTECHNOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES'
BANGALORE - 560 065

CERTIFICATE

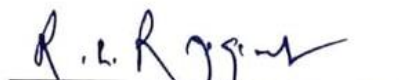
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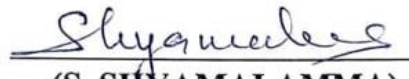

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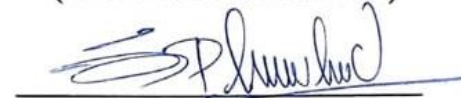
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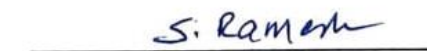
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External Examiner

ACKNOWLEDGEMENT

It is always a nostalgic feeling whenever one glances back to the days of hard work, tension and the need of the hour to excel. One would not achieve whatever he can, without the help, support, encouragement and the wishes of the near and dear ones. I owe them a lot and it is always a very difficult task expressing and putting into words the sense of gratitude I feel towards them. I wish to remember and acknowledge all who made this thesis possible.

*I take this opportunity to express my feeling, profound heartfelt gratitude to the respectable Chairman of Advisory Committee, **Dr. R. L. Ravikumar**, Professor (Retd.), Department of Plant Biotechnology, UAS, GKVK, Bengaluru for his prodigious guidance, valuable suggestions, constant supervision, encouragement and help, besides personal concern bestowed during the course of investigation which have all made it possible for the successful completion of my thesis.*

*I wish my sincere thanks members of my Advisory Committee to **Dr. S. Shyamamma**, Professor and Head, Department of Plant Biotechnology, **Dr. P. S. Benherlal**, Ass. Professor, Department of Plant Biotechnology, **Dr. M. S. Sheshshayee**, Professor and Head, Department of Crop Physiology, **Dr. S. Ramesh**, Professor, Department of Crop genetics and Plant Breeding, UAS, GKVK, Bengaluru, for their constant supervision, invaluable guidance and the facilities extended during the course of this investigation.*

*I take this auspicious opportunity to express my sincere gratitude and profound reverence to professors **Dr. K. M. Harinikumar**, **Dr. Anitha Peter**, **Dr. Veena S. Anil**, **Dr. Nagesha N.**, **Dr. Mohan Chavan**, **Dr. Bhavani P** and **Mrs. Poornima R.** for their valuable suggestions and guidance during my research programme.*

*Every result described in this thesis was accomplished with the help and support of my fellow lab-mates. I express deep sense of heartfelt gratitude to my fellow lab-mates and beloved seniors **Dr. Ashutosh**, **Dr. Sunil**, **Dr. Shyam**, **Dr. Meghana**, **Dr. Naflath**, **Dr. Neetha** and **Dr. Veeresh** for their invaluable suggestions and their timely assistance*

at all stages of my research and for their candid help. Also, I would like to thank my beloved juniors **Saumya, Varsha** and **Akshay** for co-operation during my research. I express my sincere thanks to **Shashank sir, Shivkumar sir, Ravikumar sir, Parinaya, Rathnamma, Jyothi, Ramakrishna** and **Aadinarayan** who helped me immensely throughout the course of investigation in the technical work, lab and field experiments.

I also have been fortunate in having affectionate friends as my classmates who always stood with me and shared my anxiety, happiness, tension and sad moments without whom life would be miserable. I am ever thankful to **Upasana, Lakshmeesha, Ramesh, Ranganath sir, Harshitha** and **Jayashree** for their constant encouragement and co-operation throughout my study and research.

With love, affection and encouragement of my beloved grandparents, parents and sisters, I would have not come up to this stage. My vocabulary has no words to express my gratitude to them. I owe my sincere thanks to **Sonali** for her moral support, keeping me up and cherishable memories throughout the journey.

Life at Bengaluru became a real joy with friends, seniors and juniors. I will always cherish their company, I wish to express my sincere thanks **Dr. Ramchandra, Dr. Swapnil, Khetaram sir, Likhith, Muttugoud, Sandeep, Veeresh, Ajmal, Ganesh, Udayan** and **Pallavi** who went above and beyond the call of duty in making things in the life run by voluntarily tackling the most heinous tasks and stepping up whenever the odd emergency arose. I express my heartfelt thanks to all beloved and respected people who helped me but could not find separate mention.

A very special gratitude goes out to all down at Indo-Switzerland collaboration in Biotechnology (**ISCB**) and **CAAST-NGT** program for providing funds to carrying out the research work. My study needs special acknowledgement to the **UGC, New Delhi**, Government of India for providing financial assistance in the form of **UGC-NFOBC fellowship** and grants for consumables which buttressed me to perform my work comfortably

At the end of my thesis, I would like to thank all those people who made this thesis possible and it is a pleasant task to express my thanks to all those who contributed in many ways to the success of this study and made it an unforgettable experience for me.

Any omission in this acknowledgement does not mean lack of gratitude.

Bengaluru

August, 2022

(ANTRE SURESH HARIBHAU)

“Genetic association mapping to identify QTLs for drought tolerance and expression analysis of selected genes under drought environment in finger millet [*Eleusine coracana* (L.) Gaertn]”

ANTRE SURESH HARIBHAU

ABSTRACT

Although the finger millet is renowned for its drought tolerance, drought stress at reproductive stage dramatically reduces the seed yield. Three hundred and fifty diverse genotypes of finger millet were evaluated for sixteen agro-morphological traits under drought stress (DS) and well watered (WW) conditions over the two years viz. summer 2019 and summer 2020 at GKVK, Bangalore. The DS at initial reproductive stage significantly affected the performance of the genotypes for seed yield and component traits. The genotype and genotype $\times$ environment interaction biplot method was used to identify high yielding and stable genotypes under WW (GE-5078, GPU-66), DS (GE-2644, GE-3605) and across WWDS (GE-2644) conditions. Stepwise multiple linear regression and principal component analysis under WW and DS indicated that, total earhead weight per plant and main earhead weight are the important traits for both the environments. Genome-wide SNPs were identified by re-sequencing of 350 genotypes and the marker trait association analysis revealed 49 SNPs under WW and 34 SNPs under DS conditions were associated with different agro morphological and drought tolerance related traits. Six each drought tolerant and drought susceptible genotypes were evaluated in replicated field trial for reproductive stage drought stress tolerance during summer 2021. The DS at reproductive stage showed significant effect on number of pollen grains per anther, pollen sterility, relative water content and chlorophyll content. Drought tolerant genotypes recorded significantly less pollen sterility and higher relative water content compared to susceptible genotypes. Two contrasting genotypes, GE-1234 and GE-4719 were used to study the expression of Sucrose synthase-1 (*SUS1*), Cell wall invertase (*INCW*), Invertase-1 (*IVR1*) and two uncharacterized genes (GRMZM2G162968 and GRMZM5G844309) in the immature anthers under DS. The expression level of *SUS1*, *INCW*, *IVR1* and GRMZM2G162968 were reduced under DS conditions and the extent of reduction was influenced by the drought tolerance of the genotypes.

August, 2022
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(R. L. Ravikumar)

**ರಾಗಿಯಲ್ಲಿ ಬರ ಸಹಿಷ್ಣುತೆಗಾಗಿ QTLs ಗಳನ್ನು ಗುರುತಿಸಲು ಅಸೋಸಿಯೇಷನ್ ಮ್ಯಾಪಿಂಗ್ ವಿಶ್ಲೇಷಣೆ
ಮತ್ತು ಬರ ಪರಿಸರದ ಒತ್ತಡದಲ್ಲಿ ಆಯ್ಕೆ ಜೀನ್‌ಗಳ ಅಭಿವ್ಯಕ್ತಿ ವಿಶ್ಲೇಷಣೆ**

[Eleusine coracana (L.) Gaertn]

ಅಂತ್ಯ ಸುರೇಶ್ ಹರಿಬಾಹು

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ರಾಗಿ ತನ್ನ ಬರ ಸಹಿಷ್ಣುತೆಗೆ ಹೆಸರುವಾಸಿಯಾಗಿದ್ದರೂ, ಸಂತಾನೋತ್ಪತ್ತಿ ಹಂತದಲ್ಲಿ ಬರಗಾಲದ ಒತ್ತಡವು ಬೀಜದ ಇಳುವರಿಯನ್ನು ಕುಂಠಿತಗೊಳಿಸುತ್ತದೆ. ಪ್ರಸ್ತುತ ಅಧ್ಯಯನದಲ್ಲಿ ಹದಿನಾರು ಅಗ್ರೋಮೊಫೋಲೋಜಿಕಲ್ ಗುಣಲಕ್ಷಣಗಳ ಮೇಲೆ ಬರಗಾಲದ ಪರಿಣಾಮವನ್ನು ೩೫೦ ಜೀನೋಟೈಪ್‌ಗಳನ್ನು ಉಪಯೋಗಿಸಿ ಎರಡು ವರ್ಷಗಳ ಕಾಲ (ಬೇಸಿಗೆ ೨೦೧೬ ಮತ್ತು ಬೇಸಿಗೆ ೨೦೧೭) ನೀರಿರುವ ಹಾಗೂ ಬರಗಾಲದ ಒತ್ತಡ ಪರಿಸ್ಥಿತಿಯಲ್ಲಿ ಅಭ್ಯಸಿಸಲಾಯಿತು. ಹೂಬಿಡುವ ಆರಂಭಿಕ ಹಂತದ ಬರಗಾಲವು ಜೀನೋಟೈಪ್ ಕಾರ್ಯಕ್ಷಮತೆಯ ಮೇಲೆ ಗಮನಾರ್ಹ ಪರಿಣಾಮ ಬೀರಿತು. ಜೀನೋಟೈಪ್ ಮತ್ತು ಜೀನೋಟೈಪ್ x ಪರಿಸರ ಪ್ರಭಾವದ ಬೈಪ್ಲಾಟ್ ವಿಧಾನವನ್ನು ಅಳವಡಿಸಿ ನೀರಿರುವ (GE-5078, GPU-66) ಬರಗಾಲದ ಒತ್ತಡ (GE-2644, GE-3605) ಹಾಗೂ ಎರಡೂ ಪರಿಸ್ಥಿತಿಗೆ (GE-2644) ಸೂಕ್ತವಾದ ಹೆಚ್ಚಿನ ಇಳುವರಿ ನೀಡುವ ಜೀನೋಟೈಪ್‌ಗಳನ್ನು ಗುರುತಿಸಲಾಯಿತು. ಗಿಡದ ಒಟ್ಟುತನ ತೂಕ ಮತ್ತು ಮುಖ್ಯ ತನತೂಕ ಗುಣಲಕ್ಷಣಗಳು ಎರಡು ಪರಿಸರಗಳಿಗೆ (ಬರ ಮತ್ತು ನೀರಿರುವ) ಬಹಳ ಪ್ರಮುಖವಾದವುಗಳೆಂದು ಸ್ಪಷ್ಟವಾಗಿ ಮಲ್ಟಿಪಲ್ ಲೀನಿಯರ್ ರಿಗ್ರೆಷನ್ ಮತ್ತು ಪ್ರಿನ್ಸಿಪಲ್ ಕಾಂಪೋನೆಂಟ್ ಅನಾಲಿಸಿಸ್ ಸೂಚಿಸಿದೆ. ಜೀನೋಮ್ ವೈಡ್ SNP ಗಳನ್ನು 350 ಜೀನೋಟೈಪ್‌ಗಳ ಮರು-ಅನುಕ್ರಮದ ಮೂಲಕ ಗುರುತಿಸಲಾಗಿದೆ. ಮಾರ್ಕರ್ ಟೈಟ್ ಅಸೋಸಿಯೇಷನ್ ವಿಶ್ಲೇಷಣೆಯು ನೀರಿರುವ ಪರಿಸ್ಥಿತಿ ಅಡಿಯಲ್ಲಿ 49 SNP ಗಳು ಮತ್ತು ಬರಗಾಲದ ಒತ್ತಡ ಪರಿಸ್ಥಿತಿಗಳಲ್ಲಿ 34 SNP ಗಳು ವಿಭಿನ್ನ ಅಗ್ರೋಮೊಫೋಲೋಜಿಕಲ್ ಗುಣಲಕ್ಷಣಗಳು ಮತ್ತು ಬರ ಸಹಿಷ್ಣುತೆಗೆ ಸಂಬಂಧಿಸಿದ ಗುಣಲಕ್ಷಣಗಳೊಂದಿಗೆ ಸಂಬಂಧ ಸೂಚಿಸಿವೆ. ೨೦೧೭ ರ ಬೇಸಿಗೆಯಲ್ಲಿ, ಸಂತಾನೋತ್ಪತ್ತಿ ಹಂತದ ಬರ ಒತ್ತಡ ಸಹಿಷ್ಣುತೆಗಾಗಿ ಪ್ರತಿ ಆರು ಬರ ಸಹಿಷ್ಣು ಮತ್ತು ಬರಕ್ಕೆ ಪೀಡಿತ ಜೀನೋಟೈಪ್‌ಗಳನ್ನು ಪುನರಾವರ್ತಿತ ಕ್ಷೇತ್ರ ಪ್ರಯೋಗದಲ್ಲಿ ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಗಿದೆ. ಸಂತಾನೋತ್ಪತ್ತಿ ಹಂತದಲ್ಲಿ ಬರಗಾಲದ ಒತ್ತಡವು ಪ್ರತಿ ಪರಾಗದ ಕಣಗಳ ಸಂಖ್ಯೆ, ಪರಾಗ ಸಂತಾನಹೀನತೆ, ಸಾಪೇಕ್ಷ ನೀರಿನ ಅಂಶ ಮತ್ತು ಕ್ಲೋರೊಫಿಲ್ ಅಂಶಗಳ ಮೇಲೆ ಗಮನಾರ್ಹ ಪರಿಣಾಮವನ್ನು ತೋರಿಸಿದೆ. ಬರ ಸಹಿಷ್ಣು ಜೀನೋಟೈಪ್‌ಗಳು ಬರಪೀಡಿತ ಜೀನೋಟೈಪ್‌ಗಳಿಗೆ ಹೋಲಿಸಿದರೆ ಕಡಿಮೆ ಪರಾಗದ ಸಂತಾನಹೀನತೆ ಮತ್ತು ಹೆಚ್ಚಿನ ಸಾಪೇಕ್ಷ ನೀರಿನ ಅಂಶವನ್ನು ಬರದ ಒತ್ತಡದಲ್ಲೂ ಹೊಂದಿವೆ. ಒಂದು ಬರ ಸಹಿಷ್ಣುತೆ (GE-1234) ಮತ್ತು ಒಂದು ಬರಪೀಡಿತ (GE-4719) ಜೀನೋಟೈಪ್‌ಗಳ ಆಪಕ್ಷವಾದ ಪರಾಗಗಳಲ್ಲಿ ಸುಕ್ರೋಸ್ ಸಿಂಥೇಸ್-1 (*SUS1*), ಸೆಲ್ ವಾಲ್ ಇನ್‌ವರ್ಟೇಸ್ (*INCW*), ಇನ್‌ವರ್ಟೇಸ್-1 (*IVR1*) ಜೀನ್‌ಗಳು ಮತ್ತು ಎರಡು ಗುರುತಿಸಲಾಗದ ಜೀನ್‌ಗಳ (GRMZM2G162968 ಮತ್ತು GRMZM2G162968) ಅಭಿವ್ಯಕ್ತಿಯನ್ನು ಬರಗಾಲದ ಒತ್ತಡದಲ್ಲಿ ಅಧ್ಯಯನ ಮಾಡಲಾಯಿತು. ಬರಗಾಲದ ಒತ್ತಡ ಪರಿಸ್ಥಿತಿಗಳಲ್ಲಿ *SUS1*, *INCW*, *IVR1* ಮತ್ತು GRMZM2G162968 ನ ಅಭಿವ್ಯಕ್ತಿ ಮಟ್ಟವು ಬರಪೀಡಿತ ಜೀನೋಟೈಪ್‌ಗಳಲ್ಲಿ ಹೆಚ್ಚು ಕಡಿಮೆಯಾಗಿದೆ. ಬರಗಾಲದ ಜೀನ್ ಅಭಿವ್ಯಕ್ತಿಯ ಕಡಿತದ ಮಟ್ಟವು ಬರಸಹಿಷ್ಣುತೆಯನ್ನು ಸೂಚಿಸುತ್ತದೆ.

ಆಗಸ್ಟ್, ೨೦೧೭

ಸಸ್ಯ ಜೈವಿಕ ತಂತ್ರಜ್ಞಾನ ವಿಭಾಗ

ಕೃಷಿ ವಿಶ್ವವಿದ್ಯಾನಿಲಯ

ಗಾ ಕೃ ವಿ ಕೇ, ಬೆಂಗಳೂರು - ೫೬೦ ೦೭೫

ಮುಖ್ಯ ಸಲಹೆಗಾರರು

(ಆರ್. ಎಲ್. ರವಿಕುಮಾರ್)

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I INTRODUCTION

Finger millet (*Eleusine coracana* (L.) Gaertn.) is a C₄ crop expected to play an important role in climate smart agriculture in order to boost food production particularly in the developing countries (Goron *et al.*, 2015; Bandyopadhyay *et al.*, 2017). Finger millet is highly nutritious crop with its composition of protein (7.3%), fat (1.3%), carbohydrates (72.6%), dietary fibre (18%), ash (3.0%), calcium (352 mg/100g) and 594 mg/g of protein (Shobana *et al.*, 2013; Chandra *et al.*, 2016; Gupta *et al.*, 2017; Sharma *et al.*, 2017). Apart from high nutritional richness and high photosynthetic efficiency, finger millet has better tolerance to biotic and abiotic stresses with vast genetic and genomic potential which provides a wide choice for developing strategies for making climate resilient staple crop (Gupta *et al.*, 2017). This proves the crucial role of finger millet in food, nutrition, and economic security, and hence it should be called “Climate-smart Nutri-cereal” rather simply “Nutri-cereal” alone. To bring millets back to the mainstream and gain benefits from their nutritious properties, the year 2018 was declared as the “National Year of Millets” by the Indian Government. Also, FAO declared that the year 2023 will be the “International Year of Millets” to increase the production and productivity of millets throughout the globe (Ashwini *et al.*, 2019).

Globally, finger millet is cultivated on an area of 4.0–4.5 million ha, with a production of ~4.5 million tonnes every year (Ceasar *et al.*, 2018) and it is widely grown in Asia and Africa. India is one of the major producer of finger millet with an area of 1.17 million hectares with a production of 2.00 million tonnes and an average productivity of 1661 kg/ha (ASSOCHAM, 2021). In particular, more than 90% of finger millet cultivation is under rain-fed (dry zone) areas in India (Davis *et al.*, 2019) and thus vulnerable for yield loss due to drought stress. Unfortunately, recurring climate events have decreased the production of all the millets with a high negative growth rate (-3.6%) backed by a decline in area under cultivation majorly in finger millet with the compound annual growth rate (CGAR) of -19.8% followed by sorghum (-17.8%) and pearl millet (-7.5%) from 2014-15 to 2019-20 (ASSOCHAM, 2021).

Drought stress is expected to become more severe and frequent in near future (Choudhary and Padaria, 2015). It also has been reported that, even a short period of

drought during critical growth stages in finger millet reduces the grain yield significantly (Udayakumar *et al.*, 1986). Terminal drought occurs regularly in the semi-arid region, destabilising the reproductive development and seed yield of finger millet (Krishnasastry *et al.*, 1982; Dinesh Kumar *et al.*, 1987; Krishna, 2010). The occurrence of water stress is unpredictable in finger millet growing regions and the impact differs at different growth stages, affecting different traits (Mude *et al.*, 2020) and ultimately reducing the yield (Talwar *et al.*, 2020). However, if crop encounter even a minor drought stress at the reproductive stage, the yield is noticeably reduced, even if the rain returns, the effect is irreversible (Saini and Lalonde, 1997). Therefore, it is important to identify genotypes with suitable phenotypic target traits at reproductive stage for yield improvement and drought tolerance in finger millet.

Drought response is polygenic in nature (Khalili *et al.*, 2016) with a complex phenotype affected by genotype and environment. To improve the breeding efficiency, researchers have to identify more useful agro-morphological traits that influence drought tolerance and seed yield. Therefore, it is necessary to have knowledge on the extent of association between seed yield and its contributing characters. However, understanding the genotype $\times$ environment interaction among the genotypes in multiple environment is very crucial for plant breeders, as it complicates the selection of promising genotypes by declining the association between genotypic and phenotypic values (Ebdon and Gauch, 2002; Yan and Tinker, 2006). Several approaches exist to analyze genotype stability, including biplots derived from additive main effects and multiplicative interaction (AMMI; Gauch, 1988) and genotype plus genotype $\times$ environment interaction (GGE; Yan *et al.*, 2000; Yan and Kang, 2002) which gained popularity. The use of two or more traits could produce superior genotype to direct selection for grain yield than single trait independently under stress environment (Yan and Frégeau-Reid, 2018; Kendal, 2019). Therefore, genotype by yield*trait (GYT) biplot approach has been used to tackle the problem of genotype evaluation on multiple traits under certain environments. This is an effective way to improve yield and drought tolerance by selecting donor genotype/s with desired yield*trait combinations in breeding programs.

The use of molecular markers is an important tool in selection and breeding programs including traits that are difficult to phenotype and have complex genetic architecture like seed yield and drought response. DNA markers are being used to tag important genes or genomic regions governing complex quantitative traits by using bi-parental or association mapping approaches (Sehgal *et al.*, 2015). The availability of SSR markers in finger millet is limited (Gimode *et al.*, 2016) and only a few reports are available on association mapping for agro-morphological traits using SSR markers (Babu *et al.*, 2014a; Babu *et al.*, 2014b). The SNPs are considered to be the ideal markers in plant genetic studies and genome analyses because of their excellent genetic attributes, such as ubiquitous distribution, co-dominant nature of inheritance, chromosome-specific location and high reproducibility (Sachidanandam *et al.*, 2001; Gupta and Rustgi, 2004). However, SNP mining becomes highly challenging in allo-tetraploid crops such as finger millet due to low polymorphism resulting from high degree of inbreeding and large numbers of homeologous SNPs, which are the result of polymorphism between the AA and BB sub-genomes of the same individual (Gimode *et al.*, 2016). But, identification of SNPs in several other polyploid crops including wheat (Abou-Elwafa and Shehzad, 2021; Saini *et al.*, 2022), oats (Canales *et al.*, 2021), cotton (Zhao *et al.*, 2022) and groundnut (Maalouf *et al.*, 2022) has been successful as a result of suitable filtering tools and stringency in mapping parameters.

Major breeding targets for finger millet have included traits such as improvement of agro-morphological characteristics, calcium accumulation, blast resistance, nitrogen use efficiency, and tolerance to phosphorus deficiency (Sood *et al.*, 2016; Gupta *et al.*, 2017). Mapping using bi-parental mapping-population has limitations of low number of segregating alleles and lower mapping resolution in finger millet (Sood *et al.*, 2016). On the other hand, association mapping utilizes the existing genetic diversity in natural germplasm populations and exploit their historic recombination events to map the genomic regions or genes. This approach, also known as linkage disequilibrium (LD) mapping, as it has a higher mapping resolution due to the use of a genetically diverse population (Buckler and Thornsberry, 2002). In finger millet, association mapping has been used to identify quantitative trait loci (QTLs) associated with different agro-morphological traits, protein and tryptophan contents as well as blast tolerance, and low

phosphorus tolerance (Babu *et al.*, 2014a; Babu *et al.*, 2014b; Babu *et al.*, 2014c; Ramakrishnan *et al.*, 2016a; Ramakrishnan *et al.*, 2017). In recent years, SNP markers used for genome-wide association mapping in finger millet for seed yield related traits (Sharma *et al.*, 2018), seed protein content (Tiwari *et al.*, 2020), minerals (iron, zinc, calcium, magnesium, potassium, and sodium) and protein (Puranik *et al.*, 2020). However, there is no study available on genome-wide association mapping for identification of SNP markers for agro-morphological traits under drought stress in finger millet.

At reproductive stage drought stress induced pollen sterility has been reported in many crops as detrimental factor in reducing grain number. Exploring the mechanisms underlying pollen fertility under drought stress conditions could assist breeding high-yielding cultivars with stress tolerance. Drought stress at reproductive stage significantly affects the development of the floral meristem and can cause a reduction of spikelet initiation and abortion of developing florets as observed in wheat (Dolferus *et al.*, 2011). Additionally, an understanding of the molecular mechanisms or regulation of gene expression in male reproductive organs is of great importance as they are highly vulnerable to moisture/drought stress. So far, there is very little information on the molecular aspects that improve drought tolerance in reproductive tissue in finger millet. Thus, understanding the variation in the expression of stress related genes in anthers among the drought stress tolerant and susceptible genotypes will give a clear-cut idea of the important genes responsible for enhancing drought tolerance in crop plants.

Taking into consideration all these points, the present work was carried out to identify drought tolerant genotypes in finger millet as well as an adequate characterization of useful agro-morphological traits for drought tolerance and their association with yield and yield components to improve the drought tolerance combined with higher productivity in finger millet. The specific objectives of the present study are:

1. Identification of DNA markers associated with QTLs for seed yield and drought stress tolerance related traits.
2. Effect of early reproductive growth stage stress on flowering, quality and quantity of pollen grains, seed number and grain yield in contrasting genotypes.
3. Understanding the expression of stress related genes in reproductive tissues under moisture stress.

II REVIEW OF LITURATURE

Finger millet (*Eleusine coracana*) is one of the most ancient crops, commonly grown in the warmer regions of the world, particularly in southern Asia and Africa as a food and fodder crop (Rachie and Peters, 1977). It is a crop with an immense importance in the areas where it is grown and it stands in unique place among cereals attributed with properties such as higher nutritional value, excellent grain storage quality, adaptability to extreme abiotic and biotic stresses and it also has the ability to thrive in marginal areas making it an important food security crop. Despite climate resilience, nutrient richness and importance as subsistence crop, very little scientific attention has been given to finger millet as compared to major cereals like rice, maize and wheat (Saha *et al.*, 2016; Ceasar *et al.*, 2018) hence called orphan/underutilized crop (Goron and Raizada, 2015; Hatakeyama *et al.*, 2018).

Development and effective utilization of available genetic and genomic resources is a very crucial step in improving the crop species for specific traits. The recent advances in sequencing technologies and the modern genomic and transcriptomic sequence analysis offer greater advantage to analyse the complex genome. The availability of whole genome sequences (WGS) and transcriptome sequences in finger millet will provide novel prospective of crop improvement (Hittalmani *et al.*, 2017 and Hatakeyama *et al.*, 2018).

Finger millet is mostly cultivated in sub marginal lands and limited moisture conditions, it is prone for recurrent drought, which affects crop growth due to moisture as well as temperature stress. The growth and development of finger millet gets affected by duration and intensity of drought (Mohanabharathi *et al.*, 2019). There is a greater need of involving diverse germplasm, good phenotyping facilities and genotyping with appropriate number and choice of molecular markers to identify robust markers, especially for seed yield and drought tolerance related traits in finger millet. Hence, the present study was envisaged to identify QTLs for seed yield and drought tolerance related traits through association mapping and expression analysis of selected genes under drought environment in finger millet. The literature on the various aspects of marker

development and their utilization in finger millet has been reviewed and presented under the following headings;

2.1 Origin of finger millet and its importance

2.2 Genetic variability studies in finger millet

2.2.1 Germplasm resources of finger millet

2.2.2 Genetic variability, heritability and genetic advance as *per cent* mean

2.3 Correlation, multiple linear regression and path analysis

2.4 Identification of drought tolerance related traits and genotypes

2.4.1 Effect of drought on agro-morphological traits

2.4.2 Drought tolerance indices (DTI)

2.4.3 Genotype $\times$ environmental interaction and biplot analysis

2.5 Reproductive stage drought stress in finger millet

2.5.1 Effect of drought stress on physiological and pollen related traits

2.5.1.1 Pollen number and sterility

2.5.1.2 SPAD chlorophyll meter reading (SCMR)

2.5.1.3 Relative water content (RWC)

2.5.1.4 Membrane leakage

2.6 Genomic resources in finger millet available for crop improvement

2.7 Identification of QTLs for seed yield and drought tolerance related traits

2.8 Association mapping in finger millet

2.9 Expression analysis of drought responsive genes in reproductive tissue

2.1 Origin of finger millet and its importance

Finger millet is an allotetraploid ($2n=4x=36$), highly self-pollinating crop belonging to the family Poaceae, sub-family Chloridoideae and tribe Chlorideae (Dida *et al.*, 2007). The *Eleusine* genus is a small, which includes around ten to eleven annual or perennial grasses. Most of the species of *Eleusine* are distributed in tropical and subtropical parts of Africa, Asia and South America. *Eleusine indica* (goose grass) is a most successful cosmopolitan grass. This species has been found introduced as far as north upto the British Isles and as far as south till New Zealand (Kennedy-O'Byrne, 1957).

The evidence of origin of finger millet is thought to date back to 3000 B. C., where the oldest archaeological sample of finger millet was discovered in Axum, a city in the northern Ethiopia (Hilu and Dewet, 1976a). The fact that *Eleusine indica* was also discovered along with *Eleusine coracana* in the debris of the excavation at Neolithic Sites in Mysore district of Karnataka, India, date back to 1800 BC, gave an impetus to claim that *Eleusine indica* could have been a probable progenitor of *Eleusine coracana* and it could have been a native of India (Hutchinson, 1965). Further, for many number of years, *Eleusine indica* was considered to be a putative parent of *Eleusine coracana*.

Subsequent, cytological and morphological studies by Channaveeriah and Hiremath (1974) and Hilu *et al.* (1979) provided the evidence in favour of African origin of finger millet, as *Eleusine coracana* generally cultivated in India, had cytogenetical and morphological features similar to that of *Eleusine africana*. Additionally, they also observed that gene flow could have occurred freely between these two species.

This strengthened the speculation and affirmed that finger millet was a native of Africa and *Eleusine corocana* could have originated directly from *Eleusine africana* through a selection and domestication for a large grain mutant. The important role of natural selection in finger millet evolution was emphasized and it was concluded that, three major events were occurred during the evolution of finger millet since from its initial domestication. Chronologically, these are (1) the movement of the crop from the highlands to the lowlands of Africa, (2) its introduction to India, and (3) its later expansion to the northern parts of India.

Therefore, it is now an established fact that *Eleusine corocana* has an African origin, considered to be domesticated some 5,000 years ago from the wild *E. corocana* subsp. *africana* ($2n = 4x = 36$) as a large grain mutant for cultivation in the highland that stretches from Ethiopia to Uganda (Channaveeriah and Hiremath, 1974). It was further confirmed from the findings of the archaeological excavation in Ethiopia dating to three millennium B.C. (Hilu *et al.*, 1979). Domesticated finger millet was subsequently brought to Southern parts of India from Africa through sea trade in around 1000 B. C. and later expansion to Northern parts of India (Hilu and Dewet, 1976b). Therefore, East Africa is considered to be the primary centre of diversity whereas, India a secondary centre of diversity of finger millet (Dida *et al.*, 2008).

Finger millet is relatively resistant to storage insect pests, which makes the crop an important source of food during a famine, as the grains can be stored for several years without insect damage and much loss due to deterioration (Latha *et al.*, 2005). Finger millet is also known for its health benefits, as it is gluten-free, rich in quality protein (5–8%), with high amounts of tryptophan, cysteine and methionine, dietary fibre (15–20%), phytochemicals, calcium and other minerals (2–3%) (Chethan and Malleshi, 2007; Upadhyaya *et al.*, 2010; Chandrashekhar *et al.*, 2012; Kumar *et al.*, 2016a; Gupta *et al.*, 2017).

In recent times, in view of the higher incidence of diabetes and obesity, it is considered as a wholesome food for diabetics (Lakshmi Kumari and Sumathi 2002). Thus, finger millet is considered as a crop of high nutritive value and has maintained high socioeconomic importance in the context of subsistence farmers of the Indian and African semiarid tropic regions (Gull *et al.*, 2014).

2.2 Genetic variability studies in finger millet

2.2.1 Germplasm resources of finger millet

In the current scenario of feeding world's burgeoning population, plant genetic resources (PGR) serve as an essential component of agricultural biodiversity which play a crucial role in crop improvement programmes for development of new cultivars. The process of crop improvement includes development of new cultivar or the improvement

of existing cultivar which lacks one or other attributes (Saha *et al.*, 2016). Wild species and many elite land races possess potential allelic richness of the genes governing for the challenging traits that can be gainfully harnessed in the breeding programme to upgrade and achieve desired objective.

Finger millet is one such important crop, which has very rich genetic diversity, which holds promise to discover new genes and traits for crop improvement. Large collections of finger millet germplasm are conserved over a long-term in various organizations across globe. Development of smaller subsets of core and mini-core collections by characterization of germplasm accession resulted in the effective utilization of trait variability in crop improvement (Upadhyaya *et al.*, 2006).

Finger millet accessions belong to the eight of *Eleusine*, such as *Eleusine coracana*, *E. indica*, *E. africana*, *E. mutiflora*, *E. tristachya*, *E. jaegeri*, *E. kigeziensis*, and *E. floccifolia* (Hilu and Dewet, 1976a). The majority of the collections are from land races of *E. coracana*, which contribute major proportion of finger millet germplasm accession conserved in gene banks (Saha *et al.*, 2016).

Currently more than 37,650 finger millet accessions are available in the gene banks at various international and national research organizations worldwide (Sood *et al.*, 2019). The International crop research Institute for the Semi-Arid tropics (ICRISAT), Patancheru, Hyderabad, India holds the global finger millet collection of about 5957 accessions, which includes 5665 landraces, 137 improved cultivars, 105 wild species and 50 breeding line. Indian subcontinent possess highest diversity of finger millet, the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, holds the largest collection of finger millet accessions (10,507) which includes 117 are exotic lines and 7 wild species. The project coordination cell on small millets in India has an exhaustive collection of 6257 accessions representing the entire global distribution of finger millet. The other institutes holding such large collection of finger millet accessions are Kenya Agriculture Research Institute (KARI), Kenya has about 2875 accessions; Institute of Bio-diversity Conservation (IBC), Ethiopia has about 2156 accessions; United states Department of Agriculture (USDA), Griffin, Georgia has about 1452 accessions; Serere Agricultural and Animal Production Research Institute (SAARI), Uganda has

about 1452 accessions; SADC Plant genetic Resources Centre, Lusaka, Zambia, has about 1037 accessions. The countries like Japan, Nepal, Srilanka, Bhutan, China, Tanzania, and Malwai also have a reasonable collection of finger millet accessions (Dwivedi *et al.*, 2012; Goron and Raizada, 2015; Saha *et al.*, 2016; Sood *et al.*, 2016; Gupta *et al.*, 2017; Ceasar *et al.*, 2018 and Sood *et al.*, 2019).

2.2.2 Genetic variability, heritability and genetic advance as *per cent* mean

The evolution of crop plants mainly depends upon the variability present in the base population. The variation among the individuals occurs due to differences in genetic composition and/or the environment in which they are raised. The morphological diversity present within finger millet is immense. For example, a range of seed colors can be produced, which are correlated with protein and calcium content (Vadivoo *et al.*, 1998). Landraces with different attributes (e.g., time to maturity, grain size, drought tolerance) are valued by farmers based on local agricultural complexities which reflects their productivity across multiple agro-economic zones (Tsehay *et al.*, 2006). The accurate assessment of genetic diversity from the gene banks around the globe is essential for genetic resource management, trait mapping and crop improvement (Wambi *et al.*, 2021). Hence, breeding for stress tolerant crops is in high demand and in this situation, it is essential to fully characterize and identify genetic variation for the stress tolerant traits in the available germplasm before using them in a breeding program (Biradar *et al.*, 2019). Therefore, screening of large germplasm for drought tolerance traits is more important in crop improvement programme to evolve varieties suitable for rainfed situations.

However, little efforts have been made in dissecting the genetic determinants of drought resistance, because of its complex nature. In this context, a better knowledge on the genetic make-up and molecular basis of primary and secondary traits that underlie the response of crops across a broad range of soil moisture is an essential prerequisite toward more effective and targeted breeding activities (Salekdeh *et al.*, 2009). However, encouraging heritability of physiological or secondary traits that are highly correlated with yield gives a good opportunity for plant breeding in drought-prone regions (Stiller *et al.*, 2005).

Swaminath (1981) studied genetic diversity among 175 African finger millet accessions and observed highest genotypic and phenotypic variances for density of seed followed by plant height, earhead weight, fodder weight and days to maturity.

Abraham *et al.* (1989) observed genetic variability in seven quantitative characters among 20 diverse genotype of finger millet. They found that phenotypic coefficient of variation and genotypic coefficient of variation were high for productive tillers per plant, grain yield per plant, 1000-grain weight and number of finger per ear.

Thakur and Saini, (1995) evaluated 90 finger millet accessions. High heritability and genetic advance were observed respectively for seed yield/plant (59 and 124%) days to flowering (80 and 17%) plant height (56 and 13%) and days to maturity (79 and 29%). Days to germination, heading and maturity showed high negative association with seed yield where as ears/plant showed high positive association.

Bendale *et al.* (2002) studied genetic variability, correlation, and path analysis in 29 phenotypically diverse genotypes of finger millet (*Eleusine coracana*) collected from different agro-climatic regions of India. Phenotypic variances were higher than genotypic variances. Phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were high for number of tillers, number of effective tillers, grain yield per plant, straw yield per plant, and weight of grains of main ear head.

John (2006) evaluated genetic variability among 20 genotypes of finger millet and observed high genotypic and phenotypic variance levels for days to 50 *per cent* flowering, plant height, days to maturity, straw yield per plant and harvest index. The traits such as 1000-grain weight, main earhead length, number of productive tillers per plant and grain yield per plant recorded lower estimates of genotypic and phenotypic variance. He reported higher estimates of GCV and PCV for number of productive tillers per plant, number of finger per main ear and total dry matter production and moderate estimates for main earhead length, test weight, harvest index and grain yield per plant while plant height, days to 50 *per cent* flowering and days to maturity recorded lower estimates of GCV and PCV.

Satish *et al.* (2007) reported genetic variability in 178 genotypes of finger millet for fifteen quantitative characters. The estimates of genotypic variances showed a considerable range of variation for most of characters. High heritability coupled with high genetic advance as per cent of mean was observed for number of tillers, productive tillers, days to 50 per cent flowering, fingers number per ear, length of ear head, length of finger, florets number per spikelet, spikelet density, ear weight, test weight, straw yield and grain yield indicating the presence of additive genetic effects for the manifestation of these characters. Plant height, days to maturity and flag leaf length exhibited high heritability with moderate genetic advance which indicates the presence of both additive and non additive genetic effects for these characters.

Ganapathy *et al.* (2011) estimated variability parameter for yield and yield related trait in 230 finger millet accessions and they observed higher estimates of PCV and GCV for productive tillers per plant and moderate estimates for the traits viz., days to 50 per cent flowering, plant height, finger numbers per main ear, finger length and grain yield per plant while the lower estimates were observed for days to maturity.

Haradari *et al.* (2012) screened 1000 finger millet germplasm and observed high PCV and GCV for plant height, number of productive tillers, grain yield per plant and low PCV and GCV for days to 50 per cent flowering and days to maturity. High heritability was observed for plant height, finger length and days to flowering.

Ulaganathan and Nirmalakumari (2013) evaluated the variability among 105 finger millet genotypes and observed high PCV and GCV estimates for the single plant grain yield and productive tillers per plant and moderate estimates for test weight, flag leaf blade length, finger number per panicle, flag leaf sheath length, finger length, flag leaf sheath width, plant height and flag leaf blade width. Lower PCV and GCV exhibited for days to 50 per cent flowering, finger width and days to maturity. Except days to 50 per cent flowering, all the characters exhibited wide range of variation and high heritability coupled with high genetic advance.

Dhanalakshmi *et al.* (2013) evaluated 622 core set of finger millet and observed high PCV and GCV estimates for days to 50 per cent flowering, plant height, number of

productive tillers per plant and low PCV and GCV for grain yield plot. High heritability was observed for all characters except number of productive tillers per plant and moderate genetic advance as *per cent* of mean was observed for plant height and days to fifty *per cent* flowering. The number of productive tillers per plant and grain yield per plot recorded high genetic advance as a *per cent* mean.

Suryanarayana *et al.* (2014) studied 35 diverse genotypes of finger millet to know the genetic variability and divergence based on seven quantitative traits that were recorded. The coefficient of variation at phenotypic (PCV) and genotypic (GCV) levels were high for seed yield per plant, productive tillers per plant and main ear length. High heritability coupled with high genetic advance observed for seed yield per plant and main ear length.

Naeem *et al.* (2015) investigate the genetic variability of plant growth and physiological characters in thirty wheat genotypes under normal and drought stress conditions. They observed higher genetic variance between the genotypes for the cell membrane stability, excised leaf water loss, relative water content, proline, sugar and glycine betain under both conditions. The study suggested that these indices can be used as direct selection criteria for wheat improvement. While excised leaf water loss and fresh root weight revealed the least variability for GCV and PCV correspond to high heritability under stress condition.

Devaliya *et al.* (2018) screened 68 diverse genotypes of finger millet for thirteen quantitative traits. Highest PCV and GCV were recorded for grain yield per plant. The characters viz., straw yield per plant, number of productive tillers per plant, main ear head length and number of fingers per ear were expressed the moderate amount of variation indicated large extent of genetic variability for these traits in the material. High heritability along with high genetic advance (% of mean) observed for for grain yield per plant, number of productive tillers per plant, straw yield per plant and main ear head length, indicating involvement of additive gene action for these traits and phenotypic selection based on these traits in the segregating generations would likely to be more effective.

Kandel *et al.* (2019) reported the significant variation in 16 finger millet genotypes for baring head, days to 50 *per cent* flowering, days to *per cent* heading, days to *per cent* maturity, finger length, flag leaf length, plant stand, plant height, number of finger, peduncle length, number of productive tiller, thousand grain weight, grain yield and straw yield. It has been proved that genetic variation based on morphological characters are very informative enough and can also be used into either selecting superior accessions or to be utilized to select parents for a breeding program under different agro-climatic region.

Anteneh *et al.* (2019) evaluated two-hundred and twenty-five finger millet germplasm accessions for seed yield and related parameters. They reported genotypic and phenotypic coefficients of variation were highest for number of earheads per plant. The highest GCV was obtained from earhead width followed by earheads per plant. Grain yield per plant and culm diameter showed relatively moderate GCV. Whereas, high PCV values were recorded for earhead width, harvest index, grain yield per plant and grain yield per plot. The estimates of broad sense heritability were highest for culm diameter and the lowest for number of earheads per plant.

Patil *et al.* (2021) evaluated 36 white finger millet accessions constituted of 27 landraces and nine released varieties to assess the genetic potential through the variability, correlation analysis among the quantitative traits. They reported moderate genotypic and phenotypic coefficient of variation found for the traits viz., number of fingers per earhead, number of productive tillers per plant, straw yield per plant, grain yield per plant, finger length, harvest index and main earhead. High heritability estimates were observed for days to 50% flowering, days to maturity, plant height, number of productive tillers per plant, number of fingers per earhead, main earhead length, finger length, 1000 grain weight, grain yield per plant, straw yield per plant and harvest index.

2.3 Correlation, multiple linear regression and path analysis

Any character in a plant is manifested as a consequence of a chain of interwoven events, each affecting the other, either directly or through other event. The association of these characters can be estimated through correlation coefficients. The measurement of

direct influence on yield and also indirect effects *via* different characters on yield estimated through path coefficient analyses enables the breeders to select better plant by accommodating most suitable component characters (Laxuman *et al.*, 2011). Multiple linear regression analyses has been used to predict yield on other traits to identify few predominantly responsible secondary traits having considerable influence on yield to be used in selection process to boost yield and for improving genotypes (Mohammadi *et al.*, 2003; Badu-Apraku *et al.*, 2012; Augustina *et al.*, 2013).

Abraham *et al.* (1989) reported that the grain yield had positive correlation with days to 50 per cent flowering, number of productive tillers per plant, number of days to maturity and 1000 grain weight. There was a positive genotypic association of yield with days to 50 per cent flowering and days to maturity. Plant height and grain yield had no significant relationship. Chaudhari (1989) also reported that the productive tillers and grain weight of main ear were the major components of grain yield.

Sharma (1998) evaluated thirty-five genotypes of finger millet (*Eleusine coracana*) for eighty yield-related traits under dry-temperate conditions. The relationship of different agro-morphological characters with seed yield was determined. Seed yield had a high positive correlation with 1000-grain weight, tillers per plant, harvest index, plant height and grains per earhead. Tillers per plant and harvest index had the highest positive effects on seed yield, although they did not contribute indirectly to increased seed yield. The characters 1000-grain weight, tillers per plant and harvest index should be taken into consideration in breeding high yielding finger millet varieties through selection.

Bendale *et al.* (2002) studied correlation and path analysis in 29 phenotypically diverse genotypes of finger millet (*Eleusine coracana*) collected from different agro-climatic regions of India. Grain yield had significant positive correlation with days to emergence of finger, days to maturity, finger length, weight of grains of main earhead, and straw yield per plant. On the basis of association and path analysis, days to emergence of finger, days to 50% flowering, finger length, finger width, and weight of grains of main ear head had significant influence on grain yield per plant in finger millet.

Kebera-Bezawele et al. (2006) reported that the grain yield per plant associated positively with productive tillers, 1000-grain weight, number of grains per spikelet and finger number and negatively associated with days to heading and maturity. The genotypic correlation and path coefficient analysis showed 1000-grain weight, finger number and productive tillers as major contributors to grain yield per plant.

Wolie and Dessalegn (2011) investigated the associations among yield components and their direct and indirect influence on the grain yield in eighty eight diverse genotypes of finger millet. The correlation analysis showed plant height, number of ears per plant, number of fingers per earhead, finger length, biomass yield, harvest index and 1000 kernel weight were positively correlated with seed yield. Phenotypic path analysis showed biomass yield and finger length, number of fingers per earhead and number of earheads per plant exerts positive direct effects on grain yield, while plant height, days to heading and days to maturity exhibited negative direct effects.

Haradari et al. (2012) evaluated base germplasm of 1000 accession of finger millet for eleven quantitative characters. Character association studies revealed that, grain yield per plant had highly positive and significant association with plant height, days to 50 per cent flowering, days to maturity and ear weight per plant. Path analysis revealed that number of productive tillers per plant, days to maturity and earhead weight per plant had direct positive effect on grain yield.

Dhamdhare et al. (2013) estimated direct and indirect effect of various component characters on yield for 29 finger millet elite genotypes. The results revealed that grain yield was found positively associated with days to 50% flowering, days to maturity, thousand grain weight, earhead weight and harvest index. Days to 50% flowering showed highly significant positive correlation with days to maturity, ear weight, straw weight and total biomass.

Shinde et al. (2014) studied forty-one genotypes of finger millet were assessed for genetic variability, heritability and character association for twelve important traits. The correlation analysis revealed that grain yield per plant was positively and significantly correlated with productive tillers per plant, plant height, finger length and number of

fingers per main earhead at both genotypic and phenotypic levels, indicating that grain yield would be improved through these characters.

Dasanayaka (2016) conducted a study to characterize randomly selected 24 finger millet genotypes. The correlation studies revealed that weight of grain per earhead was highly significant and positively associated with days to flowering, flag leaf width, flag leaf length, plant height, culm thickness, finger length, finger width, days to maturity and weight of sun dried ears, flag leaf width, flag leaf length, plant height, culm thickness, finger length, finger width, days to maturity and weight of sun dried ears highlighting increasing any one of them would lead to improvement of other character.

Negi *et al.* (2017) screened 35 diverse genotypes of finger millet for fourteen quantitative traits related seed yield. The analysis of variance revealed highly significant differences among all thirty-five genotypes with a wide range of mean values for different characters. Grain yield per plant exhibited very strong positive association with biological yield per plant, harvest index, number of productive tillers per plant, 1000 seed weight and peduncle length at phenotypic and genotypic level. Path-coefficient analysis identified biological yield per plant and harvest index as major direct contributors towards grain yield per plant at phenotypic and genotypic level.

Gohel and Chaudhari (2018) evaluated 30 different genotypes of finger millet for growth, yield and seed quality parameters. The correlation study indicated that the grain weight per main ear, harvest index, number of productive tillers per plant, biological yield per plant and panicle length had positive association towards grain yield per plant at phenotypic and genotypic level. Thus these traits could be considered for grain yield selection. Similarly, path coefficient analysis revealed that grain weight per main ear (0.534) had the highest direct positive effect towards the grain yield followed by number of productive tillers per plant (0.353), panicle length (0.166), days to flowering (0.133), harvest index (0.124), biological yield per plant (0.081), leaf blade length (0.011). The characters identified above merit due consideration in formulating effective selection strategy in finger millet for developing high yielding varieties.

Kandel *et al.* (2019) reported the grain yield had positive and significant phenotypic association with baring head, finger length, number of fingers, flag leaf length, peduncle length, productive tiller, thousand kernel weight, plant stand, straw yield and thus selection within this is traits importance for grain yield improvement.

Patil *et al.* (2021) evaluated 36 white finger millet accessions constituted of 27 landraces and nine released varieties to assess the genetic potential through the variability, correlation analysis among the quantitative traits. The study showed that grain yield per plant was found to be highly significant and positively correlated with plant height, number of productive tillers per plant, 1000 grain weight, straw yield per plant and harvest index at both genotypic and phenotypic levels and finger width had highly significant correlation with grain yield per plant at genotypic level indicating that these attributes were mainly influencing the grain yield in finger millet.

Krishna *et al.* (2021) suggested the combination of traits viz., productive tillers, mean ear weight and threshing percentage are important in achieving 96.6 *per cent* grain yield under well watered condition and; 96.8 *per cent* in drought stress condition with their residuals less than 0.034 and 0.032 respectively, implies the best fit and significance of these traits in determining the grain yield in finger millet genotypes. Therefore, in selection of tolerant varieties for drought stress condition, productive tillers, mean ear weight and threshing ratio in addition to finger width were predicted as important traits. Overall, the study demonstrated that drought stress from ear emergence to grain filling (36 days) decreases the grain yield by 36.6 *per cent*.

2.4 Identification of drought tolerance related traits and genotypes

In the semiarid tropics, where there is a soil-water-limited ceiling for the cropping period length, finger millet often faces intermittent drought (Krishna, 2010). In the African and southern Asian environments, finger millet is affected more by the intermittent drought followed by the terminal drought affecting the reproductive growth (Krishnasastry *et al.*, 1982; Dinesh Kumar *et al.*, 1987). Intermittent drought is common in the semiarid tropical regions and expected to turn more erratic as after effects of global warming (Ludlow and Muchow, 1990; Urrea *et al.*, 2009). The fluctuating environmental

cues, such as rainfall and temperature, plants are ultimately affected severely for growth and yield. To overcome this bottleneck, a focused trait-based breeding approach has been proposed and is being widely adapted (Reynolds and Langridge, 2016).

2.4.1 Effect of drought on agro-morphological traits

Maqsood and Ali (2007) screened finger millet landraces to investigate the effects of environmental stress on the growth, development and yield. The study demonstrated that drought has a significant influence on the vegetative and reproductive growth of finger millet. There was a decline in leaf production as a result of drought. Similarly there was a notable restriction in canopy size under the drought treatment due to reduction in leaf production and leaf numbers. Dry biomass and grain yield reduced significantly under drought stress environment.

Krishnamurthy *et al.* (2016) evaluated minicore collection of sixty nine finger millet germplasm in mini-lysimeters for the variation in shoot biomass as total water use and transpiration efficiency under terminal drought-stress and well-watered environments. Results from this study indicated that drought stress significantly influenced all the traits in both the years. It also reduced the total shoot biomass, the amount of water transpired, and increased the transpiration efficiency. They found that contribution of total water use to shoot biomass under drought was minor but that of transpiration efficiency was large. A huge reduction in grain yield than shoot biomass recorded suggested that drought affected the reproductive growth much more severely than vegetative growth.

Talwar *et al.* (2020) evaluated a set of thirty eight accessions of finger millet in both field and mini-lysimeters under both well-watered and water-stressed conditions. Approximately 2-fold variations in shoot biomass and ~9-fold variations in grain yield under water stressed conditions were recorded. Drought stress significantly influenced all the yield parameters (shoot biomass, grain yield and harvest index) in trials irrespective of whether conducted in field or mini-lysimeters. The results also indicated that reproductive growth was more sensitive to water stress than the vegetative growth.

Mude *et al.* (2020) assessed the drought tolerance capacities of four finger millet genotypes based on their physiological and biochemical characteristics at three different phenological stages. Drought stress significantly affected root length, shoot length, root/shoot ratio, and plant height. The study confirmed the significant reduction in shoot growth and minimal effect on root growth under drought stress. The total chlorophyll and carotenoid contents were also decreased under drought stress. Kholova *et al.* (2011) also reported reduction in total chlorophyll content in drought stress condition in pearl millet, and was due to a significant decrease in both chlorophyll *a* (Chl *a*) and *b* (Chl *b*).

Krishna *et al.* (2021) imposed drought stress in sixteen released varieties of finger millet for 36 days from earhead emergence to grain filling under field condition. The result indicated the reduction in grain yield by 36.6% with decreased physiological and yield contributing traits. Among the agronomic traits, productive tillers, finger width, mean ear size and threshing percentage recorded equal and positive contribution towards the grain yield under drought stress.

2.4.2 Drought tolerance indices (DTI)

Breeding for drought tolerance by selecting purely for grain yield is difficult, because the heritability of yield under stress environment is low due to large genotype by environment interaction as compared to non-stress environments (Kirigiwi *et al.*, 2004). Achieving a yield increase under stress environments has been recognized to be a difficult challenge, while progress in yield has been much higher in favorable environments (Richards *et al.*, 2002). Thus, drought indices which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes (Mitra 2001; Sio-Se *et al.*, 2006; Talebi *et al.*, 2009). The relative yield performance of genotypes in drought and non-stress environments appear to be a common starting point for identifying desirable genotypes for unpredictable conditions (Mohammadi *et al.*, 2012; Nouri *et al.*, 2011). Several drought tolerance indices used in different crops to identify high yielding and stable drought tolerant genotypes across the environments are reviewed below.

Ashraf *et al.* (2015) in bread wheat and Yousefi (2015) in barely reported significant and positive correlation between grain yield under drought (Ys) and non-stress (Yp) conditions with drought tolerance index (STI), geometric mean productivity (GMP), mean productivity (MP), yield index (YI), harmonic mean (HM), and drought resistance index (DRI) indicated that high correlation of indices with both Ys and Yp are most appropriate for identifying stress tolerant genotypes.

Mohammadi (2016) examined the repeatability, similarity and accuracy of drought tolerance indices under different levels of drought stress (mild, moderate, severe) in durum wheat. Several yield-based drought tolerance indices including stress tolerance index (STI), geometric mean productivity (GMP), mean productivity (MP), tolerance index (TOL), stress susceptible index (SSI), yield stability index (YSI) and yield index (YI) were calculated based on the different levels of stress. Coefficient of variation and correlations between the estimates of drought tolerance indices indicated that MP followed by STI and GMP showed moderate repeatability with high accuracy and concluded that these indices are accurate identifying drought tolerant genotypes.

Ali and Sadek (2016) screened forty nine wheat lines to identify drought-tolerant lines using several drought indices. Results from the study showed that grain yield under stress and non-stress environments were highly correlated with the mean productivity (MP), the geometric mean productivity (GMP), stress tolerance index (STI), yield index (YI), harmonic mean (HM), drought resistance index (DRI), and modified stress tolerance index (STI). The MP, GMP and STI were considered as the best indices for the selection of the relatively tolerant lines.

Lapuimakuni *et al.* (2018) evaluated eight foxtail millet genotypes under both drought and normal watering conditions. Based on the correlation, principal component analysis, and cluster analysis, yield index (YI) and harmonic mean (HM) were considered the best indices for the selection of drought-tolerant foxtail millet genotypes.

Ferede *et al.* (2020) evaluated drought tolerance in sixty four tef [*Eragrostis Tef* (zucc.) Trotter] genotypes using drought tolerance indices. Drought tolerance indices including drought susceptibility index (SSI), drought tolerance index (STI), geometric

mean productivity (GMP), mean productivity (MP), yield index (YI), yield stability index (YSI), drought resistance index (DRI), abiotic tolerance index (ATI), stress susceptibility percentage index (SSPI), harmonic mean (HM) were estimated from grain yield under drought (Y_s) and non-stress (Y_p) conditions. They found that grain yield under both conditions had significantly positive correlation with STI, GMP, MP, YI, DRI, and HM, indicating that these indices would be useful for selection of genotypes with high grain yield under drought stressed and non-stressed conditions.

2.4.3 Genotype × environmental interaction and biplot analysis

Grain yield is a complex trait and is genetically governed by many quantitative genes with small additive effects. Hence, the expression of this is generally affected by genotype, environment, and genotype × environment interaction. Complex genotype × environment interaction can be understood in simpler and effective manner by use of graphics based biplot methodology (Gabriel, 1971). The scatter plot visualization using two factors to explain underlying relationships and interactions is called as biplot. However, genotype evaluation faces two key challenges. The first is genotype by environment interaction for a key trait, and the second is unfavorable associations among key traits (Yan, 2001; Yan, 2002). Several studies of genotype plus genotype-by-environment (GGE) biplot based stability analysis have been conducted in field for understanding genotype × environment interaction (Balestre *et al.*, 2009; Shiri 2013; Boshev *et al.*, 2014; Faria *et al.*, 2017).

Yan *et al.* (2000) and Yan (2001) developed genotype plus genotype-by-environment (GGE) biplot to visually analyze the genotype by environment (GGE) data, and genotype by trait (GT) data in an individual environment or across environment of multi-environment trial data. They reported that GGE biplot was originally designed for graphical analysis of multi-environment trial data and it was later extended to graphical analysis of other types of two-way data, such as genotype by trait data and other types of two-way data (Gurmu *et al.*, 2009).

Rakshit *et al.* (2012) investigated performance of ten Indian sorghum hybrids over 12 locations across the rainy seasons using GGE biplot analysis. The results showed that

location attributed higher proportion of the variation in the data (59.3–89.9%), while genotype contributed only 3.9–16.8% of total variation. Genotype $\times$ location interaction contributed 5.8–25.7% of total variation. They also identified superior hybrids for grain yield, fodder yield and for harvest index using biplot graphical approach effectively.

Choudhary *et al.* (2019) evaluated thirteen baby corn hybrids were for green ear yield, baby corn yield and green fodder yield over eight locations (environments) in kharif seasons of 2015 and 2016 using GGE biplot analysis. The results revealed a higher proportion of the variation in the data is attributable to the environment while genotype contributed less to the total variation. Superior stable hybrids for green ear yield, baby corn yield and green fodder yield could be identified using a biplot graphical approach effectively. ‘Which won where’ plot for each of the traits partitioned testing locations into three mega-environments with different winning genotypes for different traits in respective mega-environments.

Kizilgeci *et al.* (2019) evaluated thirteen spring durum wheat genotypes in four environmental conditions in consecutive two growing seasons in the year 2013-14 and 2014-15. The stability and superiority of genotypes and favorable testing environments were described by using ANOVA and GGE biplot analysis (genotype, genotype $\times$ environment). Genotype, environment and GEI (genotype $\times$ environment interaction) was found to be highly significant for multiple traits. The results of the study showed that GGE biplot analysis can be used as a good tool to identity of the most suitable environment in terms of all the characteristics as well as to identify the best genotypes.

Woyann *et al.* (2020) compare the multitrait selection indexes based on factor analysis and ideotype-design (FAI-BLUP), GGE biplot, and grain yield $\times$ trait index (GYT), and proposes the use of predicted genetic values together with the GYT index (best linear unbiased prediction used in grain yield*trait index, GYT-BLUP) based on the yield data of 35 homozygous soybean lines. The result suggested that combined use of FAI-BLUP and GYT-BLUP: GYT-BLUP was highly important for grain yield (generally the most important trait), which is related to all other traits are related with it. FAI-BLUP provided similar weights for the traits. They recommend the combined different approaches that can provide better answers to the breeders in selection programm.

Anuradha *et al.* (2022) evaluated sixty finger millet varieties released across the country over six consecutive rainy seasons. The genotype $\times$ environment interaction was found to be significant during these seasons showed that considerable variation existed among different genotypes within environment. Furthermore, the Additive Main effects and Multiplicative Interaction (AMMI) analysis asserted that genotypes and the genotype $\times$ environment interaction effects accounted for approximately 89% of the total variation. Strong positive associations were also observed in an estimated set of eleven stability parameters which were chosen to identify stable genotypes.

2.5 Reproductive stage drought stress in finger millet

2.5.1 Effect of drought stress on physiological and pollen related traits

Finger millet crop purely depends on rainfall for its water supply under rainfed condition; drought is the most severe abiotic stress that limits crop growth and productivity in rainfed condition. Even in areas of arid and semiarid regions which do not undergo water availability problems so far are expected to experience prolonged periods of drought. Limited arable land and ever-increasing population have created an unprecedented demand for fresh water (Tardieu, 2012). Moreover, increased atmosphere temperature and heat wave incidences are expected to negatively impact crop yield and food security (IPCC, 2013).

It has also been observed that drought stress at the reproductive stage is the most prevalent in drought-prone rainfed agriculture, at least simply because in most rainfed ecosystem the crop season's rains diminish toward flowering and harvest time. Therefore, irrespective of biomass production up to flowering, continuance of water uptake and water use into the reproductive growth stage is crucial for reproductive success and the desired levels of drought tolerance (Merah, 2001; Kato *et al.*, 2008).

The arid and semiarid lands of most developing countries are facing a problem of water scarcity, which affects the type and performance of crops grown in those areas. Drought is one of the major abiotic stresses affecting plant growth and reducing crop productivity. It has been estimated that, 60 *per cent* of the crop yield loss can be attributed to abiotic stresses, especially drought. A complete yield loss was reported in

two finger millet landraces subjected to drought after 4 weeks of sowing. Undesirable effects of drought have been reported not only on the productivity of crops but also on their nutritional quality including grain mineral and protein content (Hadebe *et al.*, 2017).

The climate is changing at an alarming rate, hence, the world is facing various environmental problems, including changing rain patterns, high certainty of drought, etc., triggering various abiotic stresses which are affecting food crops in one way or another, which directly affects the global food supply (Pandian and Ramesh, 2018).

2.5.1.1 Pollen number and sterility

In self-pollinating crops a critical factor controlling grain number is pollen development during the young microspore stage. It has been reported that drought stress and moderate water-deficit stress at this stage of development can dramatically reduce grain number (Saini *et al.*, 1984; Dorion *et al.*, 1996), which has been linked to pollen sterility during the early reproductive stages, whereas ovary fertility remained unaffected (Ji *et al.*, 2010b). Yield reduction is more pronounced when abiotic stresses are centred around reproductive organ development (flowering), pollination and kernel setting (Dolferus *et al.*, 2013). Drought is one such abiotic stress that has a profound effect on yield when crop reproductive stage coincides with drought stress.

McWilliams, (2003) reported the sensitivity to drought stress is greater during reproductive stages than during the vegetative stage in sorghum. They also observed that water stress during reproductive stages stopped the development of pollen and ovules, thus prevented fertilization and also induced premature abortion of fertilized ovules.

The drought induced at the pre-anthesis stage shortened the time to anthesis while that applied after anthesis reduced the period of grain filling in cereals (Estrada-Campuzano *et al.*, 2008). The process of the grain filling in cereals is controlled by four major enzymes, *i.e.*, Sucrose Synthase, Starch Synthase, Starch Branching Enzyme, and Adenosine Diphosphate Glucose Pyrophosphorylase (Taiz and Zeiger, 2006). A decreased activity of these enzymes has been reported under the drought conditions which have a negative impact on the yield of major cereal (Ahmadi and Baker, 2001). The exposure of plants to drought stress at the flowering resulted in complete sterility in

pearl millet (*Pennisetum glaucum* L.) which is usually due to the disturbed assimilate movement to the developing ear (Yadav *et al.*, 2004).

Previous studies have shown that the maintenance of tapetum activity and anther sink strength play central roles in well-coordinated pollen development (Clément *et al.*, 1996; Nguyen *et al.*, 2009). Water-deficit stress causes developmental defects in the tapetum and a lack of starch accumulation in pollen grains (Ji *et al.*, 2010a). In both wheat and rice, it has been reported that male sterility may occur when carbohydrate metabolism is significantly affected thereby disrupting starch accumulation in the pollen grains (Dorion *et al.*, 1996; Sheoran and Saini, 1996).

The importance of ABA in controlling grain number and the maintenance of pollen sink strength possibly relies on its functional roles in sugar signaling (Laby *et al.*, 2000; Rook *et al.*, 2001) and the regulation of *cell wall invertase* genes which are known to affect starch accumulation and pollen fertility (Oliver *et al.*, 2007; Ji *et al.*, 2011).

Dong *et al.* (2017) subjected two wheat cultivars to different levels of polyethylene glycol (PEG)-induced drought stress at the young microspore stage to understand the possible links between pollen fertility and stress tolerance. The results indicated that, in both cultivars, higher grain number reduction was observed under condition of lower water availability. Overall, the drought tolerant cultivar (Jinmai47) exhibited less grain number reduction than the drought sensitive cultivar (Shiluan02-1) under all stress conditions. Compared with Shiluan02-1, Jinmai47 exhibited superior physiological performance in terms of leaf photosynthetic rate, ear carbohydrate accumulation, pollen sink strength, pollen development and fertility under stress.

The reproductive stages of crops are more vulnerable to drought, high temperature and combined stress than the vegetative stages (Sehgal *et al.*, 2017; Choukri *et al.*, 2020). During anthesis and flowering periods, both stresses led to fertilization failures because of reduced pollen and ovule function and inhibited pollen development and sterility in legumes (Sita *et al.*, 2017; Kumari *et al.*, 2021) and cereals (Fabian *et al.*, 2019; Zahra *et al.*, 2021). They also reported that functionality of female and male reproductive parts was reduced under combined stress condition by 34% and 66%, respectively.

2.5.1.2 SPAD chlorophyll meter reading (SCMR)

Chlorophylls are the main part of photosynthetic apparatus and that determine the yield. The chlorophyll meter or SPAD meter is a simple portable diagnostic tool that can measure the greenness or relative chlorophyll content of leaves (Kariya *et al.*, 1982). SPAD chlorophyll meter reading (SCMR) is an indication of the light-transmittance characteristics of the leaf which is dependent on the leaf chlorophyll content. Maximum chlorophyll content is a desirable characteristic because it indicates a low degree of photoinhibition of photosynthetic apparatus, therefore reducing carbohydrate losses for grain growth (Farquhar *et al.*, 1989).

Chlorophyll (Chl) is an important photosynthetic pigment that largely determines photosynthetic capacity and growth of a plant (Marini, 1986). Under moisture stress decrease in relative water content reduces chlorophyll content mainly due to deterioration of thylakoid membrane and accelerated rate of breakdown of chlorophyll pigment (Wahid *et al.*, 2007; Jaleel *et al.*, 2009; Tiwary *et al.*, 2016).

Leaf chlorophyll content has a positive relationship with leaf chloroplast content, photosynthetic status of the plant, plant metabolism and photosynthetic rate (Guo and Li, 1996). Several studies in several crops have indicated that higher chlorophyll content (*i.e.*, stay green trait) contributes to improved yield under drought conditions (Benbella and Paulsen, 1998; Borrell *et al.*, 2000; Haussmann *et al.*, 2002; Verma *et al.*, 2004).

SCMR is an indirect measure of chlorophyll pigment, which determines the photosynthetic capacity (Barutcular *et al.*, 2015; Croft and Chen 2018). It was also reported that, a decrease in chlorophyll content resulted in decreased photosynthetic rate by more than 50% and consequent biomass and yield (Anitha *et al.*, 2019).

Mishra *et al.* (2019) evaluated responses of 130 indigenous rice landraces to drought stress for physiological parameters. The results from their study suggested that chlorophyll degradation was more pronounced in the sensitive cultivar and that was possibly attributable to free radicals generated under drought stress. This was further supported by Sarkar, *et al.* (2013), Mishra and Panda (2017) and Mathobo *et al.* (2017).

Mohanabharathi *et al.* (2019) imposed the drought stress in finger millet genotypes by withholding the water for 8 days from 35th to 42th day. The significant reduction recorded in SPAD values under drought stress as compared to control conditions. Similarly, Franca *et al.* (2000) reported the reduction in photosynthetic rate as a common response of plants to water deficit stress environment. The response could be attributed to either stomata closure or metabolic impairment relative water content was significantly reduced under drought condition.

Krishna *et al.* (2021) observed the significant decrease in SCMR value under drought stress condition (20 days after stress imposition) from 42.6 to 33.8 in finger millet genotypes. Their study recorded a 35.5% contribution of SCMR towards grain yield under well watered conditions. In another study, positive relationship was observed between SCMR and grain yield. However, it also has been reported that the differences in SCMR value less than 10.0 between varieties have no significant influence on productivity traits (Aparna, 2016).

2.5.1.3 Relative water content (RWC)

The relative water content (RWC) of leaves is an important physiological tolerance character directly related to soil water content (Lawlor and Cornic, 2002). RWC plays an important role in drought stress tolerance as it defines the ability of plant to maintain higher leaf water status to maintain turgidity and yield stability under moisture stress conditions (Mullan and Pietragalla, 2012).

Aslam *et al.* (2006) assessed sixty diverse maize genotypes for water deficit tolerance under four moisture levels in pots. They screened for survival rate, stomatal conductance and relative cell membrane injury. At low moisture level (40 % of field capacity) high survival rate was observed in ten maize accessions which were selected as water deficit tolerant. While, at mild stress (80 % of field capacity) ten maize accessions which showed lowest survival rate were selected as sensitive to water stress.

Mukami *et al.* (2019) investigated the physiological and biochemical responses of six finger millet varieties under mannitol-induced drought stress. The results showed that increased levels of drought stress seriously decreased germination and early seedling

growth of finger millet varieties. Under irrigated conditions, all varieties maintained high RWC levels. However, when exposed to progressively greater mannitol concentrations, all varieties exhibited reduction in RWC and total chlorophyll content values.

2.5.1.4 Membrane leakage

The drought and heat stress have similar effect on the plant cell, damaging the selective permeability of the plasma membrane. Thus, the cell cannot maintain its internal composition (Barnabás *et al.*, 2007). The damage affects plant growth and development (Bajji *et al.*, 2002). Measurement of solute leakage from the plant tissue was used to estimate the damage to the cell membrane caused by drought (Tardieu *et al.*, 2012; Wang *et al.*, 2016) and heat (Reynolds *et al.*, 1994; Suzuki *et al.*, 2014; Jamali *et al.*, 2015) under field conditions.

Membrane damage *via* ROS induced lipid peroxidation contributes to the loss of cellular integrity, inactivation of membrane enzymes and to some extent even of cytoplasmic proteins. Peroxidation of membrane lipids, especially the unsaturated lipids, is one of the main causes for loss of membrane integrity leading to increased electrolyte leakage and is related to the extent of free radical generation in plants. Reduced electrolyte leakage under drought stress reflects better membrane integrity and tolerance towards oxidative stress (Liu *et al.*, 2011).

Bhatt *et al.* (2011) investigated the impact of drought stress on five finger millet varieties representing contrasting areas of Indian sub-continent. The genotypes recorded up to 480 % increase in electrolyte leakage under stress as compare to control conditions.

Sudan *et al.* (2015) subjected the 45 days old seedlings of finger millet to drought stress. The exposure of plants to stresses led to an increase in the electrolyte leakage from the leaf tissues of affected plants. In case of drought induced ROS production, the signs of membrane damage appeared on 4th day after exposure to stress.

2.6 Genomic resources in finger millet available crop improvement

Finger millet has rich diversity and large collections are conserved in long-term storage in various gene banks across the globe. The availability of diverse genetic

resources is a prerequisite for genetic improvement and manipulation of any crop (Babu *et al.*, 2014a). More importantly, many of these are cross-compatible with the domesticated species, which offer greater opportunities for improvement for tolerance to the climatic stresses (Saha *et al.*, 2017).

2.6.1 Finger millet genome sequence

Sequencing and analysis of genomes through use of high throughput DNA sequencing and bioinformatic tools enable to assemble and analyse the function and structure of entire genomes. The proven adaptability to harsh conditions and climate resilience characteristic of finger millet have shown numerous advantages over major cereals in terms of nutritional quality, stress adaptation and health benefits revealing finger millet has an indispensable repository of novel genes for crop improvement, which can be exploited in crop improvement programmes (Sood *et al.*, 2016). Although rapid strides have been made in allele mining in model crops and major cereals, the progress in finger millet genomics is lacking (Sood *et al.*, 2019).

The availability of whole genome and transcriptome sequences of finger millet provides an useful insight and novel perspectives for crop improvement (Hittalmani *et al.*, 2017; Pandian and Ramesh, 2018). The major problem encountered in genome assembly of polyploid species is large genome size and presence of repetitive DNA sequences, which pose a major challenges in functional annotation of genome assembly (Hittalmani *et al.*, 2017; Hatakeyama *et al.*, 2018 and Appels *et al.*, 2018).

The whole genome sequence of finger millet genotype ML-365 was sequenced by Hittalmani *et al.* (2017) using Illumina and Sequencing by Oligonucleotide Ligation and Detection (SOLiD) sequencing technologies covering approximately about 82 *per cent* of the estimated genome size, which spanned a length of 1196 Mb. This study also revealed that the genome sizes of *E. coracana* subsp. *coracana* and *E. coracana* subsp. *africana* were relatively similar. The genome assembly of ML-365 has generated around 45 GB paired end and 21 GB mate-pair data. The whole genome assembly of ML-365 consisted of 525,759 scaffolds with N50 length of 23.73 Kb and average scaffold length was about 2275 bp. About 85,243 genes involved in different molecular and cellular process were

identified. The greater co-linearity of finger millet genome was found to be associated with foxtail millet and rice (Hittalmani *et al.*, 2017).

Hatakeyama *et al.* (2018) sequenced and assembled whole genome of finger millet variety: PR202, IC: 479099 via a novel multiple hybrid assembly workflow combining next generation sequence with single-molecule sequencing which was followed by whole-genome optical mapping using Bionano Irys® system. The multiple hybrid workflow yielded genome assembly of 1.2 Gb, while the genome size measured by flow cytometry was found 1.5 Gb, the genome assembly consisted of 1897 scaffolds with an N50 length >2.6 Mb and detection of 96.5 *per cent* of single-copy orthologs. They have predicted around 62,348 genes using MAKER software, which are twice in number when compared to the 35,679 genes identified in diploid rice assembly.

2.6.2 Molecular markers for assessing genetic diversity in finger millet

Genetic diversity refers to the variations within the individual gene loci/among alleles of a gene, or gene combinations, between individual plants or between plant populations (Sood *et al.*, 2016). There is huge variability in morphological diversity in finger millet. Genetic diversity provides the opportunity to utilize various genomic resources and technologies to manipulate desirable traits. Genetic diversity is being studied using molecular markers such as random amplification of polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP), Restriction fragment length polymorphism (RFLP), inter-simple sequence repeats (ISSR), simple sequence repeats (SSR), expressed-sequenced tags (EST), etc. provide unique opportunities to quickly and accurately access the genetic variability in a germplasm or any population. Substantial degree of the genetic variation investigated among the cereal grasses may be because of genome-wide coverage of the SSR markers in eukaryotes. Microsatellites or SSRs domains are highly conserved during course of evolution and demonstrate cross-transferable phenomenon to remove the boundary of taxonomic limitations (Singh *et al.*, 2019).

The foundation of molecular breeding in finger millet was laid by RAPD markers, which were initially the default choice to characterize finger millet germplasm. Many

efforts were made to identify origin of cultivated species *E. coracana* subsp. *Caracana*. The A genome of the cultivated crop were identified as *E. coracana* subsp. *africana* along with *E. indica* as one of the genome donors and B genome is unknown (Salimath *et al.*, 1995).

The RAPD markers were used for genetic diversity studies which shows higher polymorphism ranging from 35 to 100 % (Fakrudin *et al.*, 2004; Babu *et al.*, 2007; Kumari and Pande, 2010; Ramakrishnan *et al.*, 2016b). The genetic diversity studies using RFLP, RAPD, and ISSR were helpful in species identification, genetic relatedness and clustering of genotypes based on similarity or geographical adaptation (Sood *et al.*, 2016).

The genomic SSR were developed by isolating di- and tri-nucleotide SSRs from random genomic *Hind*III, *Pst*I, and *Sal*I libraries of finger millet accession PI 321125 following hybridization of 18,432 double-gridded colonies with mixtures of di [(AG)¹⁵/(AC)¹⁵] and tri [(AAG)¹⁰/(AAC)¹⁰/(ATC)¹⁰, (AGC)¹⁰/(AGG)¹⁰, (CCG)¹⁰] nucleotides (Dida *et al.*, 2007). They identified 82 SSR markers and developed the first skeleton genetic map of finger millet using genomic SSR, RFLP, AFLP, and EST markers. The linkage map was developed with 721 cM on the A genome, 787 cM on the B genome and covered all the 18 finger millet chromosomes.

Dida *et al.* (2008) developed another 45 genomic SSR markers to evaluate genetic variation among 79 finger millet accessions belonging to Africa and Asia. This study predicted the origin of genotypes and also substantiated the theory that finger millet was domesticated in Africa before being introduced to India. Most of the studies were limited to a small set of germplasm collections (Arya *et al.*, 2013; De Villiers *et al.*, 2015), local germplasm of restricted area (Panwar *et al.*, 2010a; Kumar *et al.*, 2012) or the number of markers used were limited where large germplasm sets were analyzed (Babu *et al.*, 2014a; Babu *et al.*, 2017; Ramakrishnan *et al.*, 2016a).

Panwar *et al.* (2010b) evaluated genetic diversity of among 52 finger millet genotypes collected from different districts of Uttarakhand using 18 RAPD, 10 pairs of SSRs and 10 pairs cytochrome P450 gene based markers. Their study revealed 49.4 per

cent 50.2 per cent and 58.7 per cent polymorphism among 52 genotypes of *E. coracana* using RAPD, SSR and cytochrome based markers. The dendrogram developed by RAPD, SSR and cytochrome P450 gene based primers analyses revealed that the genotypes are grouped in different clusters according to their calcium content as high, medium and low.

Kumar *et al.* (2012) analysed 52 genotypes of *Eleusine coracana* using SSR, RAPD-PCR for their variation. A total of 21 RAPD and 24 SSR primers were assayed for their specificity in detecting genetic variability in *E. coracana*, of which 20 RAPD and 21 SSR primers were highly reproducible and were found suitable for use in PCR analysis. The 20 polymorphic RAPD primers generated 56 different loci and SSR generated 168 scorable alleles, with an average of 8 bands per primer.

Arya *et al.* (2014) used a set of 14 polymorphic genomic SSR and three genic SSR markers were to study the genetic diversity of Indian and African finger millet accessions. The result showed greater magnitude of diversity was reported in African finger millet accessions. This study concluded that geographical adaptation of finger millet genotypes to highlands and lowlands in African before its movement to India.

Obidiegwu *et al.* (2014) mined sequence information at NCBI database for developing EST-SSRs markers and selected three markers (UH-Ec-931; UH-Ec-956 and UH-Ec-958) based on pre-testing of polymorphism among 5 accessions. Further they used, three EST-SSR markers to characterize randomly selected 48 accessions from the pool of finger millet core set. The PIC of the developed markers was in the range 0.6741 for marker UH-Ec-931 to 0.7658 for marker UH-Ec-958, with a mean PIC value of 0.7171. Marker UH-Ec-958 generated 13 alleles per locus while marker UH-Ec-956 generated 20 alleles per locus.

Nirgude *et al.* (2014) developed 36 EST-SSR primers for the opaque2 modifiers of calcium transporters and 20 anchored-SSR primers from the nucleotide sequences of calcium transporters and calmodulin for estimating the genetic diversity for grain protein and calcium contents across 103 finger millet genotypes. From among, 36 opaque2 modifiers primers, 15 primers (68.23 % polymorphism) were polymorphic establishing average gene diversity value of 0.29 with an average PIC value of 0.17. The diversity

analysis based on anchored SSR loci revealed an average gene diversity value of 0.21. The opaque2 modifiers specific EST-SSRs could be able to differentiate the finger millet genotypes into high, medium and low protein containing genotypes.

Ramados (2014) studied physiological responses of 83 finger millet genotypes for drought tolerance and identified IE 7079 as the most sensitive accession and IE 5091 as the most tolerant. The gene expression analysis was carried out by using RNA sequencing. In this study, out of 3376 putative microsatellites primer pairs developed, 288 were prioritized based on several criteria including a preference for amplicons with the highest proportion of SSR length compared to total amplicon length. The 10.76 *per cent* polymorphism was observed among 83 accessions generating a total number of 152 alleles with an average of 4.9 alleles per locus and mean PIC of 0.49.

Kumar *et al.* (2015b) developed anchored SSR primers from the microsatellites identified within nucleotide sequences of different calcium transporters and sensors (calcium exchangers, calcium channels, calcium ATPase and calcium sensors like calmodulin) of different cereals (finger millet, rice, maize, wheat and barley) from NCBI database. The microsatellite identification was done using web based “SSRIT” tool and anchored SSR primers were designed using Primer 3. They also analysed 113 genotypes of finger millet having calcium content up to 450 mg/100 g of seed using 23 anchored SSR primers. Out of 23, 14 anchored SSRs were found to be polymorphic generating 83 alleles with a mean PIC value of 0.18 and average genetic diversity of SSR loci was about 0.22.

Kumar *et al.* (2016b) sequenced transcriptome of developing spikes of two finger millet genotypes differing in grain calcium content using Illumina HiSeq 2000 Genome analyser. In addition, they also mined transcript assembly of contrasting genotypes for microsatellite identification and SNP mining using perl script of “MISA” software and “SAM” tools software package respectively. A total of 24,748 and 21,276 SSRs were identified in the high and low grain calcium genotypes, respectively. The number of SNPs detected per transcript was highly variable, a total of 43,928 and 36,652 SNPs were detected in the contrasting genotypes. This study also revealed, at the protein level, 74.32

and 75.39 % finger millet transcripts have a significant similarity with the proteins of rice and sorghum respectively.

Gimode *et al.* (2016) sequenced and assembled reads of two cultivated finger millet genotypes KNE755 and KNE796 23285 into a reference FASTA file containing 6,810,971 nodes/contigs spanning 207,197,804 bp and generated 23285 SNPs. Out of which, 92 SNP markers were validated further for genetic diversity in cultivated and wild species of finger millet. Out of 92, 80 SNP markers were polymorphic. However, SNP markers also resulted in a low PIC value of 0.29 revealing narrow genetic base of finger millet as reported with SSRs.

Apart from SSRs, two recent studies have also appeared on SNP discovery in finger millet. Kumar *et al.* (2016b) identified 23,000 SNPs through genotyping by sequencing (GBS) of 113 diverse finger millet genotypes. A model-based population structure analysis reveals the presence of three subpopulations among the finger millet accessions, which are in parallel with the results of phylogenetic analysis. A total of 1128 gene ontology (GO) terms were assigned to SNP-carrying genes for three main categories: biological process, cellular component, and molecular function.

2.7 Identification of QTLs for seed yield and drought tolerance related traits

2.7.1 Population structure analysis

Undetected population stratification can cause spurious genotype-phenotype associations in candidate-gene approaches and indirect association *via* linkage disequilibrium mapping as well (Jorde, 1995). Further spurious associations between genotype and phenotype caused by population stratification must be detected among unrelated individuals in association studies to reduce Type I errors. Clustering techniques are one approach to identify stratified populations. STRUCTURE is the widely used software in genetic studies to analyze differences in the distribution of genetic variants among populations with a Bayesian iterative algorithm by placing samples into groups whose members share similar patterns of variation (Pritchard *et al.*, 2000).

Crop improvement through marker assisted breeding relies on the genetic diversity and population structure; therefore, it is an essential step to study the genetic polymorphisms using DNA markers. The primary variable that produces genotype-phenotype misleading relationship can be due to population structure or genetic background. Depending upon the crop's genetic origin, mutation rate, or admixtures can affect its probability of having any allele that varies in frequency across groups, as well as the probability of having any varying phenotype. Genomic constitution of different plant genetic resources can be accurately and finely evaluated through determining the population structure with different DNA markers (Nachimuthu *et al.*, 2015).

Dida *et al.* (2008) studied the population structure of 79 finger millet accessions (*E. coracana*) from 11 African and five Asian countries, including 14 wild *E. coracana* subsp. *africana* lines collected from Uganda and Kenya. They used 45 genomic SSR markers distributed across the finger millet genome. Phylogenetic and population structure analyses showed that the *Eleusine* germplasm formed three largely distinct subpopulations, representing *E. coracana* subsp. *africana*, *E. coracana* subsp. *coracana* originating from Africa and *E. coracana* subsp. *coracana* originating from Asia.

Bharathi, (2011) found four subpopulations while analyzing large finger millet germplasm of 990 collections. The sub-population 1 and sub-population 2 had a mixture of East Africa and South Asian accessions while sub-population 3 contained maximum number of South Asian accessions, and sub-population 4 had a mixture of all other geographical origins. Similarly, Babu *et al.* (2014a) characterized a subset of germplasm representing minicore collection and few Indian genotypes and concluded the existence of two sub-populations which were in correspondence with the geographical origin of the accessions.

Almost in all studies admixtures were observed in the sub-populations. More than 80% exotic alleles in 16 genotypes of the North Western Himalayan region of India and 30 % Indian alleles were observed in exotic lines in sub-population 1 (Babu *et al.*, 2014a). The occurrence of admixtures could be attributed to *Eleusine* gene flow between India and Africa and higher admixture in Indian or African genotypes probably could be the result of intensive inter-crossing between Indian and African germplasm.

The structure analysis using molecular markers clearly finger millet gene pool has 2-4 sub-populations, and *Eleusine coracana* subsp. *africana* forms a separate group. These reviews conclude that the separation of accessions into different sub-populations was assigned mainly based on their geographical adaptation (Dida *et al.*, 2008; Bharathi, 2011; Babu *et al.*, 2014b). However, in the study of Kumar *et al.* (2015b), the structure analysis did not classify the 113 Asian *coracana* accessions for calcium content variation and distributed them in all four sub-populations without biased distribution toward any particular subpopulation.

Lee *et al.* (2017) used 12 SSR markers in 76 finger millet accession which were genetically diverse and were clustered into two subpopulations which had an admixture of alleles. Pandian *et al.* (2018b) used genic SSR to analyze the genetic diversity of finger millet populations from different geographical origins. Mixed grouping pattern of finger millet genotypes was highlighted in the STRUCTURE software-based population structure. Bayesian model based structure analysis showed the maximum DK value of $K = 3$ which illustrating the 90 genotypes of finger millet were divided into three subpopulations. The subpopulations had a greater admixture of alleles and no pure line was observed. The structure analysis also clearly assigned the individuals into subpopulations according to their geographic origin.

2.7.2 Kinship co-efficient matrix (K matrix)

Kinship or relatedness is estimated by the “coefficient of relatedness”, which is defined as the probability that the alleles of a locus chosen at random from two individuals are identical by descent. Kinship or relatedness can have a similar effect as population structure on association mapping analysis *i.e.*, identification of spurious associations. In association mapping analysis, a matrix showing the relationship of all individuals of the association mapping population is used to account for effects due to kinship. Mixed models using variance component approaches that account for kinship estimates have been exploited in animal research for over two decades (Henderson, 1984; George *et al.*, 2000). Arbelbide *et al.* (2006) developed a mixed model for self-pollinating plants that accounted for multiple location effects and kinship based on pedigree records.

The complex traits are usually controlled by multiple QTLs, hence the primary goal of QTL mapping is to find associated markers for each QTL. To achieve this goal, many association studies fit markers into a linear model as fixed effects. Any QTL not associated with a marker will lead to the residual error, results in the reducing statistical power. As a result, QTL not physically linked to a marker may cause spurious associations between phenotypes and that marker due to factors, such as selection, population admixture or family structure. Therefore, the structured association approach can partially correct for these false associations by using a Q-matrix of population membership estimates. Additional improvement can be made by incorporating multiple background QTLs as a random effect in a mixed mode, which takes into account covariances due to relatedness. The average relationship between individuals or lines can be estimated by kinship (K) calculated either from pedigrees or a suitable number of random markers across the entire genome. A composite approach, Q+K, that combines information from both Q and K, has been shown to be superior to these former methods (Yu and Buckler, 2006).

2.7.3 Genome wide association mapping

Linkage and association mapping are the two most common methods used for identifying QTL conditioning complex traits; through these methods genes/QTL have been identified in different plant species (Yang *et al.*, 2011; Zhang *et al.*, 2016; Sharma *et al.*, 2018). Since the development of bi-parental mapping populations for small millets is very difficult, the classical linkage analysis of QTL for traits associated with climatic stresses is limited.

Association mapping (AM), also known as linkage disequilibrium (LD) mapping, is a method used to identify marker alleles associated with phenotypic traits. It makes use of ancestral recombination present in the gene pool of a species to identify marker/trait associations. Rare alleles are difficult to be detected in AM and to get higher alleles frequency that includes the genetic diversity of the crop species. AM panels must be suitably chosen. AM considers the use of panels with diverse cultivars for the purpose of recording more recombination events that contribute to a higher resolution to find regions associated with trait than linkage mapping (Zhu *et al.*, 2008).

The term LD refers to the non-random association between two markers or two genes/QTLs or between a gene/ QTL and a marker locus (Gupta *et al.*, 2005). LD commonly found in natural populations between loci which have sufficient to dissipate the initial disequilibrium. When a population in LD mates at random, the amount of disequilibrium progressively declines with succeeding generation. Thereby the degree of LD is therefore dependent on both the recombination fraction and the time in generation. LD of a given population depends on a very large number of factors.

The SNPs are considered to be the ideal markers in plant genetic studies and genome analyses because of their excellent genetic attributes, such as ubiquitous distribution, co-dominant nature of inheritance, chromosome-specific location and high reproducibility (Gupta *et al.*, 2001). SNP mining becomes highly challenging in allo-tetraploid crops such as finger millet due to low polymorphism level resulting from high degree of inbreeding and large numbers of homeologous SNPs, which are the result of polymorphism between the A and B sub-genomes of the same individual (Gimode *et al.*, 2016). But, identification of SNPs in several other polyploid crops including wheat, cotton, oats and groundnut has been successful as a result of suitable filtering tools and stringency in mapping parameters (Clevenger and Ozias-Akins, 2015).

2.8 Association mapping in finger millet

Association mapping based on linkage disequilibrium (LD), however, exploits historical recombination and targets multiple alleles at individual loci to detect marker-phenotype associations to identify genomic regions linked to a wider range of phenotypic traits, and is expected to provide higher resolution (Buckler and Thornsberry, 2002). Although, conventional bi-parental mapping-population-based QTL mapping approach has shown its promise in identifying loci for many important traits in major crop plant species, it is constrained by its low resolution (10–30 cM) and the limited number of segregating alleles at any locus (Flint-Garcia *et al.*, 2005).

The availability of plenty of landraces and diverse germplasm of finger millet indicates the possibility to dissect and associate highly useful and agronomically important alleles to traits. There is only one report of linkage mapping in finger millet

that too with an inter-specific population of *Eleusine coracana* subsp. *coracana* cv. Okhale 1 and its wild progenitor *E. coracana* subsp. *africana* accession MD 20 (Dida *et al.*, 2007). Furthermore, there is very little progress toward identification of QTLs for important traits through association mapping in finger millet crop.

Association analysis using phenotypic data of agro-morphological traits on finger millet reference set (300 germplasm accessions) and genotypic data of 20 SSR markers revealed 15, 22, and 14 QTLs in three environments and 11 QTLs in a combined analysis. Inconsistent associations between the agronomic traits and markers were mainly due to a limited number of random and non-trait specific markers (Bharathi, 2011). Two markers UGEP8 in LG3 and UGEP56 in LG9 showed a consistent association for days to 50% flowering indicating relatively tight linkage between the trait and marker.

In another study, 46 genomic SSR markers were used to identify 4 agro-morphological traits such as basal tiller number, days to 50% flowering, flag leaf blade width, and plant height in 190 finger millet genotypes (Babu *et al.*, 2014a). In the same year, two QTLs (OM5 and FM8) were identified for tryptophan content and one QTL (FMO2EST1) for protein content in the aforementioned genotypes of finger millet using 120 SSR markers and these QTLs were linked to opaque2 modifiers (Opm) gene (Babu *et al.*, 2014c).

Babu *et al.* (2014b) used 104 SSR markers to screen 190 FM genotypes and via general linear model (GLM) and mixed linear model (MLM) approach and identified four QTLs for finger blast and one QTL for neck blast resistance in finger millet. The rice genomic marker RM 262 and genic marker FMBLEST32 were linked to finger blast disease resistance at a P-value of 0.007 and explained a low phenotypic variance of 10% and 8% respectively. The genomic marker UGEP81 was associated to finger blast resistance at a P-value of 0.009 and explained 7.5% of phenotypic variance. The QTLs for neck blast was associated with the genomic SSR marker UGEP18 at a P-value of 0.01 which explained 11% of phenotypic variance.

Finger millet is specifically Ca-rich compared to other cereals. It is also a locally well-adapted semi-arid crop that is expected to drive food security for the developing

world under climatic change-related stresses. Hence, the identification of QTL controlling nutrient signaling and Ca accumulation traits will be essential for improving finger millet. A total of 9 QTL associated with Ca content were identified in 113 genotypes of finger millet using 14 anchored-SSR markers that were designed from calcium transporters and sensors (Kumar *et al.*, 2015b).

Through association analysis, using a global collection of 238 genotypes, and 85 genic and non-genic (SSR) markers, two genomic SSR markers (UGEP78 and UGEP60) were reported to be significantly associated with grain calcium content (GCC) (Yadav *et al.*, 2017). UGEP60 marker is linked to QTL for linkage group 1B of finger millet, which is also syntenous with rice chromosome 1 harboring a QTL for calcium content.

Based on association mapping of 128 finger millet genotypes from 18 countries identified two QTL (qLRDW.1 and qLRDW.2) to be associated with root dry weight, under low P, using 72 SSR markers (Ramakrishnan *et al.*, 2017). The same authors reported two other QTL, viz., qHSDW.1 and qHRL.1, under P-sufficient conditions, which affected shoot dry weight and root length. The four reported QTL, viz., qLRDW.1, qLRDW.2, qHSDW.1, and qHRL.1, were linked to UGEP19, UGEP68, UGEP13, and UGEP90 markers, respectively. Most of the work in this area has been directed toward identifying genomic regions of interest to facilitate marker-assisted selection. The majority of the traits that confer tolerance to climatic stresses such as drought, salinity, disease, nutrient starvation tolerance are complex and governed by QTL (Ramakrishnan *et al.*, 2016a; Ceasar *et al.*, 2018; Sharma *et al.*, 2018).

Sharma *et al.* (2018) used 113 diverse finger millet genotypes for genome wide association mapping of important agro-morphological traits using SNPs. The marker trait associations were determined using single locus single trait (SLST), multi-locus mixed model (MLMM) and multi-trait mixed model (MTMM) approaches. They reported 109 novel SNPs associated with important agro-morphological traits. Out of which, five SNP sequences showed homology to candidate genes of *Oryza sativa* (Rice) and *Setaria italica* (Foxtail millet), which play an important role in flowering, maturity and grain yield.

Puranik *et al.* (2020) used an assembly of 190 diverse finger millet genotypes and evaluated for six minerals (iron, zinc, calcium, magnesium, potassium, and sodium) and protein content. A combination of genotyping-by-sequencing (GBS) and genome-wide association study (GWAS) was applied to identify marker-trait associations (MTAs). They observed a wide range of variation was for all the traits. GBS generated 169,365 single-nucleotide polymorphisms and three subpopulations were identified. GWAS, using general linear and mixed model approaches to correct for population structure and genetic relatedness, identified 418 common markers ($p\text{-value} \leq 10^{-3}$, $\text{FDR} < 0.1$) linked with mineral content.

Tiwari *et al.* (2020) conducted genotyping of 113 diverse finger millet genotypes was carried out for understanding the complex genetic architecture of SPC (seed protein content) and related traits like DM (days to maturity) and GY (grain yield). A set of 2977 genome-wide SNPs identified through genotyping by sequencing were used for MTA analysis. GLM resulted in 7, 10 and 9, while MLM found 4, 9 and 7 MTA's for SPC, DM and GY, respectively across the locations using TASSEL pipeline. Among GLM and MLM 4, 7 and 6 MTA's were found common for SPC, DM and GY, respectively. Another pipeline, GAPIT identified 12 MTA's each for SPC, DM and 11 MTA's for GY across the locations. NCBI blast of common SNP markers for all three traits resulted in 5, 3 and 5 most effective genome regions associated with SPC, DM and GY.

Backiyalakshmi *et al.* (2021) studied the global finger millet germplasm diversity panel of 314 accessions by genotyping using the DArTseq approach to assess genetic diversity and population structure. They obtained 33,884 high-quality single nucleotide polymorphism (SNP) markers on 306 accessions after filtering. Finger millet germplasm showed considerable genetic diversity and the mean polymorphic information content. Also, the study revealed that, finger millet diversity is structured based on a geographical region of origin, while the racial structure made negligible contribution to population structure.

Brhane *et al.* (2022) used genotyping-by-sequencing (GBS) method to identify novel single nucleotide polymorphism (SNP) markers and genotype 288 finger millet accessions collected from Ethiopia and Zimbabwe. The grouping of the 288 accessions

into seven populations based on geographic proximity and the potential for germplasm exchange revealed a narrow range of observed heterozygosity (H_o ; 0.09–0.11) and expected heterozygosity (H_e) that ranged over two fold, from 0.11 to 0.26. The analysis of molecular variance (AMOVA) revealed a highly significant genetic differentiation among groups of accessions classified based on the geographic region, country of origin, days to flowering, panicle type, and Al tolerance ($p < 0.01$).

2.9 Expression analysis of drought responsive genes in reproductive tissue

Reproductive stage is sensitive to moisture stress and pollen development in particular is considered a limiting step in crop productivity (Parish *et al.*, 2012). The understanding of differences in transcription levels at the reproductive stage between drought-tolerant and drought-sensitive genotypes under drought conditions may, therefore, aid in identifying genes important in enhancing drought tolerance.

Crop plants, to cope up with moisture stress or drought, have developed special measures at morphological, physiological, biochemical, cellular and molecular levels. At molecular level, the substantial change in the metabolism under moisture stress is mainly attributed to extensive transcriptome rebuilding and gene regulation. Many studies have identified the expression of genes related to dehydrins (Kosova *et al.*, 2014), transporters (Aharon *et al.*, 2003; Li *et al.*, 2015b), transcription factors (Hu *et al.*, 2006; Nakashima *et al.*, 2009) starch biosynthesis (Jin *et al.*, 2013) *etc.*, under moisture stress conditions. Although, many molecular elements have been identified, the gene network of drought stress response is still not fully elucidated (Zhu, 2002).

Pollen grains of moisture stress affected anthers have little or no starch. This reduction in starch accumulation correlated with the reduction in the level of ADP glucose pyrophosphorylase (AGP) in wheat anthers under moisture stress (Lalonde *et al.*, 1997). Huang *et al.* (2000) reported accumulation of pollen specific and desiccation associated LLA23 proteins and mRNAs in pollen grains of *Lilium longiflorum* upon treating them with abscisic acid (ABA) and polyethylene glycol (PEG)-8000 during germination.

Cell wall and vacuolar invertases play an important role in carbon allocation to developing organs. In rice anthers, upon inducing moisture stress before anthesis, vacuolar invertases (*OsVIN2*) were up-regulated while cell wall invertases (*OsCIN1*) were down regulated (Ji *et al.*, 2005). In wheat anthers, the expression analysis of two cell wall (*Ivr1*, *Ivr3*) and one vacuolar (*Ivr5*) invertase in anthers under moisture stress revealed that water deficit during meiosis down-regulated *Ivr1* and *Ivr5* enzymes without affecting *Ivr3* expression in anthers (Koonjul *et al.*, 2005; Ji *et al.*, 2010a). Similar results were also observed in rice where *OsCIN1* level was repressed and the expression of sucrose transporter *OsSUT5* and monosaccharide transporter gene *OsMST7* were significantly increased in stressed anthers at the microspore and vacuolated microspore stages (Nguyen *et al.*, 2010).

Low sucrose synthase activity during starch accumulation in pollen of moisture stressed anthers of wheat at meiosis was reported by Dorion *et al.* (1996). Similar results were also reported by Sheoran and Saini (1996) where sucrose synthase activity was lower in moisture stressed anthers of rice except during late microspore stage. The expression of genes in immature tassel of maize during meiosis under moisture stress was studied by Zhuang *et al.* (2007). They reported that out of three isoforms of sucrose synthase viz., *Sus1*, *Sus2* and *Sus3*, expression of only *Sus1* and *Sus2* were repressed while *Sus3* expression was induced under moisture stress. Under combined heat and drought stress, increased expression of genes encoding sugar transporter (*MST8*), sugar metabolism enzymes (stachyose synthase and sucrose synthase) and a cell wall invertase (*INV4*) were observed in heat tolerant N22 compared to heat susceptible Moroberekan cultivar where high expression of carbon starved anthers were observed (Li *et al.*, 2015b; Zuther *et al.*, 2015).

Jin *et al.* (2013) examined for floral development and transcriptomic changes in rice subjected to reproductive stage drought stress. The results showed that male fertility was dramatically affected, with differing pollen viability in flowers of the same panicle due to aberrant anther development under water stress. Examination of local starch distribution revealed that starch accumulated abnormally in terms of position and abundance in anthers of water-stressed plants. Microarray analysis using florets of

different sizes identified >1000 drought-responsive genes, most of which were specifically regulated in only one or two particular sizes of florets, suggesting developmental stage-dependent responses to drought. Genes known to be involved in tapetum and/or microspore development, cell wall formation or expansion, and starch synthesis

Li *et al.* (2021) combined the transcriptomic (RNA-seq) and proteomic (iTRAQ) technologies to investigate the finger millet samples treated with drought at different stages to determine drought response mechanism. A total of 80,602 differentially expressed genes (DEGs) and 3,009 differentially expressed proteins (DEPs) were identified in the transcriptomic and proteomic levels, respectively. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis identified that DEGs-DEPs which were drought tolerance-related were enriched in hydrolase activity, glycosyl bond formation, oxidoreductase activity, carbohydrate binding and biosynthesis of unsaturated fatty acids.

III MATERIAL AND METHODS

Finger millet is one of the orphan crop lacking in genomic information for crop improvement. A limited success has been achieved to improve the crop through the traditional breeding approaches. Plant genetic resources provide the foundation for improving agricultural resilience to biotic and abiotic challenges caused by climate change. Genomics has potentially helped the characterization of genetic architecture of plant genomes and establishing the relationship between genome and the traits. Utilization of finger millet genetic and genomic resources is one of the approaches that could provide sustainable crop improvement by increasing the productivity of the crop. Hence, the research work has been framed to screen the finger millet germplasm for seed yield related traits under drought stress conditions and to identify QTLs for drought tolerance related traits. In addition, expression analysis of selected genes under drought stress environment was also carried out in contrasting genotypes to identify important genes associated with drought tolerance.

The details of the experiments conducted in present study are briefly described in this chapter. The following four experiments were conducted,

3.1 Evaluation of association mapping panel and identification of drought tolerant genotypes

3.2 Genome wide association mapping and identification of QTLs for seed yield and traits associated with drought tolerance

3.3 Effect of early reproductive growth stage stress on flowering, quality and quantity of pollen grains in contrasting finger millet genotypes

3.4 Expression of a few stress related genes in the reproductive tissue under moisture stress

3.1 Evaluation of association mapping panel and identification of drought tolerant genotypes

3.1.1 Experimental material

An association mapping panel (AMP) of finger millet [*Eleusine coracana* (L.) Gaertn] consisting of 350 genotypes representing the geographical and agro-

morphological diversity of the world germplasm has been assembled. A “core” collection consisting of 623 finger millet accessions representing global diversity (Upadhyaya *et al.*, 2010) was grown in the field and phenotyped for 18 quantitative traits. Molecular diversity analysis of the 623 core accessions was also performed using SSR primers. Then phenotypic and molecular data of the 623 accessions (2015) was used to develop a “diversity panel” of 350 finger millet accessions, representing the wide diversity present in the original core panel (Reddy *et al.*, 2022). The assembled genotype represents different geographical finger millet growing regions of the world (Appendix I).

3.1.2 Experimental location

The field experiments were conducted at Department of Plant Biotechnology, GKVK, Bangalore, which is located at 13° 05'N;77° 34'E with an altitude of 930 m above sea level. The 350 genotypes were evaluated for sixteen agro-morphological traits related to seed yield and drought tolerance under drought stress (DS) and well watered (WW) conditions over the two years.

3.1.3 Phenotyping of association mapping panel

Three hundred and fifty genotypes were grown under field conditions during summer season of 2018-19 and 2019-20. In each year, the genotypes were grown in two separate blocks; one for well watered or no stress (WW) environment and another for drought stress (DS) condition. The two blocks were separated by 3 meter gap to avoid soil moisture drift to the stress block. In each main block (WW/DS) the genotypes were grown in a single replication under augmented design. During summer 2019, each main block (WW/DS) was divided into five sub-blocks accommodating seventy genotypes and six check varieties (*viz.* KMR-340, GPU-28, ML-365, MR-1, MR-6 and INDAF-7) per sub block. During summer 2020, each main block (WW/DS) was divided into seven sub-blocks. Each sub block accommodated with fifty test genotypes and five check varieties (*viz.* GPU-28, ML-365, MR-1, MR-6 and INDAF-7). The genotypes were sown in the month of December-2018 for the summer 2018-19 experiment and in January-2019 for the summer 2019-20 experiment.

In both the years, the seeds were sown in plastic cups and grown in the greenhouse for 21 days and manually transplanted in the field. Each genotype was planted in a single row of 1.5 meter with the spacing of 30 cm between rows and 10 cm between plants within the row. The recommended dose of NPK 50:40:25(kg ha⁻¹) was applied in a split dose. Half of the recommended dose of nitrogen and full dose of P and K was given as basal dose at the time of transplanting and the remaining 50% N (urea) was provided as top dressing at 30 days after transplanting. An insecticide spray of Imidachloprid (0.5 ml/L) was given to the crop to avoid sucking insect pests. Manual weeding was undertaken twice within 30 days after transplanting. Irrigation was applied to both WW and DS blocks regularly every two days till 57th day after sowing when the majority of genotypes reached the flowering stage. Drought stress was imposed during flowering by withholding the for 20 days from 57th day after sowing for the DS block, while regular irrigation was continued for the WW block. After 20 days of stress treatment, irrigation was provided to both the blocks equally at two days of interval till maturity. The two years (2019 and 2020) and two blocks (WW and DS) are coded as WW19, DS19, WW20 and DS20 respectively.

3.1.4 Observations recorded on agro-morphological traits

Five plants per genotype were chosen randomly for recording the observations on seed yield and agro-morphological traits. Observations on days to flowering (DTF), plant height at reproductive stage (PHS), plant height at harvest (PHH), total number of tillers per plant (TLP), number of productive tillers per plant (PTLP), culm length (CL), number of earheads per plant (EHP) and main earhead length (EHL) were recorded on the field grown plants. The main earhead weight (EHW), total earhead weight per plant (EHWP), finger numbers on main earhead (FNEH), finger length (FL), finger width (FW), test weight (TW), total biomass (BM) and seed yield per plant (SYP) were measured after harvesting the individual plant at maturity and details are given in table 3.1.

Table 3.1 Description of seed yield and agro-morphological traits recorded in field conditions

Sl. No.	Trait	Abbreviation	Description
1.	Days to flowering	DTF	Number of days taken for flowering in 50% of the plants in each genotypes
2.	Plant height at reproductive stage (cm)	PHS	Height measured in cm from the ground level to the tip of the inflorescence of the main earhead at the end of stress period (76 th DAS)
3.	Plant height at harvest (cm)	PHH	Height measured in cm on main tiller from the ground level to the tip of the main earhead immediately after harvesting at the maturity
4.	Total number of tillers per plant	TLP	Number of basal tillers per plant was counted
5.	Total number of productive tillers per plant	PTLP	Number of basal tillers which bears mature earheads per plant was counted
6.	Culm length (cm)	CL	Length measured in cm from first internode from the top to the tip of the finger on main earhead at the maturity
7.	Number of earheads per plant	EHP	The number of earheads was counted on a plant at maturity
8.	Main earhead length (cm)	EHL	Length measured in cm from base of thumb finger to the tip of the longest finger on main earhead
9.	Main earhead weight (g)	EHW	Weight in grams of main earhead after harvesting and drying to uniform moisture content
10.	Total earhead weight per plant (g)	EHWP	Weight in grams of all the earheads from a plant after harvesting and drying to uniform moisture content
11.	Finger numbers per earhead	FNEH	Number of fingers on main earhead were counted
12.	Finger length (cm)	FL	Length from the base of the longest finger on the main earhead to the tip was measured in cm
13.	Finger width (cm)	FW	Width measured in cm at the widest point of the longest finger on the main earhead
14.	Test weight (g)	TW	Weight in grams of dried, threshed and randomly selected 1000 seeds from each genotype
15.	Total biomass (g)	BM	After maturity, five plans were harvested at the ground level along with earheads; dried completely and weighed
16.	Seed yield per plant (g)	SYP	Weight in grams of seeds after threshing all the earheads of a plant

3.1.5 Statistical Analysis

In the present experiment, the data set contains a large number of variables and genotypes, there is often a serious amount of overlap between variables and genotypes. Therefore, we have applied different statistical approaches to reduce the large number of variables into a small number of important variables which makes clear interpretations as well as selection of trait donor genotypes in individual environment as well as across environments. The mean values of sixteen quantitative agro-morphological traits were subjected to statistical analysis. Different statistical methods as given below were used to assess the genetic variability in the population, to identify drought tolerant genotypes and to identify important traits for drought tolerance.

- i) Analysis of variance and estimation of genetic variability parameters
- ii) Correlation between seed yield and agro-morphological traits
- iii) Stepwise multiple linear regression analysis for determination of important traits for productivity and drought tolerance
- iv) Identification of important traits and genotypes for seed yield and drought tolerance
 - a) Principal component analysis (PCA)
 - b) PCA-biplot analysis
 - c) Genotype by Yield*Trait (GYT) analysis
 - d) Genotype plus Genotype-by-Environment (GGE) analysis

The details of statistical analysis are given below,

3.1.5.1 Analysis of variance

Analysis of variance (ANOVA) was carried out by following augmented design using package ‘augmentedRCBD’ (v0.1.5) in R studio (Aravind *et al.*, 2021) (v1.1.463) to partition the total phenotypic variation among the genotypes and check entries into sources such as ‘Blocks’, ‘ Genotypes + check entries’, ‘Genotypes’, ‘check entries’ and

‘Genotypes vs check entries’. The ANOVA was done separately for WW and DS environment in each year.

Table 3.2 Structure of analysis of variance (ANOVA) as per augmented design
(Federer, 1956)

Source of variation	df	MSS	‘F’ ratio
Blocks(b)	(b-1)	MSS(b)	MSS(b)/ EMSS
Entries (e) (Genotypes + check varieties)	(e-1)	MSS(e)	MSS(e)/ EMSS
Genotypes (g)	(g-1)	MSS(g)	MSS(g)/ EMSS
Check varieties (c)	(c-1)	MSS(c)	MSS(c)/ EMSS
Genotypes vs Check varieties	(g-1) (c-1)	MSS(gc)	MSS(gc)/ EMSS
Error	(c-1) (b-1)	EMSS	

Where,

b = number of blocks

e = number of entries

g = number of genotype accessions

c = number of check varieties

The effect of each block (B_j) was computed as, $B_j = X_j - X$

Where,

X_j = Trait means of check entries in j^{th} block

X = Trait mean of all the checks in all the blocks

B_j was used to adjust the trait means of the accessions relevant to the block. Thus, trait means of each accession evaluated in j^{th} block was adjusted by subtracting the block effect B_j of the j^{th} block from the actual trait value of the accession.

3.1.5.2 Estimation of genetic variability parameters

Genetic variability among the genotypes was assessed by determining mean, range, phenotypic coefficient of variability (PCV) and genetic coefficient of variability (GCV) (Burton and De vane, 1953) to enable comparison of phenotypic and genotypic variability across the traits. Broad sense heritability (h^2_{bs}) (Lush, 1949) and expected genetic advance on mean (GAM) in *per cent* was estimated (Johnson *et al.*, 1955) using mean trait values of each genotype. The statistical analysis was done separately for WW and DS conditions for each year.

a. Mean

Arithmetic mean is defined as the sum of all observations divided by the total number of individuals added (n), was calculated by following formula;

$$\text{Mean} = \Sigma X_i/n$$

Where,

X_i = i^{th} observation of a population

n = Total number of observations

Mean was obtained for each trait under WW and DS conditions separately for each year.

b. Range

The minimum and maximum values observed on the basis of all accessions for a particular trait indicate range for a given trait.

c. Standard error (SE)

It is a measure of uncontrolled variation present in a sample, which is estimated by dividing the standard deviation by the square root of the number of observations.

$$SE = SD / \sqrt{n}$$

Where,

SD = Standard deviation

n = Number of observations

d. Variance

It is expressed as the sum of squares of the deviations of all observations of the population from its mean and divided by n-1 where n is the number of observations.

$$\sigma^2 = \sum_{i=1}^n (X_i - \bar{X})^2 / n - 1$$

Where,

σ^2 = Variance

X_i = Value of each genotype

$\bar{X}$ = Arithmetic mean of population

n = Number of genotypes

e. Phenotypic and Genotypic Co-efficient of Variation (PCV and GCV)

Both phenotypic and genotypic co-efficient of variation for all the traits were estimated using the formula of Burton and De vane (1953),

Phenotypic Co-efficient of Variation: $PCV (\%) = [(\sqrt{\sigma^2_p}) / \bar{X}] \times 100$

Genotypic Co-efficient of Variation: $GCV (\%) = [(\sqrt{\sigma^2_g}) / \bar{X}] \times 100$

Where,

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

$\bar{X}$ = Mean

PCV and GCV were classified as shown below by following the methods suggested by Robinson *et al.* (1949);

0-10%	- Low
10-20%	- Moderate
>20%	- High

f. Heritability in broad sense (h^2_{bs})

Heritability (broad sense) was estimated for all the traits as the ratio of genotypic variance to the total variance as suggested by Johnson *et al.* (1955);

$$h^2_{bs} = (\sigma^2_g / \sigma^2_p) \times 100$$

Where,

h^2_{bs} = Heritability (broad sense) expressed in *per cent*

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

The heritability was categorized as suggested by Robinson *et al.* (1949);

0-30%	- Low
30-60%	- Moderate
60-90%	- High

h. Genetic Advance as *per cent* mean (GAM)

The Genetic Advance was predicted by using the formula as given below, (Lush, 1949; Johnson *et al.*, 1955)

$$GA = h^2 \times \sigma_p \times k$$

Where,

GA as *per cent* mean (GAM) = GA/Trait mean $\times$ 100

h^2 = Heritability in broad sense

σ_p = Phenotypic standard deviation

k = Selection differential at 5% (2.06)

The GA as *per cent* mean (GAM) was categorized as suggested by Johnson *et al.* (1955) and the same is given below:

0-10%	- Low
10-20%	- Moderate
20% and above	- High

3.1.5.3 Correlation between seed yield and agro-morphological traits

The data obtained on sixteen agro morphological traits were analyzed for Pearson's correlation of coefficients (r) in order to determine the relationship between seed yield and agro-morphological traits under WW and DS conditions separately in each year. The analysis based on variance and co-variance components suggested by Weber and Moorthy (1952). and was computed by using statistical package “ggcorrplot” in R studio (v1.1.463). The degree of association among the traits was determined by their correlation coefficients (r).

$$r_{xy} = \frac{\text{Cov}(xy)}{\sigma_x \sigma_y}$$

Where,

r_{xy} = Correlation coefficient between x and y

$\text{Cov}(xy)$ = Covariance of x and y

σ_x = Standard deviation of x

σ_y = Standard deviation of y

3.1.5.4 Stepwise multiple linear regression analysis for determination of important traits for productivity and drought tolerance

Stepwise multiple linear regression was used to understand the cause of variation and functional relationship between dependent and independent variables. It is often used to determine the important variables contributing to dependent variables. In the present

study, stepwise multiple linear regression (with backward elimination) was used to identify major traits contributing to the seed yield (dependent variable) using statistical software SPSS (v16.0.0). To achieve this stepwise multiple linear regression analyses carried out using two models. In the first model all fifteen traits were used as independent variables and were regressed on dependent variable seed yield. The model represented below,

$$Y = a + \sum_{i=1}^n b_i X_i + \varepsilon$$

Where,

Y = dependent variable (seed yield), a = intercept (constant), X_i = independent variables (different plant characteristics) viz. DTF, PHS, PHH, TLP, PTLT, CL, EHP, EHL, EHW, EHWP, FNEH, FL, FW, TW and BM. ε = error term, b_i = slopes associated with respective independent variables X_i .

In order to identify the contribution of major independent variables on seed yield; a improved better fit model was constructed by stepwise multiple regression with backward elimination where regression analysis was carried out by stepwise elimination of an independent variable in each step with non-significant regression co-efficient ($P > 0.05$). The second model was:

$$Y = a + b_1 X_1 + b_2 X_2 + b_3 X_3 + \dots + b_n X_n + \varepsilon$$

Where,

Y = dependent variable (seed yield), a = intercept (constant), independent variables $X_1, X_2, X_3, \dots, X_n$, $b_1, b_2, b_3, \dots, b_n$ are regression coefficient or slope of the respective independent variables, ε = error term, ($n = 1 \dots 15$), viz. DTF, PHS, PHH, TLP, PTLT, CL, EHP, EHL, EHW, EHWP, FNEH, FL, FW, TW and BM.

3.1.5.5 Identification of important traits and genotypes for seed yield and drought tolerance

3.1.5.5 (a) Principal component analysis (PCA)

Principal component analysis (PCA) applied to reduce a large series of data into smaller number of components by looking for groups that have very strong inter-correlation in a set of variables and each component explained *per cent* variation to the total variability. It is efficient in finding the components that maximize the variance. The first principal component is the largest contributor to the total variation in the population followed by subsequent components. The criteria used by Clifford and Stephenson (1975) and corroborated by Guei *et al.* (2005), suggested that the first two or three principal components are often the most important in reflecting the variation patterns among accessions, and the characters associated with these are more useful in differentiating the accessions. Thus it is useful for genetic improvement of important traits having larger contributions to the variability rather than going for all the characters under study. It is also useful to group the genotypes based on components. In the present study, PCA was performed with all sixteen traits for WW and DS conditions in each year separately. The contribution by each trait to the PC1 and PC2 under WW and DS condition in each year was used to identify the major traits contributing to higher variability.

Computational procedures

PCA selects linear combinations of the attributes, which retains the highest proportion of unexplained variability between the units. These linear combinations (the principle components) can explain the relationship between units and attributes and by restriction; the dimensionality of the data is reduced. The major axis or vector is called the principal component 1 (PC 1) and subsequent orthogonal axes are numbered sequentially (PC 2, PC 3...PC n). Each PC axis is defined in terms of linear transformation of the original variable score. Coefficients of each transformation equation form a set of eigen values exceeding 1.0 and the corresponding PC has inherently more information than any single variable alone. A significant PC interprets the percentage of the total variation in the data set.

Pearson (1901) and Hotelling (1933) described the variation in a set of multivariate data in terms of a set of uncorrelated variables. The data matrix of 'n' observation on 'p' correlated variables $x_1, x_2, \dots, x_p$. PCA looks for a transformation of the x_i into 'p' new variables Y_i that are uncorrelated.

Looking for a transformation of the data matrix X ($n \times p$) such that

$$Y = \delta^T X = \delta_1 X + \delta_2 X + \dots + \delta_p X_p$$

Where, $\delta = (\delta_1, \delta_2, \dots, \delta_p)^T$ is a column vector of weights with $\delta^2 + \delta_p^2 + \dots = 1$

Maximize the variance of the projection of the observations on the Y variables

Find δ so that $\text{Var}(\delta^T X) = \delta^T \text{Var}(X)$ is maximal

The matrix $C = \text{Var}(X)$ is the covariance matrix of the X_i variables

Corresponding to the largest γ is given by the eigen vector δ . The direction of eigen value of matrix C . The second vector that is orthogonal (uncorrelated) to the first is the one that has the second highest variance which comes to be the eigen vector corresponding to the second eigen value and so on. New variables Y_i that are linear combinations of the original variables (x_i): $Y_i = a_{i1}x_1 + a_{i2}x_2 + \dots + a_{ip}x_p$; $i=1 \dots p$. The new variables Y_i are derived in decreasing order of importance; they are called 'principal components' calculating eigen values and eigen vectors are found by solving the equation λ . The PCA was performed using statistical software Past (v4.09).

3.1.5.5 (b) PCA-Biplot analysis

PCA-biplot is one of the most effective multivariate analyses used evaluate the traits interaction, genotypic performance and to dissect the correlation between the traits. Thus PCA-biplot analysis was performed by using first two principal components (PC) which were computed based on correlation matrix using data from sixteen agromorphological traits under WW and DS conditions in each year separately using Past (v4.09) software. It has been previously reported that in PCA-biplot, the cosine of the angle between the trait vectors approximates the correlation between them, where an acute angle ($<90^\circ$) represents positive correlation, angle of $>90^\circ$ indicates a negative

correlation, while equivalent to 90° angle denotes traits are independent of each other. This was utilized to select and group the traits based on their contribution to the principal components under WW and DS conditions in each year.

3.1.5.5 (c) Genotype by Yield*Trait (GYT) analysis

A genotype by yield*trait (GYT) biplot approach used to tackle the problem of genotype evaluation on multiple traits under certain environments and it helps in genotype selection based on multiple traits. It is based on the following conceptualization: 1) Yield is the most important trait and all other target traits are important only when combined with high yield. 2) The superiority of a genotype should be judged by its levels in combining yield with other target traits, rather than by its levels in individual traits. GYT biplot has become a popular data visualization tool in multi-trait evolution.

The mean data of sixteen agro morphological traits for each of the 350 genotypes screened under WW and DS conditions in each year separately were used in GYT analysis. The data for each genotype by trait (GT) first transformed into a genotype by seed yield*trait (GYT) and then displayed in a GYT biplot. Since all yield-trait combinations had yield as a component, it allowed genotypes to be graphically and meaningfully ranked based on their yield-trait combinations on GYT biplot. The average tester coordination (ATC) view of the GYT biplot is employed to rank genotypes based on their overall superiority across the yield-trait combinations and to show their trait profiles (*i.e.* strengths and weaknesses), which served as the basis for genotype evaluation. In this mode, the economic value of the level of the trait is determined which is dependent on the level of seed yield. The GYT biplot graphically ranks genotypes based on their superiority in combining yield with other traits in each of the environments. The superior genotypes for seed yield with trait combinations were identified under WW, DS and combined WWDS conditions separately in each year. The analysis was performed as suggested by Yan and Frégeau-Reid, (2018) and the same is given below. `

Data standardization

The GT table or the GYT table was standardised so that the mean for each trait or yield-trait combination becomes 0 and the variance becomes unit. The standardization was performed as:

$$P_{ij} = T_{ij} - \bar{T}_{ij} / S_j$$

Where,

P_{ij} is the standardized value of genotype i for trait or yield-trait combination j in the standardized table, T_{ij} is the original value of genotype i for trait or yield-trait combination j in the GT or GYT table, $\bar{T}_j$ is the mean across genotypes for trait or yield-trait combination j , and s_j is the standard deviation for trait or yield-trait combination j . The GYT analysis was performed under WW, DS and across all the four (WWDS) conditions separately with all the recorded traits using R studio (v1.1.463) with the statistical package “metan” (Olivoto and Lúcio, 2020).

3.1.5.5 (d) Genotype plus Genotype-by-Environment (GGE) analysis

A genotype main effect plus genotype $\times$ environment interaction (GGE) biplot based on data recorded on different traits under certain environments allows visualization of (i) the which- won-where pattern of the genotypes with respect to the environment (ii) the ranking of genotypes based on both mean performance and stability across the environment. The GGE biplot methods are most popular and useful in identifying stable and high yielding genotypes. It is biplot technique based on genotype $\times$ environment interaction estimated variance and predicting several values and select genotypes that are adapted to the target environment. The important traits for yield and drought tolerance viz. seed yield, total earhead weight per plant and main earhead weight which were identified through stepwise multiple linear regression (section 3.1.5.4) were used for GGE biplot analysis. The mean data of individual 350 genotypes of these traits under WW and DS conditions in each year were used to identify best suitable genotypes for WW, DS and across all the four (WWDS) environments. The analysis was performed by statistical package “metan” using R software (v4.1.2) (Olivoto and Lúcio, 2020). The

ideal genotypes with high mean performance and high stability for these traits across the environments were identified by ranking of genotypes biplot. All the GGE biplots were based on singular-value partitioning of genotype (SVP = 1), environment-centered (centering = 2), and standard deviation-standardized (scaling = 1). GGE biplots exhibit which genotype(s) has (have) the highest yield and stability performance in which environment(s) (Yan and Kang 2002) and the high yielding and stable (or ideal) genotype/s under WW, DS and across well watered and drought stress (WWDS) conditions were identified.

3.1.5.6 Determination of drought tolerance indices (DTI) and selection of genotypes

Drought tolerance is a complex trait influenced by combinations of physiological and productivity related traits (Tuberosa, 2012). The relative yield performance of genotypes in drought and non-stress environments appears to be a common starting point for identifying desirable genotypes for unpredictable conditions (Mohammadi *et al.*, 2012; Nouri *et al.*, 2011). Drought tolerance is defined by Hall (1993) as the relative yield of a genotype compared to other genotypes subjected to similar kind of drought stress. Drought susceptibility of a genotype is often measured as a function of reduction in yield under drought stress (Blum, 2010) whilst the values are confounded with differential yield potential of genotypes (Ramirez and Kelly, 1998). Several drought indices which provide a measure of drought tolerance, based on loss of yield under drought conditions in comparisons to normal conditions have been used for screening drought tolerant genotypes (Mau *et al.*, 2019; Ferede *et al.*, 2020).

The commonly used drought tolerance indices are mean productivity (MP), yield stability index (YSI), geometric mean productivity (GMP), yield index (YI), harmonic mean (HM) and drought resistance index (DRI) to identify drought tolerant genotypes under drought stress conditions (Table 3.3). These indices consider the ability and performance of the genotypes under stress and non stress environments. All indices were estimated based on the extent of reduction in seed yield per plant of the genotypes under DS environment to those evaluated under WW environment separately in each year.

Table 3.3 Different drought tolerance indices computed among the finger millet genotypes

Sl. No.	Drought indices	Formula	Description	Reference
1	Geometric Mean Productivity (GMP)	$GMP = \sqrt{Y_p * Y_s}$	Genotypes with high value of this index are more desirable	Schneider <i>et al.</i> , (1997)
2	Mean Productivity (MP)	$MP = Y_s + Y_p / 2$	Genotypes with high value of this index are more desirable	Rosielle and Hamblin (1981)
3	Harmonic Mean (HM)	$HM = 2(Y_p * Y_s) / (Y_p + Y_s)$	Genotypes with high value of this index are more desirable	Jafari <i>et al.</i> , (2012)
4	Drought Resistance Index (DRI)	$DRI = Y_s(Y_s / Y_p) / Y_{\bar{p}}$	Genotypes with high value of this index are suitable for drought stress condition	Lan, 1998
5	Yield Index (YI)	$YI = Y_s / Y_{\bar{s}}$	Genotypes with high value of this index are suitable for drought stress condition	Gavuzzi <i>et al.</i> , (1997)
6	Yield Stability Index (YSI)	$YSI = Y_s / Y_p$	Genotypes with high values are regarded as stable genotypes under stress and non-stress conditions	Bousslama and Schapaugh (1984)

Where,

Y_s : grain yield under DS for each genotype Y_p : grain yield under WW for each genotype

$Y_{\bar{s}}$: average yield of all genotypes under DS $Y_{\bar{p}}$: average yield of all genotypes under WW

For each DTI the overall mean (μ) and standard deviation (σ) was determined for each year separately and the genotypes were grouped as given below,

DTI value of a genotype	Group
$\mu+2\sigma$ and above	- Tolerant
$\mu+\sigma$ to $\mu+2\sigma$	- Moderately tolerant
$\mu+\sigma$ to $\mu-\sigma$	- Moderately susceptible
$\mu-\sigma$ to $\mu-2\sigma$	- Susceptible
$\mu-2\sigma$ and less	- Highly susceptible

The genotype which falls in the tolerant group at least in two different DTI in each year was considered as stable tolerant genotype. Similarly, the genotype which falls in a highly susceptible group at least in two different drought tolerant indices in each year was considered as drought susceptible genotype.

3.2 Genome wide association mapping and identification of QTLs for seed yield and traits associated with drought tolerance

3.2.1 Genotyping and population structure analysis of AMP using SSR markers

Breeding experiments rely on the genetic diversity and population structure; therefore it is an essential step to study the genetic polymorphisms using various markers. Previously, several studies have been carried out for analyzing the genetic diversity using various markers (Babu *et al.*, 2007; Dida *et al.*, 2008; Panwar *et al.*, 2010a; Babu *et al.*, 2014a). The analysis of population structure allows to identify sub-populations in the original population. The population structure was carried out using SSR and SNP markers independently.

3.2.1.1 DNA extraction from 350 genotypes

The 350 genotypes were grown in plastic cups under greenhouse conditions. The young leaves were collected from the fifteen days old seedlings and the genomic DNA

was extracted by following Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle, 1987). The following steps were used to extract the DNA.

1. Two grams of fresh young leaves from seedling were crushed in liquid nitrogen in a pre-cooled pestle and mortar to make fine powder.
2. The ground powder was transferred to a 2 ml sterile polypropylene tube containing 1 ml of extraction buffer pre warmed to 65°C.
3. The samples were incubated at 65°C for 30 minutes with intermittent mixing of tubes every 15 minutes.
4. After bringing back tubes to room temperature, equal volume (1 ml) of chloroform: isoamyl alcohol (CIA) (24:1 v/v) was added to each tube containing sample and extraction buffer and mixed by inverting (did not vortex).
5. Tubes were then centrifuged at 10000 rpm for 10 minutes.
6. Upper aqueous phase was carefully transferred to the new sterile tube (step four was repeated once again if the upper layer did not look clear). Then equal volume and pre-chilled isopropanol was added and mixed gently.
7. Tubes were kept at -20°C overnight and centrifuged at 10000 rpm for 15 minutes to pellet the DNA.
8. The supernatant was decanted and the pellet was washed with 1 ml of 70 per cent ethanol and then air dried completely for 1-2 hour (there should not be any alcohol trace).
9. The DNA pellet was suspended in 40-50 µl of TE buffer in sterile centrifuge tube. When DNA was fully dissolved, 1 µl of RNase (stock 10 mg/ml) per 100 µl volume of sample was added (Final RNase concentration 100 µg/ml) and incubated at 37°C for one hour or at room temperature overnight.

10. DNA samples were stored in -20°C.

3.2.1.2 DNA quality and quantity estimation

The extracted DNA samples were loaded on 0.8 % (w/v) agarose gel, prepared in 1X TAE buffer (For 250 ml 50X TAE - 60.5g of Tris Base, 14.25 ml glacial acetic acid and 25 ml of 0.5 M EDTA) containing 0.5 µl/10 ml ethidium bromide (10mg/ml). This was followed by visualization and documentation using Alpha digidoc 1000 gel documentation system (Alpha Innotech Corporation, USA). Further, the quality of DNA was assessed by checking for the shearing of DNA and contamination with RNA. Total DNA was quantified by biophotometer at $A_{260/280}$ and the DNA stocks of the samples were diluted to required 50 ng/µl for genotyping by SSR markers.

3.2.1.3 Polymerase chain reaction

Fifteen genomic SSR markers which were developed using whole genome sequence of finger millet in our laboratory were used for the study. The markers represented di, tri and tetra-nucleotide repeats from different scaffolds of whole genome (Table 3.4). The PCR was carried out in a 10µl reaction mixture consisting of template DNA and other components required for amplification of the SSR priming regions of genomic DNA (Table 3.5). Polymerase chain reaction (PCR) for all the primer pairs was performed in 10µl volume by following PCR profile (Table 3.6) in a Thermal Cycler (Master cycler gradient, Eppendorf, Hamburg, Germany). The PCR products from each tube along with 1µl of loading dye (6X) was loaded to 3.5% agarose gel containing ethidium bromide and were separated electrophoretically. The amplification products were examined under UV light and photographed using Alpha digidoc 1000 gel documentation system (Alpha Innotech Corporation, USA).

Table 3.4 List of SSR primers used to study genetic diversity and population structure in finger millet

Sl No.	Primer name	SSR motif	Forward (5'-3')	Reverse (5'-3')	Annealing temp (°C)	Expected amplicon size (bp)
1.	UASBFM-19	(AT)34	ATGGTTGTGGAGAA TTGAACCT	AGAAACCTCGG GTATGAAATGA	55.9	170
2.	UASBFM-31	(G)15	ATGATCTCGATGGCT CTTTGAT	AAATGATACAT GGTGCCTCACA	52.0	190
3.	UASBFM-24	(ATG)23	GCCAATGATGATGA AGATGATG	CTTGTTACCCCTT GCCATTTAGC	52.0	220
4.	UASBFM-32	(GAT)12	CTTCTAAACCCAATC GTCCAAT	AGGCTTGCTTG ATCTTGATTCT	52.0	380
5.	UASBFM-33	(AG)21	ATTTTCTCCTCCCTTT CTCACC	TTCTACAACITGG ATGGATACCG	52.0	290
6.	UASBFM-35	(TC)10	TTACCCCTGACACAC TCACAAG	CACGATGCAGA GTTGGAAATAA	55.0	280
7.	UASBFM-251	(ATAG)20	GTCTTAAGAGCGAA TGTGATGGC	TGGCACTCATGT GTAGATGTCC	55.0	140
8.	UASBFM-252	(CT)29	ACAACCCCGTACAT GTAGAACG	TGTCAGGTTCG AGTGAAGTTGG	55.0	190
9.	UASBFM-253	(AT)27	GCTTTTCGTTGATTTC TGGACTCC	AGGCCATTTAC CTGTGATTTTAC C	55.0	220
10.	UASBFM-256	(CAA)12	ACTACCCATGCTACA ACTGTGG	CATTGGTGAGT TCACTTGGGC	55.0	180
11.	UASBFM-22	(A)15	TACACCGGAAGAAA GCCACTAT	TGGTGATGGG AAGAAATAACT	50.8	230
12.	UASBFM-25	(GA)27	AGAACCCATAGGAG GACGATG	TTCTCTCTCTCT TTGACGACCC	58.1	160
13.	UASBFM-26	(AC)24	GAAACACCGAGTCA AAACAGAA	CTCCTCCTACAC CAAACCTCCAC	58.1	320
14.	UASBFM-20	(TGA)7	AGAAGCATAGGAAA GGTGAAGC	CAAGCATTTTA GGAAGTGAAGC	54.6	300
15.	UASBFM-261	(ATA)18	GCCAATGATGATGA AGATGATG	CTTGTTACCCCTT GCCATTTAGC	50.8	140

Table 3.5 PCR reaction mixture used for amplification of finger mille DNA using SSR primers

Components	Concentration per μl	Quantity (μl/reaction)
Primers (F + R) (Eurofins, Genomics India Pvt. Ltd.)	10pmol	1.0 μ l
dNTP's 2.5 mM each (GeNei™, Bangalore)	10mM	0.4 μ l
<i>Taq</i> buffer A with 15mM MgCl ₂ (GeNei™, Bangalore)	10X	1.0 μ l
<i>Taq</i> polymerase (GeNei™, Bangalore)	1U	0.3 μ l
Template DNA	50ng	1.0 μ l
Nuclease free water (Sigma Aldrich)	-	6.3 μ l
Total	-	10.0 μ l

Table 3.6 PCR conditions used for amplification of finger millet DNA

Steps	Temperature (°C)	Duration (min)	Cycles
Initial denaturation	95 °C	5	1
Denaturation	94 °C	1	} 35-40
Annealing	55* °C	45 sec	
Extension	72 °C	1	
Final extension	72 °C	8	1
Incubation (pause) 4 °C			

*Depending on the primer

3.2.1.4 Data scoring and statistical analysis

Gel scoring was done by considering the banding pattern of individual genotype. Each PCR amplified product was treated as an independent locus in each genotype. The bands were scored based on their amplicon size in base pairs (bp) and also scored based on present (1) or absent (0) and thus obtained allelic data was subjected to statistical analysis. The dendrogram was constructed based on the allelic information obtained through polymorphic SSR markers used in this study.

The major allele frequency, total number of alleles (N_a), number of effective alleles (N_e), Shannon's information index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), and polymorphic information content (PIC) (Anderson *et al.*, 1993) were calculated using PowerMarker (v3.25). Along with this, GenAlEx software (v6.5)(Peakall and Smouse, 2012) was used to estimate analysis of molecular variance (AMOVA) to assess the statistical distribution of gene diversity and estimate the variance components of the populations *i.e.* among populations and within populations. The Euclidean genetic distance was applied to construct a neighbor joining tree based on the unweighted pair-group method of mathematical averages (UPGMA) as implemented in DARWIN software (v6.0.021) (Perrier and Jacquemoud-Collet, 2006).

3.2.1.5 Population structure analysis using SSR markers

Population structure analysis using SSR data was performed using STRUCTURE (v.2.3.4) software (Pritchard *et al.* 2000). The parameters of the software were set to default, specifying K value 1 to 10, with each K repeated for 5 times. The parameter for clustering were set with initial burning period of 100,000 and Markov Chain Monte Carlo (MCMC) iteration to 1000,000 specifying K = 1 to 10 to generate a STRUCTURE 'Q' matrix. The results obtained from the STRUCTURE analysis were subjected to Evanno "Delta-K" method (Evanno *et al.* 2005) in the STRUCTURE Harvester software (Earl and VonHoldt, 2012) to establish an optimum value of K value *i.e.* number of sub-populations present in the association mapping panel.

3.2.2. Genotyping and population structure analysis of AMP using SNP markers

The panel of 350 finger millet genotypes was resequenced by assembly with Illumina and new Bionano Saphyr optical genome/methylation mapping system (Bionano ver3.0) with 3-5x Illumina coverage. The resequencing of the genotypes and identification of SNPs were carried out as a part of Indo-Swiss Collaboration in Biotechnology Program (ISCB) on finger millet. The genome resequencing and SNP identification were carried at Functional Genomics Center Zurich, Zurich, Switzerland. The chairperson of my advisory committee (Dr. R. L. Ravikumar) is one of the principal investigators in this collaborative project and the data was made available for the present study. The details of SNP mining as provided by Functional Genomics Center Zurich, Zurich, Switzerland is given below.

The genome was separated as A and B sub-genome and aligned with the help of high coverage of the PR-202 genome as a reference. The SNP calling was performed with Genome Analysis ToolKit (GATK) haplotype caller and GATK joint genotyping. The genome wide comparison revealed a total of 80,82,179 SNPs (516Mb) in sub-genome A and 90,71,360 SNPs (650Mb) in sub-genome B. Further SNPs were subjected to hard filtering with custom script for discarding false positives. The SNP calling was imputed by BEAGLE application by using high coverage variant call format (vcf) as a reference to get high quality SNPs. SNPs were filtered out based on missing percentage (<50% missing), homozygosity (inbreeding $F \geq 0.5$) and minor allele frequency (MAF $\geq 5\%$). The graphical linkage disequilibrium (LD) decay was imputed by PopLDdecay v3.41 software. Thus after PLINK filtering and quality check (QC) a total of 32,04,543 SNPs from sub-genome A and 22,71,900 SNP's from sub-genome B were used to study maker trait association studies for sixteen agro-morphological traits recorded under WW and DS conditions in each year separately.

3.2.2.1 Population Structure (Q-matrix)

Population structure was assessed by STRUCTURE (v2.3.4) software (Prichard *et al.* 2000), based on Bayesian clustering method. The models were tested for K-values ranging from 1 to 10, with ten iterations each, with a burn-in period of 1,00,000 and Markov Chain Monte Carlo (MCMC) repeats of 1,00,000. The software STRUCTURE

provides an option to use both admixture models as well as no admixture model. Since the pedigree of the finger millet accessions was unknown, we used admixture linkage model with correlated allele frequency for identification of population structure.

3.2.2.2 Kinship Co-efficient (K-matrix)

Kinship matrix among genotypes was calculated using an R implementation available as part of GAPIT (Genome Association and Prediction Integrated tool) software libraries (Lipka *et al.*, 2012).

3.2.2.3 Genome-wide association analysis

The genotypic data (SNP) stored in HapMap format and the phenotypic data on sixteen agro-morphological traits *viz.* days to flowering, plant height at reproductive stage, plant height at harvest, total number of tillers per plant, number of productive tillers per plant, culm length, number of earheads per plant, main earhead length, main earhead weigh, total earhead weight per plant, finger numbers per earhead, finger length, finger width, test weight, total biomass, seed yield per plant in all the four environments *viz.* WW19, DS19, WW20 and DS20 were used for GWAS. The analysis was carried out for each environment and year separately. The details related to phenotyping of 350 genotypes, experimental location and sixteen agro-morphological traits are given in section 3.1.2, 3.1.3 and 3.1.4 respectively. GWAS was performed using statistical package an R package ‘GAPIT’ to get marker-trait association (MTA) throughout the genome. The Mixed Linear Model (MLM) was used to identify significant MTA in GWAS. MLM is commonly used and most effective method for controlling false positives in MTA in GWAS. This model simultaneously incorporates both population structure (Q) and kinship matrix (K). Kinship was calculated using a R implementation included in the GAPIT package libraries (Lipka, *et al.*, 2012). The graphical LD decay was imputed by PopLDdecay (v3.41) software. The GAPIT gives the results in the form of tables and graphs, *i.e.*, PCA graphs, Manhattan plot, Q-Q plot, etc. The Bonferroni threshold for significant associations between SNPs and trait was set as $p < 1.826 \times 10^{-7}$ ($p = 1/n$, where n = the number of SNP markers used; $-\log_{10} (1/54,76,443) = 6.73$) which also has been widely adopted in literature (Liu *et al.*, 2016; Huang *et al.*, 2018) used on

the False Discovery Rate (FDR) corrected p-values for eliminating false positives. The trait-wise significantly associated SNPs under WW and DS conditions in each year were identified separately and represented on Manhattan plot.

3.3 Effect of early reproductive growth stage stress on flowering, quality and quantity of pollen grains in contrasting finger millet genotypes

3.3.1 Plant material

Six drought tolerant genotypes viz. GE-1234, GE-1260, GE-1531, GE-5118, GE-2268, GE-909 and six drought susceptible genotypes viz. GE-1080, GE-1417, GE-1641, GE-2439, GE-4172 and GE-4719 based on drought tolerant indices (DTI) from the previous study (section 3.1.5.6) were selected for the present experiment. Briefly, six DTI were estimated for all the 350 genotypes based on the extent of reduction in seed yield per plant of each genotype under DS environment to the same as evaluated under WW environment separately in each year. For each DTI, the overall mean (μ) and standard deviation (σ) of 350 genotypes was determined and then further grouped into five groups viz. tolerant, moderately tolerant, moderately susceptible, susceptible and highly susceptible. The genotype which falls in the tolerant group as at least two different DTI in each year were considered as stable tolerant genotype. Similarly, the genotype which falls in a highly susceptible group at least in two different drought tolerant indices in each year was considered as drought susceptible genotype. Based on this, six drought tolerant and six drought susceptible genotypes were selected for this study.

3.3.2. Experimental layout

The seedlings of 12 genotypes were raised in the greenhouse and 21 days old seedlings were transplanted manually in the main field. The experimental field at Department of Plant Biotechnology was divided into two main blocks - one for well watered (control-WW) and another for water stress treatment (DS). The main blocks were separated by 3 meter to avoid soil moisture drift to the stress block. In each block, 12 genotypes were grown in two replications by following Randomized Complete Block Design (RCBD). Each genotype was planted in a single row of 1.5 meter with the spacing of 30 cm between rows and 10 cm between plants within the row. The recommended

dose of NPK 50:40:25 (kg ha⁻¹) was applied in a split dose. Half of the recommended dose of nitrogen and full dose of P and K was given as basal dose at the time of transplanting and the remaining 50% N (urea) was provided as top dressing at 30 days after transplanting. An insecticide spray of Imidachloprid (0.5 ml/L) was given to the crop to avoid sucking insect pests. Manual weeding was undertaken twice within 30 days after transplanting. Irrigation was applied to both WW and DS blocks regularly every two days till 47th day after sowing when genotypes reached to the early flowering stage. Drought stress was imposed during early reproductive stage by withholding the for 20 days from 47th day after sowing for the DS block, while regular irrigation was continued for the WW block. At the end of the stress period, the observations on physiological and pollen related traits were recorded as given below.

3.3.2.2 Observation recorded

a. Leaf Relative Water content (RWC)

Leaf relative water content was estimated one day before withdrawal of water stress treatment *i.e.* 66th day from drought-stressed and well-watered block following the standard protocol given by Agami (2013). Five plants per genotype per treatment were randomly selected to estimate the leaf relative water content. The fourth leaf from the top of each plant was collected and twenty-five leaf discs of 0.8 cm size were collected from the leaves and weighed immediately to record the fresh weight (FW). The leaf discs were hydrated to full turgidity by immersing them in distilled water for 4 hours. After 4 hours, the leaf discs were blotted to remove excess water from the surface and turgid weight (TW) was recorded. The leaf discs were then dried in an oven at 70 °C for 24 hours to record the final dry weight (DW). The RWC was calculated using the following formula,

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

Where,

FW = Fresh weight (g)

DW = Dry weight (g)

TW = Turgid weight (g)

b. SPAD chlorophyll meter reading (SCMR)

The relative leaf chlorophyll content of individual plants was measured using a hand held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan). The observation for SPAD value was recorded on the fourth leaf from top of the plant for all the genotypes and treatment. Five plants per genotype per treatment were selected and then the mean of five plants was determined.

c. Membrane leakage (%)

The cell membrane integrity was tested on leaf discs and relative injury to the membranes in terms of electrolyte leakage. The third leaf from the top was collected on 66th day after sowing and 0.1 gram fresh leaf discs of 10 mm size were used per plant of each genotype per treatment. The samples were incubated in 10 ml of distilled water filled in a glass test tube with flat bottom screw cap. The tubes were continuously shaken using a platform shaker for three hours. The supernatant water with electrolyte leakage was used to record absorbance at 273 nm (initial absorbance, Ia), using UV 1800 visible spectrophotometer. The same tubes were then transferred to a hot water bath (90 °C) for 30 minutes and the final absorbance value of the supernatant water were recorded at 273 nm (final absorbance, Fa) using a spectrophotometer. The cell content leakage was calculated by the following formula given below (Leopold *et al.*, 1981).

$$\text{Membrane leakage (\%)} = (Ia / Fa) \times 100$$

Where,

Ia = Initial absorbance

Fa = Final absorbance

d. Number of pollen grains per anther

Number of pollen grains produced per anther was counted in both drought stressed and well-watered plants. A finger from a main earhead of five random plants in each genotype where anthers were about to dehiscence the following day were collected. The samples were collected in the morning between 8.30-9.30am in a petri dish and brought

to the laboratory immediately and incubated at 70 °C in an oven for 24 hours. After incubation, one spikelet each was collected from the upper, middle and lower position of the finger separately. Each spikelet was dissected carefully by removing outer glume, lemma and palea. One anther from each position of the spikelet was carefully removed and transferred to a 1.5 ml microfuge tube containing 0.1 ml of 1% tween-20 solution and incubated overnight at room temperature. The tubes were then sonicated at 70 amplitude for 30-45 seconds to release the pollen grains into the solution. The sample was mixed thoroughly to ensure uniform distribution of pollen grains in the solution. From each tube, three replications of 5 µl sample was drawn and dispensed on a glass slide and the total number of pollen grains in 5 µl sample was counted using projection microscope EUROMEX-HOLLAND, Model- CMEX DC.300X at a magnification of 40X and the total number of pollen grains in a total volume of 0.1 ml was determined as number of pollen grains per anther.

e. Pollen sterility (%)

The some pollen grains in the sample which was used to count the number of pollen grains per anther were used to determine the pollen sterility. The fully circular (turgid), non-transparent pollen grains were counted in each sample and were considered as fertile pollen grains while the irregular shaped (flaccid), transparent pollen grains were considered as sterile pollen grains (Mohapatra *et al.*, 2020; Singh *et al.*, 2020a). The total number of fertile and sterile pollen grains in 5 µl sample was counted and the number of sterile pollen grains per anther was determined. The pollen sterility percentage was calculated as below,

$$\text{Pollen sterility (\%)} = \frac{\text{Number of sterile pollen grains per anther}}{\text{Total number of pollen grains per per anther}} \times 100$$

3.3.2.3 Statistical analysis

The mean and standard errors were calculated for RWC, SCMR value, membrane leakage, total number of pollen grains per anther and pollen sterility for drought stressed

and well-watered plants separately. In addition, the data was analysed using two factor (factorial RCBD), where genotypes were considered as a one factor, and two main treatments (*i.e.* well watered and drought stress) were considered as second factor for analysis. The analysis of variance was performed using mean values of the genotypes for recorded traits using SPSS (v16.0.0).

3.4 Expression of a few stress related genes in the reproductive tissues under moisture stress

3.4.1 Plant material

Two contrasting genotypes GE-4719 (drought susceptible) and GE-1234 (drought tolerant) were identified from earlier experiments were selected for the gene expression study.

3.4.2 Stress treatment

The two genotypes GE-4719 and GE-1234 were grown in the greenhouse in the pots during May 2021. Ten plants per genotype were grown in pots (30 ×20 ×15 cm) filled with 05 kg of potting mixture (soil + FYM in 1:1 ratio) under greenhouse conditions. The plants were grown till reproductive stage without any stress by regular watering to pots. Out of ten plants, five plants from each genotype were subjected to drought stress during the early reproductive stage and remaining five pots were watered regularly; served as control. To induce drought stress, the watering was stopped on 47th day after sowing (DAS) for 20 days in both the genotypes. The water status of the soil in the stressed pots was monitored using soil moisture indicator developed by Sugarcane Breeding Institute, Indian Council of Agricultural Research (ICAR-SBI), Coimbatore and marketed by Tech Source Solution, Bengaluru. The sensor rods of the instrument were inserted to a depth of 15-20 cm as the roots of finger millet are detained within 30 cm from the soil surface. The switch of the indicator was pressed and held till the LED stabilized at a particular colour, thus indicating soil moisture content as given in table 3.7.

Table 3.7 Reading soil moisture content using soil moisture indicator as described by ICARSBI Coimbatore

Colour of the LED (10)	Soil moisture status	Inference
Blue (3 levels)	Ample moisture	No need for irrigation at all
Green (3 levels)	Sufficient moisture	Immediate irrigation may not be necessary
Orange (1 levels)	Low moisture	Irrigation advisable
Red (3 levels)	Very low moisture	Immediate irrigation

Based on the colour of the LED glow, soil moisture content was determined and the stress pots were given little water for the survival of plants during drought stress. For drought stressed plants, the colour of the LED glow was maintained at orange throughout the day. When the LED glow reached first red, 100 ml of water was given to the plants to ensure their survival, yet maintaining low soil moisture content as indicated by orange LED glow. This status was maintained during the whole stress period of 20 days. On the other hand, for well watered plants, the pots were watered regularly to maintain the LED glow at blue throughout the experiment.

3.4.3 Collection of developing anthers

For RNA extraction, the immature earheads were collected on 60th DAS in case of WW plants of both the genotypes. However, drought stress has delayed flowering in both the genotypes. Therefore, immature earheads were removed for RNA extraction on 65th DAS from GE-4719 and on 67th DAS from GE-1234. The collected earheads were immediately kept in liquid nitrogen and brought to the laboratory. The main earhead was used for collection of immature earhead. Two plants each from each genotype were used for both the treatments. The spikelets from each immature earhead were dissected under laminar air flow by maintaining them under ice-cold conditions. The developing anthers were separated carefully from the each spikelet with the help of scalpel and sharp pointed forceps. The anthers from spikelet of the same earhead were pooled for RNA extraction.

For each genotype, two biological and two technical replications for both WW and DS conditions were prepared for RNA extraction. The RNA extraction was performed using NucleoSpin® RNA Plant kit as per manufacturer's instructions.

3.4.4 RNA extraction from developing anthers

The total RNA from 20 mg (approx.) of freshly collected anthers for each treatment and genotype was extracted using NucleoSpin® RNA Plant kit. The working place and materials (pestles, mortars, microfuge tubes and micro-tips etc.) used for RNA isolation were treated with 0.1% diethylpyrocarbonate (DEPC) to avoid degradation and contamination of RNA by RNase. The total RNA extracted by following the manufacturer's instructions.

RNA isolation protocol

1. Homogenise sample: 20 mg tissues were grinded under liquid N₂.
2. Lyse cells: Buffer RA1 350 µl and 3.5 µl β-mercaptoethanol (β-ME) added to 100 mg tissue and vortexed vigorously.
3. Filtrate lysate: The NucleoSpin® Filter (violet ring) filter were placed in NucleoSpin® Filter in a Collection Tube (2 ml) to clear the lysate by filtration and were centrifuged for 1 min at 11,000 xg. The filtrate was transferred to a new 1.5 ml microcentrifuge tube.
4. Adjust RNA binding conditions: The NucleoSpin® Filter discarded and 350 µL ethanol (70 %) added to the homogenised lysate and mixed by pipetting up and down (5 times). Collected flow-through transferred into a new 1.5 ml microcentrifuge tube, 350µl ethanol (70 %) added and mixed by vortexing (2 x 5 seconds).
5. Bind RNA: The lysate loaded in a Collection Tube of NucleoSpin® RNA Plant Column (light blue ring) for each preparation and centrifuged for 30 seconds at 11,000 xg. Then the column was placed in a new Collection Tube (2 ml).

6. Desalt silica membrane: 350 µl MDB (Membrane Desalting Buffer) added into the NucleoSpin® RNA Plant Column (light blue ring) placed in Collection Tube (2 ml) and centrifuged at 11,000 *xg* for 1 min to dry the membrane. Salt removal will make the following rDNase digest much more effective.
7. Digest DNA: DNase reaction mixture prepared in a sterile 1.5 ml microcentrifuge tube: For each isolation, 10 µl reconstituted rDNase added to 90 µl Reaction Buffer for rDNase and mixed by flicking the tube. 95µl DNase reaction mixture applied directly onto the center of the silica membrane of the column and was incubated at room temperature for 15 min.
8. Wash and dry silica membrane
 - 1st wash: 200 µl Buffer RAW2 added to the NucleoSpin® RNA Plant Column and centrifuge for 30 seconds at 11,000 *xg*. The column into a new Collection Tube (2 ml) was placed.
 - 2nd wash: 600 µl Buffer RA3 added to the NucleoSpin® RNA Plant Column. Centrifuge for 30 seconds at 11,000 *xg*. The flow-through discarded and the column placed back into the Collection Tube.
 - 3rd wash: 250 µl Buffer RA3 added to the NucleoSpin® RNA Plant Column and centrifuged for 2 min at 11,000 *xg* to dry the membrane completely. The column was placed into a nuclease-free Collection Tube (1.5 ml).
9. Elute RNA: The RNA eluted in 60 µl RNase-free H₂O and centrifuged at 11,000 *xg* for 1 min.

3.4.5 Reverse transcription PCR

Complementary DNA (cDNA) was synthesized from isolated RNA as a template using PrimeScript™ RT Reagent Kit (Perfect Real Time) supplied by TaKaRa Bio. Inc. as per manufacturer's instructions. The following reaction mixture on ice was prepared for reverse transcription,

Reagent per reaction	Volume	Final concentration
5X PrimeScript Buffer (for Real Time)	2.0 µl	1X
PrimeScript RT Enzyme Mix I	0.5 µl	-
Oligo dT Primer (50 µM)	0.5 µl	25 pmol
Random 6 mers (100 µM)	0.5 µl	50 pmol
total RNA (500 ng)	1.0 µl	500ng
RNase Free dH ₂ O	5.5 µl	-
Total	10 µl	-

2. The reaction mixture was incubated in a thermal cycler under the following condition.

37 °C = 15 min (Reverse transcription)

85 °C = 5 sec (Inactivation of reverse transcriptase with heat treatment)

4 °C = Hold

The cDNA concentration of all the samples were measured using Biophotometer (Eppendorf plus) and normalized for further studies.

3.4.6 Semi quantitative PCR of selected genes

For expression analysis, the genes related to pollen development from available literature viz. Sucrose synthase1 (*SUS1*) (Kakumanu *et al.*, 2012), Cell wall invertase (*INCW*), Invertase-1 (*IVR1*) (Ji *et al.*, 2010b), GRMZM2G162968 and GRMZM5G844309 (Zhang *et al.*, 2017) were selected for the present study. The relative gene expression of the selected genes was studied in DS and WW anthers of both the genotypes. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene was used as reference gene (Zhang *et al.*, 2017). The gene specific primers were synthesized from Eurofins, Genomics India Pvt. Ltd. listed in table 3.8.

To standardize the number of cycles for *SUS1*, *INCW*, *IVR1*, GRMZM2G162968 and GRMZM5G844309 genes pre-PCR of different cycles (24, 26, 28, and 30) was

performed and 30 cycles were selected. The amplicons of 30 cycles were clearly visible on an agarose gel and easily quantified in comparison to the reference gene. Semi quantitative PCR for gene specific primers was carried out in master cycler gradient (Eppendorf, Hamburg, Germany) (Table 3.9). The standard programme was the same as mentioned in the table 3.6 except for number of cycles and annealing temperature.

Table 3.8 List of gene of gene specific primers used in gene expression studies

Sr. No.	Gene	Gene specific primer (5'-3')	Annealing temperature (°C)	Amplicon size (bp)
1	<i>SUS1</i> (Sucrose synthase1)	F- CCTGTCCATCTACTTCCCGTA R- GATTGGCTTGTTCTGTCGT	55 °C	280
2	<i>INCW</i> (Cell wall invertase)	F- CTGAGAGCAAAGTCGGTCAC R- CTTCAGGCCCCGTTTCATGAC	55 °C	250
3	<i>IVR1</i> (Invertase-1)	F- GTGCTCCTGCTCGTCCTC R- AGCCAACAACCTACCGTTTCG	55 °C	160
4	GRMZM2G162968 (Uncharacterised gene)	F- TTGCCCGCCGTATCTTTTCT R- CGCTTATCTTCGGCAAAGCC	53 °C	220
5	GRMZM5G844309 (Uncharacterised gene)	F- AGATGCAGAGATGTCCGTGC R- GCTCCCTCTTCTGAAAGCAGT	53.5 °C	290
6	<i>GAPDH</i> (Reference gene)	F- CCCTTCATCACCACGGACTAC R- AACCTTCTTGGCACCACCT	55 °C	300

Table 3.9 The components of PCR reaction mixture used for gene expression studies

Components	Concentration per µl	Quantity (µl/reaction)
Primers (F + R) (Eurofins, Genomics India Pvt. Ltd.)	10 pmol	0.8µl
dNTP's 2.5 mM each(GeNei™, Bangalore)	10 mM	0.4 µl
<i>Taq</i> buffer A with 15mM MgCl ₂ (GeNei™, Bangalore)	10 X	1.0 µl
<i>Taq</i> polymerase (GeNei™, Bangalore)	1U	0.3 µl
cDNA	100 ng	1.0 µl
Nuclease free water (Sigma Aldrich)	-	6.5 µl
Total	-	10 µl

The amplification products were resolved by electrophoresis on 1.5% agarose gel with 100bp DNA ladder and visualized by using Alpha digidoc 1000 gel documentation system (Alpha Innotech Corporation, USA). The relative expression of *SUS1*, *INCW*, *IVR1*, GRMZM2G162968 and GRMZM5G844309 genes were compared between drought tolerant and susceptible genotype and was calculated by comparing the band intensity of amplified products of the gene specific primer with band intensity of *GAPDH* gene using image J software (Schneider *et al.*, 2012). The gene to *GAPDH* ratio was calculated based on the band intensity values obtained from the image J software.

IV RESULTS AND DISCUSSION

Finger millet is a hardy crop in nature due to its tolerance to biotic and abiotic stresses. It is mainly grown under rainfed conditions where occurrence of drought stress for 15-20 days is a common phenomenon which limits the productivity. However, studies have showed that drought stress for 25 to 30 days invariably occurs during reproductive stages of crop growth every year and decreases the grain yield significantly (Maqsood and Azam Ali, 2007). Hence, there is a greater need of genetic resources with diverse germplasm and extent of variability. It is a well known fact that the phenotypic expression of any character is resultant of the interactions between genotype and environment, therefore to study the genetic nature of a trait, core/mini core/association mapping panel populations serve as a valuable source. It will also enable greater understanding of the nature of a gene or QTL controlling the trait of interest. Development and precise phenotyping of mapping population for desired traits are prerequisites for identifying DNA markers associated with genomic regions controlling target traits especially for seed yield and drought tolerance related traits in finger millet. In this direction, the present study was envisaged to identify QTLs for seed yield and drought tolerance related traits through association mapping panel of 350 genotypes and expression analysis of selected genes under drought environment in contrasting finger millet genotypes.

The present experimental data set has a very large number of variables, genotypes and four environments (well watered and drought stress environments in 2019-20 and 2020-21. This makes it difficult to assess the importance of variables and genotypes across environments individually. When data set contains a large number of variables and genotypes, there is often a serious amount of overlap between variables and genotypes. Therefore, we have applied different statistical approaches to reduce the large number of variables into a small number of important variables which makes clear interpretations as well as selection of trait donor genotypes in individual environment as well as across environments. In this direction, the results of the present study are discussed under following headings:

4.1 Evaluation of association mapping panel and identification of drought tolerant genotypes

4.1.1 Genetic variability for seed yield and yield related traits

4.1.2 Correlation between seed yield and agro-morphological traits

4.1.3 Stepwise multiple linear regression analysis to determine important traits for productivity and drought tolerance

4.1.4 Identification of important traits and genotypes for seed yield and drought tolerance

4.1.4.1 Principal component analysis (PCA)

4.1.4.2 Genotype by Yield*Trait (GYT) analysis

4.1.4.3 Genotype plus Genotype-by-Environment (GGE) analysis

4.1.5 Determination of drought tolerance indices (DTI) and selection of genotypes

4.2 Genome wide association mapping and identification of QTLs for seed yield and traits associated with drought tolerance

4.2.1 Genetic diversity and population structure analysis using SSR and SNP markers

4.2.2 Genome wide association analysis using SNP markers

4.3 Effect of early reproductive growth stage moisture stress on flowering, quality and quantity of pollen grains in contrasting finger millet genotypes

4.4 Expression of a few stress related genes in the reproductive tissues under moisture stress in contrasting genotypes

4.1 Evaluation of association mapping panel and identification of drought tolerant genotypes

4.1.1 Genetic variability for seed yield and yield related traits

4.1.1.1 Analysis of variance (ANOVA)

The evolution of crop plant primarily depends on the existence of variability and the diversity in the population. Variability in broad sense refers to the presence of

differences among the individuals of a plant population, which may arise due to differences either in the genetic constitution of an individual within a population or in the agro-climatic environment in which they are grown. Since, the beginning of the systematic plant breeding, the existence of natural genetic variability and diversity between individuals within a population has been extensively studied and harnessed in the improvement of crop species for its wider adaptability, higher yield as well as resistance to biotic and abiotic stress (Bhandari *et al.*, 2017).

Analysis of variance (ANOVA) for the sixteen quantitative traits revealed significant mean sum of squares attributable to ‘Genotypes’, ‘Checks’ and ‘Checks vs Genotypes’ for most of the traits evaluated under WW and DS conditions during 2019 (Table 4.1; Plate 4.1a) and 2020 (Table 4.2; Plate 4.1b). Highly significant variability was observed in the genotypes for days to flowering, plant height at reproductive stage, plant height at harvest, total number of tillers per plant, number of productive tillers per plant, culm length, number of earheads per plant, main earhead length, main earhead weigh, total earhead weight per plant, finger numbers per earhead, finger length, finger width, test weight, total biomass, seed yield per plant under WW conditions in both the years. Except plant height at reproductive stage, plant height at harvest, culm length and finger width during DS19 condition and except plant height at harvest, total number of productive tillers per plant, culm length and total earhead weight per plant during DS20. The remaining traits showed significant differences among the genotypes. The genotypes recorded significant differences for majority of the traits under both WW and DS conditions in both the years suggesting the usefulness of the assembled population for further studies to identify drought tolerant genotypes and useful traits for suitable to DS and WW conditions in finger millet. Earlier studies by Negi *et al.* (2017), Sharma *et al.* (2018), Devaliya *et al.* (2018), Singamsetti *et al.* (2018), Kandel *et al.* (2019), Sapkal *et al.* (2019), Chavan *et al.* (2020) and Patil *et al.* (2021) recorded wide range of variation and significant differences in a diverse set finger millet genotypes for plant height, total number of tillers per plant, number of productive tillers per plant, numbers of fingers per earhead, main ear head length, finger length, finger width, total biomass, test weight and grain yield per plant. The results are further supported by the studies in different crops

Table 4.1 Analysis of variance for seed yield and yield related traits in finger millet association mapping panel under well watered (WW) and drought stress (DS) condition during summer 2019

Meansumofsquares(MSS)2019																		
Source of variation	df		DTF	PHS	PHH	TLP	PTLP	CL	EHP	EHL	EHW	EHWP	FNEH	FL	FW	TW	BM	SYP
Blocks	4	WW	0.92	88.51	61.92	0.33	0.32	2.40	0.50*	0.21	2.29	23.21	0.13	0.52	0.002	0.93**	371.94	2.17
		DS	0.80	89.17	238.78	0.78**	0.53**	5.31	0.89**	0.20	7.60	16.16	0.76	0.6	0.02	0.05	90.41	14.50
Entries (Genotypes + Checks)	355	WW	43.62**	171.37**	202.63**	1.38**	0.89**	8.39**	1.86**	3.82**	5.17	24.27	2.37**	2.68**	0.01**	0.4**	2148.76**	17.33
		DS	69.32**	110.58	168.16	0.95**	0.44**	7.17	0.99**	3.31**	3.17	17.43	2**	2.13**	0.03	0.29**	1396.81**	10.74
Checks	5	WW	67.71**	72.05	205.41*	0.59*	0.30	27.1**	0.21	3.66**	7.32*	15.50*	3.45**	1.63**	0.02**	0.55*	1935.45**	12.75
		DS	99.31**	306.54**	172.08	0.17	0.24	12.03	0.14	3.75**	2.43*	40.63*	2.30*	2.44**	0.06*	0.27*	1703.97**	15.48
Genotypes	349	WW	42.79**	173.26**	200.84**	1.33**	0.86**	8.04**	1.8**	3.52**	4.68*	23.67*	2.34**	2.46**	0.01**	0.39*	2119.09**	17.22*
		DS	68.45**	108.06	165.06	0.94**	0.44**	7.07	0.97**	3.07**	2.97*	16.52*	1.97**	1.94**	0.03	0.28**	1356.12**	10.39*
Checks vs. Genotypes	1	WW	210.78**	6.22	814.74**	22.25**	11.90**	34.7**	33.17**	111.92**	164.94**	280.18**	8.92**	84.51**	0.07**	2.37**	13569.85**	78.10*
		DS	222.72**	12.20	1230.67**	6.52**	2.45**	16.98	9.12**	84**	75.72**	217.38**	11.74**	69.49**	0.05	2.52**	14061.53**	109.08**
Error	20	WW	0.80	44.99	56.39	0.20	0.14	3.22	0.15	0.58	2.93	22.41	0.25	0.34	0.0014	0.16	437.71	16.12
		DS	0.78	65.39	100.81	0.14	0.11	5.07	0.13	0.57	3.04	15.58	0.57	0.49	0.02	0.08	256.62	5.91

* $P \leq 0.05$; ** $P \leq 0.01$

Days to flowering (DTF), Plant height at reproductive stage (PHS), Plant height at harvest (PHH), Total number of tillers per plant (TLP), Total number of productive tillers per plant (PTLP), Culm length (CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

Table 4.2 Analysis of variance for seed yield and yield related traits in finger millet association mapping panel under well watered (WW) and drought stress (DS) condition during summer 2020

Source of variation	df	Mean sum of squares (MSS) 2020																
			DTF	PHS	PHH	TLP	PTLP	CL	EHP	EHL	EHW	EHWP	FNEH	FL	FW	TW	BM	SYP
Blocks	6	WW	0.63*	185.75**	136.65	0.15	0.43	1694*	0.51	0.61	0.32	13.45	0.25	0.65	0.11	0.43**	171.51	1.36
		DS	11.45	27.39	111.48	0.22	0.71	797	0.69	1.21	2.14	11.22	0.38	0.64	0.01	0.06	163.59	3.94
Entries (Genotypes + Checks)	354	WW	36.91**	165.91**	237.78**	0.9**	1.23**	1063*	1.77**	4.01**	5.01**	48.47**	1.76**	3.02**	0.09	0.39**	1937.2**	18.68**
		DS	61.59**	100.8**	165.36	0.6**	0.94	12.33	2.74**	3.39**	3.52**	39.49	1.52*	2.51**	0.04**	0.36**	1368.81**	14.93*
Checks	4	WW	545**	521	609.38**	0.56*	0.81*	12.35	1.54*	7.95**	11.27**	102.19**	4.24**	7.07**	0.08	0.61**	2658.25**	72.26**
		DS	65.67**	375.63**	138.9	0.06	0.28	0.34	1.2	9.41**	12.83**	92.33*	4.2**	5.6**	0.01	0.26**	1644.94**	38.24**
Genotypes	349	WW	36.13**	167.63**	233.06**	0.88**	1.23**	10.3*	1.75**	3.69**	4.67**	45.64**	1.71**	2.69**	0.09	0.38**	1893.82**	16.78**
		DS	59.93**	97.91**	165.23	0.6**	0.95	11.84	2.73**	3.05**	3.21*	37.22	1.47*	2.19**	0.04**	0.35**	1321.08**	12.59*
Checks vs. Genotypes	1	WW	235.9**	20.71	398.86*	12.05**	5.13**	119.26**	7.9**	101.52**	99.73**	818.86**	8.91**	99.17**	0.23	3.41**	14191.49**	465.78**
		DS	627.25**	10.43	314.86	6**	0.74	232.57**	11.69**	96.45**	76.09**	620.83**	7.77**	100.37**	0.01	4.74**	16923.52**	738.71**
Error	24	WW	0.2	42.82	92.72	0.15	0.24	5.4	0.38	0.5	1.43	12.27	0.23	0.46	0.1	0.1	279.01	6.43
		DS	7.64	38.25	130.05	0.11	0.69	8.66	1.14	1.09	1.49	2.27	0.75	0.79	0.01	0.02	226.3	8.06

* P ≤ 0.05; ** P ≤ 0.01

Days to flowering (DTF), Plant height at reproductive stage (PHS), Plant height at harvest (PHH), Total number of tillers per plant (TLP), Total number of productive tillers per plant (PTLP), Culm length (CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

involving drought stress and well watered environments for example viz. Singh *et al.* (2014) in diverse set of genotypes in wheat, Mishra *et al.* (2019) in rice and Haseeb *et al.* (2020) in maize where highly significant variation among the genotypes recorded for the traits studied under well irrigated and drought stress conditions. The choice of mapping population (association mapping panel) selected for the investigation of QTL identification for seed yield and drought tolerance related traits is appropriate. This was further supported by the fact that the range observed was also wide for all the sixteen agro-morphological traits evaluated under WW and DS conditions.

4.1.1.2 Mean, range and components of variation

The nature and magnitude of variation in all sixteen quantitative traits were assessed by mean, range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance as *per cent* mean (GAM) for each year under WW and DS conditions (Table 4.3 and 4.4).

The existence of wide range for each trait under WW and DS condition indicated the presence of considerable variation among the genotypes. The seed yield per plant varied from 4.38 to 25.96 g with a mean of 14.33 g during WW19 and from 5.98 to 30.00 g with a mean of 16.03 g in WW20 conditions. On the other hand, seed yield per plant varied from 2.58 to 19.06 g with a mean of 8.39 g in DS19 and from 2.64 to 22.26 g with a mean of 10.32 g in DS20 conditions. The results indicated that the mean of seed yield per plant consistently reduce under DS environment compared to WW condition in both the years. For example, the mean value for seed yield per plant recorded was 14.33 g under WW19 which was reduced to 8.39 g under DS 19 causing yield reduction of 41.45 *per cent*. On the other hand, the mean value for seed yield per plant recorded was 16.03 g under WW20 which was reduced to 10.32 g under DS20 with the yield reduction of 35.62 *per cent* (Table 4.3 and 4.4). Similarly, drought had a significant effect on other traits like total number of tillers per plant, number of productive tillers per plant, plant height at reproductive stage, plant height at harvest, culm length, number of earheads per plant, main earhead length, main earhead weight, total earhead weight per plant, finger numbers per earhead, finger length, finger width, test weight and total biomass recorded

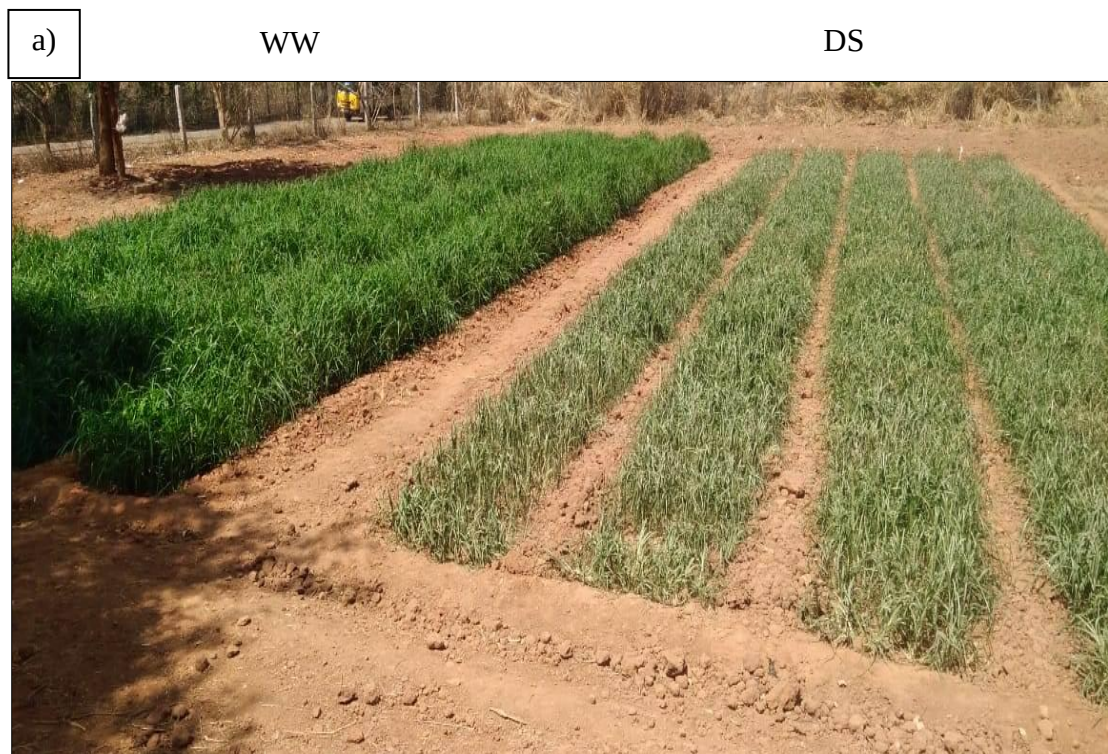


Plate 4.1 Field view of well watered (WW) and drought stressed (DS) plot of 350 finger millet genotypes . a) Summer-2018-19 b) Summer 2019-20

Table 4.3 Estimates of mean, range, variability, heritability and genetic advance as *per cent* of mean for grain yield and yield components in finger millet genotypes under well watered (WW) conditions during 2019 and 2020

Traits	Mean $\pm$ SE		Range (min-max)		Coefficient of variability				Heritability (%) (h^2_{ls})		Genetic advance on mean % (GAM)	
	2019	2020	2019	2020	GCV (%)		PCV (%)		2019	2020	2019	2020
					2019	2020	2019	2020				
DTF	77.46 $\pm$ 0.14	69.10 $\pm$ 0.13	61.00-96.00	52.00-90.00	8.40	8.83	8.48	8.85	98.14	99.45	17.17	18.17
PHS	75.67 $\pm$ 0.19	76.93 $\pm$ 0.19	34.00-112.20	35.40-112.00	14.55	14.86	16.91	17.23	74.03	74.46	25.82	26.46
PHH	88.96 $\pm$ 0.20	97.30 $\pm$ 0.21	44.80-135.40	49.60-138.60	13.32	12.79	15.71	16.48	71.92	60.21	23.31	20.48
TLP	3.93 $\pm$ 0.06	4.63 $\pm$ 0.05	2.40-8.60	2.40-9.20	26.27	18.14	28.47	19.89	85.15	83.18	50.01	34.13
PTLP	3.50 $\pm$ 0.05	3.81 $\pm$ 0.06	2.20-7.40	1.40-9.60	23.18	24.9	25.31	27.84	83.82	80.05	43.77	45.97
CL	24.33 $\pm$ 0.09	29.90 $\pm$ 0.10	17.20-33.40	19.20-39.20	8.99	7.79	11.61	11.30	59.97	47.60	14.37	11.09
EHP	4.01 $\pm$ 0.06	3.88 $\pm$ 0.06	2.40-10.00	1.40-9.60	29.65	28.41	31.01	32.09	91.43	78.34	58.49	51.87
EHL	8.53 $\pm$ 0.07	8.25 $\pm$ 0.07	4.32-16.62	3.82-18.98	20.33	22.38	22.24	24.07	83.63	86.44	38.36	42.92
EHW	6.47 $\pm$ 0.08	6.02 $\pm$ 0.08	1.40-12.88	1.08-13.80	21.92	32.74	35.81	39.32	37.48	69.34	27.69	56.24
EHWP	19.15 $\pm$ 0.12	19.94 $\pm$ 0.14	6.78-32.64	4.20-41.26	25.78	28.82	25.05	33.70	75.33	73.12	52.75	50.84
FNEH	7.53 $\pm$ 0.07	7.42 $\pm$ 0.06	4.00-15.00	4.20-14.00	19.26	17.12	20.40	18.38	89.15	86.76	37.52	32.89
FL	6.30 $\pm$ 0.07	6.44 $\pm$ 0.07	3.32-14.24	3.14-15.54	23.61	24.07	25.45	26.41	86.03	83.10	45.18	45.27
FW	0.94 $\pm$ 0.02	0.88 $\pm$ 0.03	0.52-1.14	0.40-1.66	10.60	10.45	11.32	32.80	87.76	85.69	20.49	19.23
TW	4.28 $\pm$ 0.04	4.29 $\pm$ 0.04	2.24-6.23	2.31-6.14	11.07	12.09	14.44	14.19	58.73	72.63	17.50	21.26
BM	234.32 $\pm$ 0.36	236.98 $\pm$ 0.35	123.80-399.40	133.0-367.30	16.96	16.50	19.04	17.87	79.34	85.27	31.16	31.44
SYP	14.33 $\pm$ 0.11	16.03 $\pm$ 0.11	4.38-25.96	5.98-30.00	17.31	19.72	29.03	25.11	66.35	61.67	33.8	31.94

Days to flowering (DTF), Plant height at reproductive stage(PHS), Plant height at harvest(PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

Table 4.4 Estimates of mean, range, variability, heritability and genetic advance as per cent of mean for grain yield and yield components in finger millet genotypes under drought stress (DS) conditions during 2019 and 2020

Traits	Mean $\pm$ SE		Range (min-max)		Coefficient of variability				Heritability (%) (h^2_{bs})		Genetic advance on mean % (GAM)	
					GCV (%)		PCV (%)					
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
DTF	81.39 $\pm$ 0.44	70.31 $\pm$ 0.41	62.00-103.00	52.00-90.00	10.10	10.28	10.16	11.00	98.86	87.25	20.72	19.80
PHS	42.16 $\pm$ 0.55	42.39 $\pm$ 0.53	19.19-81.45	22.80-73.40	15.49	18.23	24.65	23.35	39.49	60.94	20.08	29.35
PHH	69.69 $\pm$ 0.69	80.02 $\pm$ 0.69	27.60-107.50	46.60-108.40	11.48	7.41	18.41	16.05	38.92	21.29	14.78	7.05
TLP	3.27 $\pm$ 0.05	4.02 $\pm$ 0.04	1.30-10.50	1.60-9.00	27.44	17.26	29.8	19.21	84.76	80.76	52.11	32.01
PTLP	2.94 $\pm$ 0.04	3.87 $\pm$ 0.05	1.35-6.05	1.00-8.90	19.44	13.23	22.46	25.16	74.91	27.64	34.70	14.35
CL	19.96 $\pm$ 0.14	26.14 $\pm$ 0.18	10.50-29.40	14.60-33.20	7.07	6.81	13.31	13.14	28.22	26.84	7.75	7.28
EHP	3.40 $\pm$ 0.05	4.47 $\pm$ 0.09	1.16-7.76	1.00-9.23	27.03	28.27	29.11	37.07	86.20	58.14	51.77	44.46
EHL	7.27 $\pm$ 0.09	7.15 $\pm$ 0.09	3.80-15.11	3.76-18.04	21.65	19.53	23.99	24.35	81.45	64.30	40.31	32.31
EHW	3.91 $\pm$ 0.09	4.17 $\pm$ 0.10	0.15-11.02	0.70-9.34	29.78	31.19	43.85	42.67	83.90	53.43	42.95	47.04
EHWP	12.67 $\pm$ 0.22	14.33 $\pm$ 0.33	1.35-24.96	2.24-35.06	27.65	26.46	31.96	42.36	35.73	39.01	33.78	34.09
FNEH	6.56 $\pm$ 0.07	6.28 $\pm$ 0.06	3.10-12.60	3.20-10.20	17.97	13.47	21.35	19.28	70.82	48.81	31.20	19.41
FL	5.31 $\pm$ 0.07	5.67 $\pm$ 0.08	1.20-12.80	2.80-15.70	22.48	20.77	26.05	25.99	74.49	63.88	40.03	34.25
FW	0.76 $\pm$ 0.01	0.85 $\pm$ 0.01	0.20-1.07	0.42-2.31	12.33	18.11	21.68	22.83	32.35	62.90	14.46	29.63
TW	3.89 $\pm$ 0.03	3.92 $\pm$ 0.03	0.07-6.05	2.09-6.36	11.66	14.55	13.68	15.07	72.59	93.12	20.49	28.96
BM	197.99 $\pm$ 1.97	199.64 $\pm$ 1.94	97.40-322.10	100.90-310.20	16.72	16.55	18.56	18.18	81.08	82.87	31.05	31.08
SYP	8.39 $\pm$ 0.17	10.32 $\pm$ 0.19	2.85-19.06	2.64-22.26	25.13	20.49	38.26	34.16	43.14	35.97	34.04	25.35

Days to flowering (DTF), Plant height at reproductive stage(PHS), Plant height at harvest(PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

consistent reduction in mean values under DS conditions as compared to WW conditions in both the years (Fig. 4.1a and 4.1b). The results indicated that, drought stress at initial reproductive stage affected the performance of the genotypes for yield and related component traits. Overall, significant variability was recorded among the genotypes for all the 16 quantitative traits in both WW and DS conditions and both the years. It was also observed that, the seed yield and its contributing traits were significantly affected by drought stress. Krishna *et al.* (2021) observed decrease in the seed yield (36.6%), total earhead weight (33.0%) and mean earhead weight (26.1%) under drought stress conditions in finger millet. The adverse impact of drought stress on reproductive growth resulted in the significant reduction in seed yield and related traits under drought stress conditions in finger millet (Krishnasastriy *et al.*, 1982; Dinesh Kumar *et al.*, 1987; Krishna, 2010). It has been recorded that, drought stress reduces the seed yield up to 18 to 25 *per cent* in finger millet (Reddy *et al.*, 2020). The yield reduction due to drought stress during early reproductive stages was also reported in other cereal crops (Dolferus *et al.*, 2011; Fischer, 2011; Liu *et al.*, 2015). Under drought stress conditions, decrease in tissue turgor leading to decreased cell division, cell expansion and reduced internode length, plant height and grain yield has been reported in finger millet (Suma 2014; Manjula, 2016). However, reduction in seed yield and yield traits depends on stress severity, duration and stage of the crop (Demirevska *et al.* 2009; Caliz *et al.*, 2015). Overall in the present study, drought stress significantly affected the growth and development of finger millet ultimately resulted in reduction of seed yield and yield related traits. The results also demonstrated that, the water stress treatment given to study the genetic variability and selection of traits and genotype suitable for drought stress environment is dependable.

4.1.1.2a Genotypic and phenotypic coefficient of variation

The estimates of GCV for total number of earheads per plant (29.65%, 28.41%) and main earhead weight (21.92%, 32.74%) were found to be highest among all the 16 agro-morphological traits under WW19 and WW20 conditions respectively (Table 4.3). Higher estimate of GCV (*i.e.* >20%) was recorded consistently for both WW19 and WW20 for total number of productive tillers per plant (23.18%, 24.90%), number of

earheads per plant (29.65%, 28.41%), main earhead length (20.33%, 22.38%), main earhead weight (21.92%, 32.74%), total earhead weight per plant (25.78%, 28.82%) and finger length (23.61%, 24.07%). Moderate estimates of GCV (10 to 20%) was observed for plant height at reproductive stage (14.55%, 14.86%), plant height at harvest (13.32%, 12.79%), finger numbers per earhead (19.26%, 17.12%), finger width (10.60%, 10.45%), test weight (11.07%, 12.09%), total biomass (16.96%, 16.50%) and seed yield per plant (17.31%, 19.72%) in both the years under WW19 and WW20 respectively. Lowest GCV (less than 10%) was recorded for days to flowering (8.40%, 8.83%) in both the years followed by culm length (8.99%, 7.79%). It is interesting to note that, the GCV observed for different traits recorded the same trend under WW conditions in both the years (Table 4.3) suggesting the influence year is not very high on the genotypic coefficient variability for 16 quantitative traits.

Under drought stress conditions, the highest estimates of GCV were recorded for main earhead weight (29.78%, 31.19%) among 16 agro-morphological traits evaluated under DS19 and DS20 conditions respectively (Table 4.4). Higher GCV estimated (*i.e.* >20%) were recorded consistently in both the years for number of earheads per plant (27.03%, 28.27%), main earhead weight (29.78%, 31.19%), total earhead weight per plant (27.65%, 26.46%), finger length (22.48%, 20.77%) and seed yield per plant (25.13%, 20.49%). Moderate estimates of GCV (10 to 20%) were recorded for days to flowering (10.10%, 10.28), plant height at reproductive stage (15.49%, 18.23%), total number of productive tillers per plant (19.44%, 13.23%), finger numbers per earhead (17.97%, 13.47%), finger width (12.33%, 18.11%), test weight (11.66%, 14.55%) and total biomass (16.72%, 16.55%) whereas, the lowest GCV (<10%) estimates were recorded for culm length (7.07%, 6.81%) in both the years. The GCV estimates under DS condition for different traits recorded were constant across years suggesting consistent performance of genotypes for all the traits over two years under DS conditions.

The estimates of PCV for main earhead weight (35.81%, 39.32%) were found to be highest among all 16 agro-morphological traits in both the years under WW19 and WW20 conditions (Table 4.3). The highest estimates of PCV(*i.e.*>20%) were recorded in total number of productive tillers per plant (25.31%,27.84%), number of earheads per

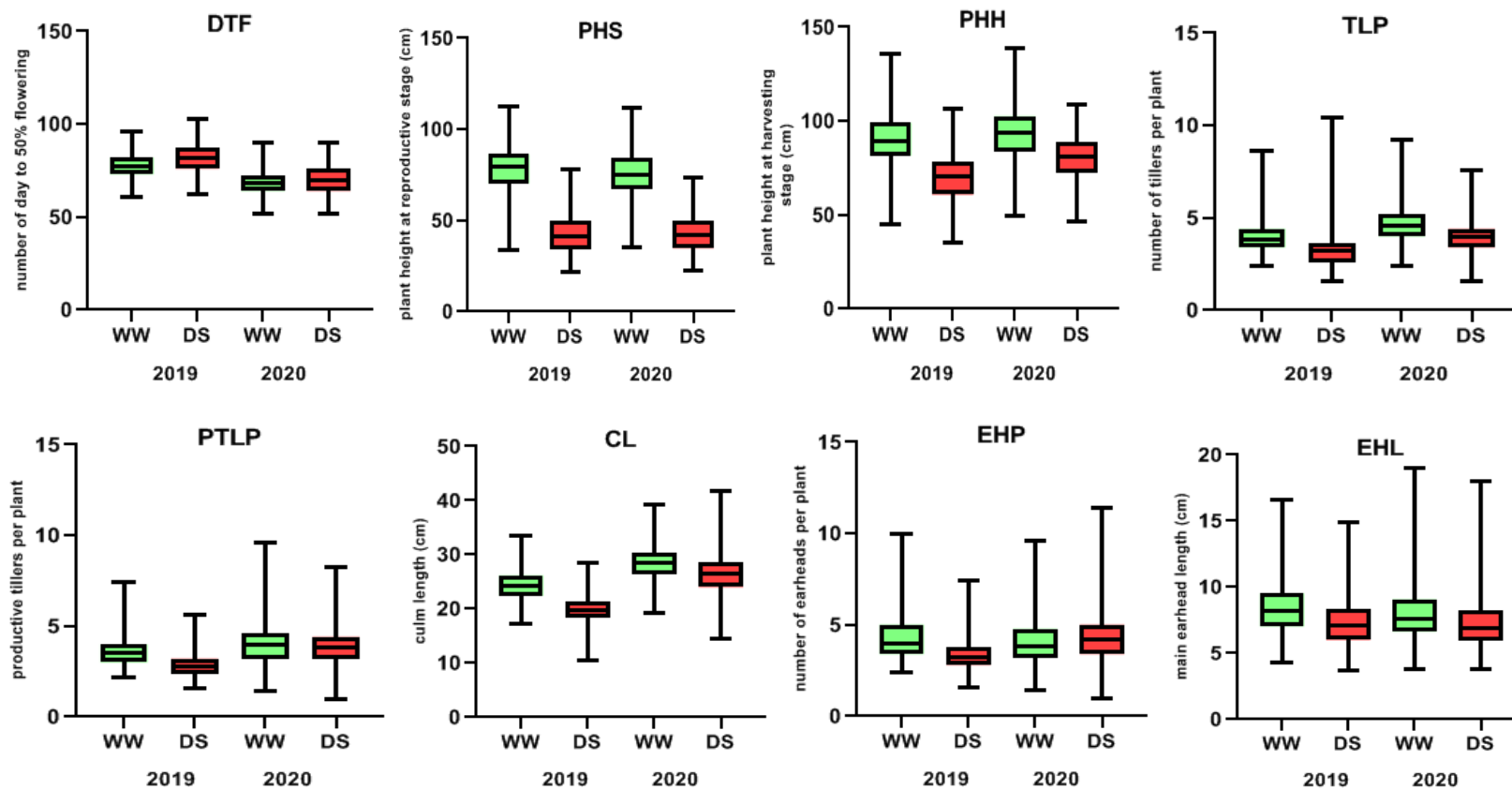


Fig. 4.1a Box plots showing the descriptive statistics viz. median (horizontal line in box), maximum and minimum values of the agro-morphological traits measured under well watered (WW) and drought stress (DS) during 2019 and 2020 on 350 finger millet genotypes. In the figure, DTF-Days to flowering, PHS- Plant height at reproductive stage, PHH- Plant height at harvest, TLP- Total number of tillers per plant, PTLP- Total number of productive tillers per plant, CL-Culm length, EHP- Number of earheads per plant, EHL- Main earhead length

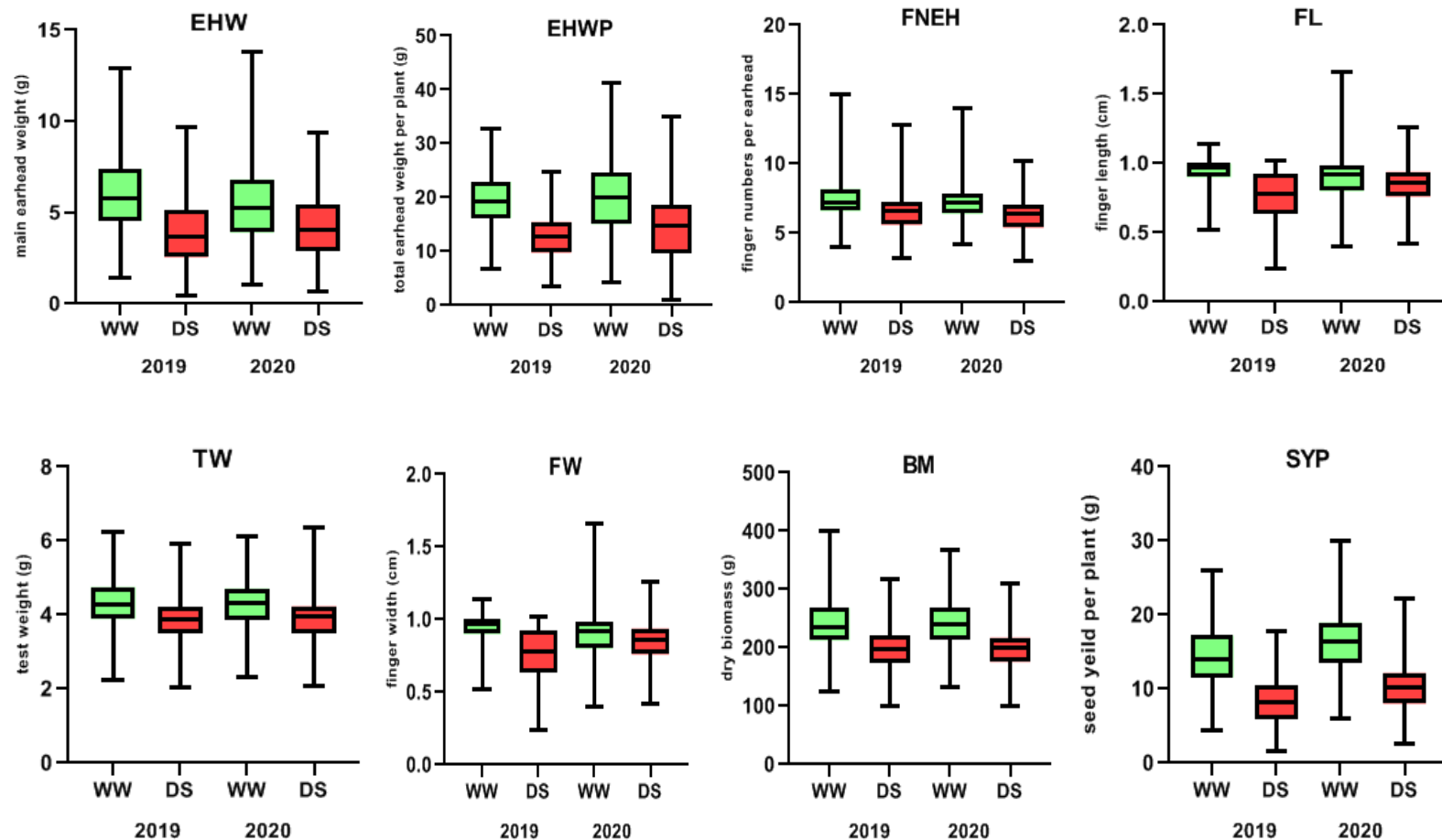


Fig. 4.1b Box plots showing the descriptive statistics viz. median (horizontal line in box), maximum and minimum values of the agro-morphological traits measured under well watered (WW) and drought stress (DS) during 2019 and 2020 on 350 finger millet genotypes. In the figure, EHW- Main earhead weight, EHWP- Total earhead weight per plant, FNEH- Finger numbers per earhead, FL- Finger length, FW- Finger width, TW- Test weight, BM- Total biomass, SYP- Seed yield per plant

plant (31.01%, 32.09%), main earhead length (22.24%, 24.07%), total earhead weight per plant (25.05%, 33.70%), finger length (25.45%, 26.41%) and seed yield per plant (29.03%, 25.11%) under WW19 and WW20 conditions respectively (Table 4.3). Moderate estimates of PCV (10-20%) observed for plant height at reproductive stage (16.91%, 17.23%), plant height at harvest (15.71%, 16.48%), culm length (11.61%, 11.30%), test weight (14.44%, 14.19%) and total biomass (19.04%, 17.87%). Lowest estimate of PCV was recorded day to flowering (8.48%, 8.85%) under WW conditions in both the years. The PCV estimates under WW condition for different traits recorded were more or less similar across the years suggesting consistent performance of genotypes for all the traits over two years under WW conditions.

Under drought stress conditions, the highest estimates of PCV recorded for main earhead weight (43.85%, 42.67%) among 16 agro-morphological traits evaluated under DS conditions in both the years respectively (Table 4.4). The other traits viz. plant height at harvest (24.65%, 23.35%), total number of productive tillers per plant (22.46%, 25.16%), number of earheads per plant (29.11%, 37.07%), main earhead length (23.99%, 24.35%), total earheads per plant (31.96%, 42.36%), finger length (26.05%, 25.99%), finger width (21.68%, 22.83%) and seed yield per plant (38.26%, 34.16%) recorded higher estimates of PCV under DS19 and DS20 conditions respectively. Moderate estimates of PCV were observed for day to flowering (10.16%, 11.00%), plant height harvesting (18.41%, 16.05%), culm length (13.31%, 13.14%), test weight (13.68%, 15.07%) and total biomass (18.56%, 18.18%). The PCV observed for different traits recorded the same trend under DS conditions in both the years suggesting the year influence is not very high on the phenotypic coefficient variability for 16 quantitative traits.

In general, GCV estimates for all sixteen traits studied under WW and DS conditions were lower than the PCV indicating the modifying effect of the environment in association with the traits at genotypic level. The higher phenotypic variance than genotypic variance may be due to the non-genetic factor which played an important role in the manifestation of these traits. Dagnachew *et al.* (2012) found higher phenotypic variance for of plant height, grain yield per plant and days to flowering in finger millet.

Reddy *et al.* (2013) also reported higher phenotypic variance than genotypic variance for yield and yield attributes of finger millet. The values of PCV and GCV showed small differences for main earhead length, total earhead weight per plant, total biomass, finger numbers per erahead, finger length, total number of tillers per plant, total number of productive tillers per plant height at harvest under WW conditions in both the years. On the other hand, main earhead length, finger numbers per erahead, finger length, test weight, total biomass showed small differences for GCV and PCV values under DS conditions in both the years suggesting the traits are stable and less affected by drought condition indicating genotypes can be improved and selected for these traits under stress condition for improvement of drought tolerance. The low difference between GCV and PCV was also reported by Rawat *et al.* (2018) for main earhead length, total earhead weight per plant, total biomass, finger numbers per erahead, finger length indicating that these traits were slightly influenced by the environment.

The GCV and PCV estimates were high (*i.e.* >20%) for the total number of earheads per plant, main earhead length, total earhead weight per plant, finger length, seed yield per plant and total number of productive tillers per plant under DS conditions in both the years indicating that these traits are more variable under DS conditions. Therefore there is a great scope for improvement of these traits by selection among the genotypes for drought tolerance. High GCV and PCV estimates for total earhead weight per plant, finger length, total number of productive tillers per plant, main earhead weight, main earhead weight were also recorded in finger millet genotypes by Kebere *et al.* (2006), John, (2007), Kadam, (2008), Lule *et al.* (2012) Karad and Patil, (2013), Suryanarayana *et al.* (2014), Devaliya *et al.* (2018) under normal conditions. Moderate estimates of GCV and PCV were recorded for plant height at harvest, finger numbers per earhead, test weight and total biomass under both WW and DS condition. The moderate to low estimates was observed for days to flowering in both WW and DS conditions in both the years. The moderate estimate for total biomass, finger numbers per earhea and plant height under normal conditions was reported by Abraham *et al.* (1989), Chunilal *et al.* (1996), Suryanarayana *et al.* (2014), and Devaliya *et al.* (2018).

4.1.1.2b Heritability

Genetic coefficient of variability together with heritability estimates would give the best picture of the amount of advance to be expected in case selection (Burton and Devane, 1953). In particular, broad sense heritability is defined as “the amount of phenotypic variance that is being credited to an effect for the whole genotype, which comprises of, sum of additive, dominance as well as epistatic effects” (Piepho and Mohring, 2007 and Schmidt *et al.*, 2019). Broad sense heritability was ranged from 37.48 (for main earhead weight) to 98.14 *per cent* (for days to flowering) and from 47.60 (for culm length) to 99.45 *per cent* (for days to flowering) under WW19 and WW20 conditions respectively (Table 4.3). Highest estimate of heritability (>60%) was recorded for days to flowering (98.14%, 99.45%) in both the years. The other traits *viz.* plant height at reproductive stage (74.03%, 74.46%), plant height at harvest (71.92%, 60.21%), total number of tillers per plant (85.15%, 83.18%), total number of productive tillers per plant (83.82%, 80.05%), number of earheads per plant (91.43%, 78.34%), main earhead length (83.63%, 86.44%), total earhead weight per plant (75.33%, 73.12%), finger number per earhead (89.15%, 86.76%), finger length (86.03%, 83.10%), finger width (87.76%, 85.69%), total biomass (79.34%, 82.27%), and seed yield per plant (66.35%, 61.37%) also recorded high broad sense heritability estimates in both the years. Moderate heritability was observed for culm length (59.97%, 47.60%) in both the years.

Under drought stress conditions, broad sense heritability was found to be ranged between 28.22 (for culm length) to 98.86 *per cent* (for days to flowering) and 21.29 (for plant height at harvest) to 93.12 *per cent* (for test weight) during DS19 and DS20 conditions respectively (Table 4.4). The highest estimates of broad sense heritability were recorded for day to flowering (98.86%) and test weight (93.12%) in DS19 and DS20 environment in both years. The other traits *viz.* total number of tillers per plant (81.16%, 80.76%), main earhead length (81.45%, 64.30%), finger length (74.49%, 63.88%), test weight (72.59%, 93.12%) and total biomass (81.08%, 82.87%) also recorded a higher estimate of broad sense heritability in both the years. Moderate estimates of heritability were recorded for total earhead weight per plant (35.73%, 39.01%) and seed yield per

plant (43.14%, 35.97%) and the lowest estimates were observed for culm length (28.22%, 26.84%) under DS in both the years.

The heritability estimates were consistently high for days to flowering, total number of tillers per plant, main earhead length, finger length, test weight and total biomass for both WW and DS conditions in both the years. The high heritability estimates for these traits under both WW and DS situation across year indicate the stable inheritance of these traits and the least influence of environmental conditions on these traits suggest that these traits respond to selection. Earlier studies also indicated high heritability for test weight, number of fingers per ear and plant height, test weight, number of productive tillers per plant, seed yield per plant, main earhead length and number of fingers per earhead in diverse set of finger genotypes (Abraham *et al.*, 1989; John, 2007; Shet *et al.*, 2010; Ganapathy *et al.*, 2011; Priyadharshini *et al.*, 2011). Similarly, high estimates of heritability for days to flowering, number of productive tillers per plant, test weight and seed yield per plant was also reported by Nandini *et al.* (2010), Shet *et al.* (2010), Haradari *et al.* (2012), Jayshree and Nagarajaiah (2013), Suryanarayana *et al.* (2014), Saundaryakumari and Singh (2015), Devaliya *et al.* (2018) and Anteneh *et al.* (2019). Dhanalakshmi *et al.* (2013) reported 53.25 *per cent* heritability for number of productive tiller, 65.07 *per cent* for grain yield per plant and 73.70 *per cent* for test weight. Most of the earlier studies were based normal environment conditions. However, in the present study high heritability for majorly was recorded under DS conditions also. Although, there was a reduction in most of the quantitative traits under drought situation, the similar pattern of heritability in WW and DS conditions suggest the large genetic variability in the alleles of all the above traits, which indicate that traits viz. high for days to flowering, total number of tillers per plant, main earhead length, finger length, test weight and total biomass seems least influenced by environmental conditions particularly drought which are the key components for any crop improvement program (Naeem *et al.*, 2015). Least variation between PCV and GCV corresponds to high heritability recorded in main earhead length, number of earheads per plant, total biomass, finger length, total number of productive tillers per plant under WW conditions. On the other hand, test weight, main earhead length, finger number per earhead, finger length and total biomass recorded least variation between PCV and GCV corresponds to high

heritability under DS conditions. Traits having fewer differences between PCV and GCV with corresponding high heritability indicated that selection would be effective for improvement in drought tolerance and yield (Khan *et al.*, 2007).

4.1.1.2c Genetic advance as *per cent* of mean

Genetic advance as *per cent* of mean (GAM) was found to be ranged between 14.37 (for culm length) to 58.49 *per cent* (for number of earheads per plant) and 11.09 (for culm length) to 56.24 *per cent* (for main earhead weight) under WW19 and WW20 conditions respectively (Table 4.3). Highest estimates (>20%) of GAM was recorded for plant height at reproductive stage (25.82%, 26.46%), plant height at harvest (23.31%, 20.48%), total number of tillers per plant (50.10%, 34.13%), total number of productive tillers per plant (43.77%, 45.97%), number of earheads per plant (58.49%, 51.87%), main earhead length (38.36%, 42.92%), main earhead weight (27.69%, 56.24%), total earhead weight per plant (52.75%, 50.84%), finger number per earhead (37.52%, 32.89%), finger length (45.18%, 45.27%), total biomass (31.16%, 31.44%) and seed yield per plant (33.80%, 31.94%) in both the years. However, the moderate estimate of GAM was recorded for days to flowering (17.17%, 18.17%), culm length (14.37%, 11.09%) under the WW conditions in both years. Rawat *et al.* (2018) recorded higher values for GAM was observed for number of fingers per earhead, days to flowering, earhead length, finger length and test weight while lower values for number of productive tillers per plant in a set of diverse finger millet genotypes. The high GAM for the traits such as seed yield per plant, productive tillers per plant and main ear length in finger millet were reported by Ganapathy *et al.* (2011), Suryanarayana *et al.* (2014) and Devaliya *et al.* (2018). In line with these results, high GAM estimates for total number of earheads per plant, seed yield per plant reported by Dagnachew *et al.* (2012).

Under drought stress, GAM was to be found to be ranged between 7.75 (for culm length) to 52.11 *per cent* (for total number of tillers per plant) and 7.05 (for plant height at harvest) to 47.04 *per cent* (main earhead weight) during DS19 and DS20 conditions respectively (Table 4.4). High estimates of GAM was recorded for plant height at reproductive stage (20.08%, 29.35%), total number of tillers per plant (52.11%, 32.01%), number of earheads per plant (51.77%, 44.46%), main earhead length (40.31%, 32.31%),

main earhead weight (42.94%, 47.04%), total earhead weight per plant (33.78%, 34.09%), finger length (40.03%, 34.25%), test weight (20.49%, 28.96%), total biomass (31.05%, 31.08%) and seed yield per plant (33.04%, 25.35%) in both the years. For culm length (7.75%, 7.28%) GAM was least in both the years.

In comparison, plant height at reproductive stage, total number of tillers per plant, number of earheads per plant, main earhead length, main earhead weight, total earhead weight per plant, finger length, test weight, total biomass and seed yield per plant were recorded high GAM under WW and DS conditions in both the years. High GAM indicated that these traits are governed by additive genes. Hence, selection of genotypes based on these traits will be rewarding for improvement in drought tolerance and seed yield in finger millet. These traits are least influenced by environmental conditions.

Estimates of heritability have a role to play in determining the effectiveness of selection for a trait, provided they are considered in conjugation with the genetic advance as per cent of mean as suggested by Johnson *et al.* (1955). Direct selection can be done through these traits for improvement of drought tolerance as well as seed yield. High heritability estimates along with the high genetic advance is helpful in predicting gain under selection (Johnson *et al.*, 1955). High heritability (*i.e.* >60%) coupled with high GAM (*i.e.* >20%) was consistently recorded for plant height at reproductive stage, plant height at harvest, number of tillers per plant, productive tillers per plant, number of earheads per plant, main earhead length, total earhead weight per plant, finger numbers per earhead, finger length, finger width, biomass and seed yield per plant under WW conditions in both the years in this study. Under DS conditions, high heritability with high GAM was observed for number of tillers per plant, main earhead length, finger length, test weight and total biomass in both the years. The number of tillers per plant, main earhead length, finger length and total biomass recorded highest GAM with high heritability under both WW and DS conditions in both the years. The high heritability associated with high genetic advance indicated, the variation was mostly due to additive gene effects. It indicates that if these characters are subjected to any selection scheme for exploiting fixable genetic variance, a wide adopted genotype can be developed. Hence, direct selection can be done through these traits for drought tolerance as well higher seed

yield. The high heritability with high GAM was reported obtained for number of productive tillers per plant (John, 2007; Karad and Patil, 2013), for seed yield per plant (Shet, 2010; Haradari *et al.*, 2012; Suryanarayana *et al.*, 2014) and for number of productive tillers per plant (Saundaryakumari and Singh, 2015). It also has been observed that, high heritability with high GAM recorded for plant height at harvest under WW conditions exhibited moderate heritability with moderate GAM under DS conditions (Table 4.3 and 4.4). This differential response could be explained by the fact that this study that used genotypes could have different sets of alleles and possibly different loci are being expressed under different environmental conditions (Eid, 2009). Low heritability for the traits under drought stress conditions as compared to non stress condition were reported in earlier studies in wheat (Collaku, 1994; Toker, 2004). The study presents the differences in estimated broad sense heritability and GAM for 16 quantitative traits under drought stress compared to non stress conditions. It strongly indicates the adaptive mechanisms for drought tolerance for the quantitative traits such as number of tillers per plant, main earhead length, finger length and total biomass which recorded high heritability and GAM under both the conditions are important for genotype selection to drought tolerance.

4.1.2 Correlation between seed yield and agro-morphological traits

Seed yield is complex trait and depends upon the interplay of several component traits (Chandra *et al.*, 2009). A clear picture of contribution of each component in the final expression of yield emerges through the study of correlation analysis. The degree of association among the traits was determined by their correlation coefficients.

Pearson's correlation coefficient analysis revealed a significant relationship among observed seed yield and seed yield component traits under WW and DS conditions. The total earhead weight per plant ($r=0.90$, $r=0.73$) followed by main earhead weight ($r=0.54$, $r=0.55$) exhibited highest positive and significant correlation with seed yield per plant under WW19 and WW20 conditions respectively (Table 4.5). Total biomass ($r=0.50$, $r=0.29$) followed by plant height at harvest ($r=0.29$, $r=0.37$), plant height at reproductive stage ($r=0.29$, $r=0.28$), days to flowering ($r=0.25$, $r=0.29$), finger

Table 4.5 Estimates of Pearson's correlation coefficients for sixteen traits in well watered (WW) environment in finger millet (WW19: below diagonal; WW20: above diagonal)

	DTF	PHS	PHH	TLP	PTLP	CL	EHP	EHL	EHW	EHWP	FNEH	FL	FW	TW	BM	SYP
DTF		0.10	0.40**	-0.28**	-0.20**	0.08	-0.30**	0.31**	0.44**	0.25**	0.29**	0.31**	0.03	0.08	0.41**	0.29**
PHS	-0.06		0.62**	-0.18**	-0.18**	0.17**	-0.24**	0.27**	0.29**	0.20**	0.17**	0.26**	0.08	0.14**	0.27**	0.28**
PHH	0.52**	0.60**		-0.29**	-0.24**	0.17**	-0.32**	0.33**	0.48**	0.31**	0.25**	0.31**	0.14**	0.14**	0.37**	0.37**
TLP	-0.51**	-0.13*	-0.37**		0.71**	-0.01	0.67**	-0.18**	-0.43**	-0.02	-0.29**	-0.18**	-0.10	-0.06	-0.25**	-0.09
PTLP	-0.47**	-0.09	-0.35**	0.91**		-0.10	0.86**	-0.14**	-0.41**	0.19**	-0.26**	-0.14**	-0.10	-0.09	-0.18**	0.08
CL	-0.22**	0.44**	0.27**	0.05	0.07		-0.10	0.07	0.17**	0.06	0.05	0.06	0.06	0.00	0.05	0.05
EHP	-0.49**	-0.09	-0.34**	0.78**	0.84**	0.09		-0.19**	-0.46**	0.13*	-0.39**	-0.19**	-0.09	-0.09	-0.18**	0.01
EHL	0.27**	0.30**	0.46**	-0.35**	-0.35**	0.15**	-0.38**		0.37**	0.24**	0.28**	0.93**	0.04	0.05	0.23**	0.18**
EHW	0.47**	0.19**	0.37**	-0.60**	-0.58**	-0.03	-0.63**	0.51**		0.55**	0.51**	0.38**	0.27**	0.12*	0.30**	0.55**
EHWP	0.26**	0.27**	0.3**	-0.14**	-0.01	0.10	-0.01	0.29**	0.57**		0.32**	0.25**	0.22**	0.04	0.14**	0.73**
FNEH	0.37**	-0.01	0.2**	-0.39**	-0.38**	-0.22	-0.48**	0.25**	0.49**	0.22**		0.22**	0.03	-0.06	0.03	0.27**
FL	0.23**	0.27**	0.41**	-0.31**	-0.34**	0.15**	-0.37**	0.90**	0.47**	0.24**	0.20**		0.01	0.04	0.21**	0.17**
FW	0.11*	0.05	0.08	-0.22**	-0.16**	0.11*	-0.11*	0.00	0.28**	0.29**	-0.17**	-0.04		0.03	0.04	0.21**
TW	0.07	0.08	0.08	-0.14**	-0.17**	0.07	-0.13*	0.01	0.11*	0.04	-0.10	-0.01	0.1		0.17**	0.13*
BM	0.50**	0.33**	0.65**	-0.19**	-0.15**	0.10	-0.17**	0.31**	0.37**	0.48**	0.07	0.26**	0.16**	0.17**		0.29**
SYP	0.25**	0.29**	0.29**	-0.13*	-0.01	0.13*	0.00	0.22**	0.54**	0.90**	0.17**	0.17**	0.29**	0.15**	0.50**	



**Significant at the 0.01 level

*Significant at the 0.05 level

Days to flowering (DTF), Plant height at reproductive stage (PHS), Plant height at harvest (PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length (CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

width ($r=0.29$, $r=0.21$), main earhead length ($r=0.22$, 0.18), finger number per earhead ($r=0.17$, $r=0.27$), finger length ($r=0.17$, $r=0.17$) and test weight ($r=0.15$, 0.13) recorded positive correlation with seed yield per plant under WW conditions in both the years (Table 4.5). However, productive tillers per plant did not show any significant relationship with seed yield per plant under WW conditions in both the years which is contradictory to earlier studies (Wolie and Dessalegn, 2011 and Suryanarayana *et al.*, 2014). The inter-correlation among the other traits suggested significant and positive correlation of total earhead weight per plant with main earhead weight ($r=0.55$, $r=0.57$) followed by total biomass ($r=0.48$, $r=0.14$) and plant height at harvest ($r=0.30$, $r=0.31$). The strong and significant positive correlation of total biomass observed with plant height at harvest ($r=0.65$, $r=0.37$) followed by days to flowering ($r=0.50$, $r=0.41$), earhead weight per plant ($r=0.48$, $r=0.14$), plant height at flowering stage ($r=0.33$, $r=0.27$), earhead length ($r=0.31$, $r=0.23$) and main earhead weight ($r=0.38$, $r=0.30$). Finger numbers per earhead found significantly and positively correlated with main earhead weight ($r=0.49$, $r=0.51$) followed by total earhead weight per plant. The significant and positive correlation for finger length observed with earhead length ($r=0.90$, $r=0.93$) followed by main earhead weight ($r=0.47$, $r=0.38$) suggesting that finger length determines the earhead length and main earhead weight. The significant and positive correlation of seed yield for finger numbers on main earhead, plant height, main earhead weight, total earhead weight per plant and main earhead length were reported by Wolie and Dessalegn (2011), Eric *et al.* (2016) and Singh *et al.* (2018) in finger millet genotypes evaluated under WW conditions. Hence selection and improvement in these traits results in improvement in seed yield.

Under DS conditions, the highest positive and significant correlation of seed yield observed with total earhead weight per plant ($r=0.89$, $r=0.53$) followed by main earhead weight ($r=0.58$, $r=0.46$) during DS19 and DS20 respectively (Table 4.6). The plant height at harvest ($r=0.47$, $r=0.36$) followed by total biomass ($r=0.43$, $r=0.029$), finger width ($r=0.37$, $r=0.29$), culm length ($r=0.26$, $r=0.29$), days to flowering ($r=0.23$, $r=0.15$), finger number per earhead ($r=0.20$, $r=0.27$) finger length ($r=0.19$, $r=0.12$) and main earhead length ($r=0.19$, $r=0.15$) recorded positive correlation except total number of productive

Table 4.6 Estimates of Pearson's correlation coefficients for sixteen traits in drought stress (DS) environment in finger millet (DS19: below diagonal; DS20: above diagonal)

	DTF	PHS	PHH	TLP	PTLP	CL	EHP	EHL	EHW	EHWP	FNEH	FL	FW	TW	BM	SYP
DTF		-0.26**	0.34**	-0.29**	-0.20**	0.31**	-0.43**	0.34**	0.49**	0.16**	0.37**	0.32**	0.21**	0.10	0.39**	0.15**
PHS	-0.52**		-0.03	-0.01	-0.07	-0.08	0.00	-0.07	-0.10	0.01	-0.11*	-0.06	-0.08	-0.06	0.03	0.01
PHH	0.30**	0.22**		-0.29**	-0.15**	0.73**	-0.17**	0.34**	0.52**	0.33**	0.23**	0.35**	0.29**	-0.01	0.37**	0.36**
TLP	-0.47**	0.04	-0.31**		0.71**	-0.18**	0.61**	-0.24**	-0.44**	-0.05	-0.28**	-0.22**	-0.19**	0.02	-0.24**	-0.15**
PTLP	-0.39**	0.02	-0.21**	0.83**		-0.10	0.70**	-0.23**	-0.33**	0.07	-0.24**	-0.21**	-0.14**	0.12*	-0.17**	-0.04
CL	-0.01	0.19**	0.60**	-0.08	0.00		-0.18**	0.48**	0.46**	0.38**	0.25**	0.47**	0.26**	0.01	0.20**	0.29**
EHP	-0.45**	0.16**	-0.18**	0.68**	0.75**	0.00		-0.34**	-0.46**	0.02	-0.45**	-0.30**	-0.13*	0.05	-0.18**	-0.04
EHL	0.40**	-0.13*	0.36**	-0.35**	-0.34**	0.15**	-0.40**		0.41**	0.24**	0.37**	0.92**	0.07	0.05	0.23**	0.15**
EHW	0.51**	-0.10	0.46**	-0.50**	-0.45**	0.16**	-0.51**	0.49**		0.50**	0.55**	0.39**	0.46**	0.06	0.39**	0.46**
EHWP	0.24**	0.08	0.47**	-0.13*	-0.05	0.24**	0.00	0.24**	0.64**		0.28**	0.23**	0.31**	0.07	0.21**	0.53**
FNEH	0.39**	-0.20**	0.14**	-0.41**	-0.32**	-0.06	-0.42**	0.32**	0.50**	0.28**		0.29**	0.04	-0.05	0.10	0.27**
FL	0.35**	-0.12*	0.31**	-0.31**	-0.32**	0.14**	-0.36**	0.90**	0.45**	0.26**	0.25**		0.07	0.05	0.22**	0.12*
FW	0.22**	0.06	0.27**	-0.29**	-0.27**	0.22**	-0.17**	0.03	0.50**	0.39**	0.03	0.05		0.02	0.16**	0.29**
TW	0.17**	-0.04	0.18**	-0.17**	-0.15**	0.15**	-0.13*	0.04	0.12*	0.07	-0.10	0.07	0.16**		0.15**	0.10
BM	0.45**	0.06	0.56**	-0.24**	-0.17**	0.17**	-0.15**	0.34**	0.42**	0.46**	0.16**	0.33**	0.26**	0.23**		0.29**
SYP	0.23**	0.07	0.47**	-0.12*	-0.02	0.26**	0.04	0.19**	0.58**	0.89**	0.20**	0.19**	0.37**	0.12*	0.43**	

**Significant at the 0.01 level

*Significant at the 0.05 level

Days to flowering (DTF), Plant height at reproductive stage(PHS), Plant height at harvest(PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

tillers plant with seed yield under DS conditions in both the years. The results indicated that, the selection and improvement of these traits resulted in improvement of seed yield per plant under drought stress environment. The inter-correlation among characters suggest that, total earhead weight per plant had significant positive correlation with main earhead weight ($r=0.64$, $r=0.50$) followed by total biomass ($r=0.46$, $r=0.21$) (Table 4.6). Thus selection of these traits plays an important role in the total earhead weight per plant and indirectly seed yield. However, negative correlation observed for main earhead weight with total number of tillers per plant ($r=-0.50$, $r=-0.44$) followed by total number of tillers per plant ($r=-0.45$, $r=-0.33$) and number of earheads per plant ($r=-0.51$, $r=-0.46$). Plant height is an important trait in determining the plant biomass production and seed yield. Plant height showed a significant positive relationship with seed yield per plant ($r=0.47$, $r=0.36$) followed by total biomass ($r=0.56$, $r=0.37$). Similarly, main earhead weight showed significant and positive correlation with finger numbers per earhead ($r=0.50$, $r=0.55$), earhead length ($r=0.49$, $r=0.41$), days to flowering ($r=0.51$, $r=0.49$) and plant height at harvest ($r=0.46$, $r=0.52$).

Biomass is an important trait which depicts the overall physiological growth of the plant and thus contributes to the seed yield. It is evident that proper growth and biomass leads to increased carbon fixation and indirectly leads to increase in seed yield. The present results are in consonance with Ravishankar (1996); Anantharaju and Meenakshi (2005); Kebera *et al.* (2006); Misra *et al.* (2008); Wolie and Dessalegn, (2011) and Suryanarayana *et al.* (2014) in finger millet. Significant positive association with seed yield per plant for test weight, number of fingers per earhead, finger length, productive tillers per plant, days to flowering and plant height were reported by Jadhav *et al.* (2015), Ashok *et al.* (2016), Jyothsna *et al.* (2016), Mahanthesha *et al.* (2018), Kandel *et al.* (2019), Sapkal *et al.* (2019) and Chavan *et al.* (2020). Plant height and total biomass had a significant positive relationship with seed yield in both the WW and DS conditions. The reason for the positive correlation between plant height and seed yield is that increasing the plant height enhances plant's capacity to accumulate sufficient reserves with biomass that are converted into seed yield (Mwadzingeni *et al.*, 2016). The positive relationship between number of fingers per earhead and seed yield has been

reported under rainfed conditions by several researchers (Negi *et al.*, 2017; Reddy *et al.*, 2019; Sapkal *et al.*, 2019).

The comparison of correlation coefficient between seed yield per plant and others traits under WW and DS conditions suggest that, total earhead weight per plant, main earhead weight, total biomass, earhead length and finger width had positive correlation under both the conditions. The results clearly indicated that the improvement in these traits is important for improvement in seed yield not only under irrigated but also drought stress conditions. Hence, selection criteria based on these component traits along with seed yield will be more useful than simply based on seed yield.

The correlation coefficient results indicated that the performance of genotypes for the traits across the years under WW and DS conditions were largely similar. However, few traits such as finger length showed significant and positive relation with plant height at reproductive stage under WW conditions; on the other hand it exhibited significantly negative correlation under DS conditions. This indicated that, the association between the traits differs under different environmental conditions and this may due to differential performance of the genotypes under WW and DS conditions. There are evidences that change in conditions can influence genetic interactions among traits as well as genetic variance in traits themselves (Eid, 2009). Exposure to drought conditions may induce negative correlation among traits because the expressions of new genes will breakdown positive correlations (Sgro and Hoffmann, 2004).

4.1.3 Stepwise multiple linear regression analysis to determine important traits for productivity and drought tolerance

In order to know the most significantly contributing traits to the variation for seed yield, the stepwise multiple linear regression was carried out for WW and DS conditions in each year separately. The analysis was carried out by considering seed yield per plant as dependent variable and fifteen traits as independent variables. Two models were selected for the study. The first model includes all the independent traits under the study and second model includes only the acceptable traits which also known as best fit model exhibiting highest contribution of independent traits to the seed yield per plant.

Analysis of variance indicated highly significant regression for the models studied under WW19 and WW220 conditions respectively. The dependent variable seed yield per plant was regressed on all fifteen predicting variables to estimate the effect of independent variables on seed yield per plant. All fifteen traits together significantly predicted seed yield per plant in both the years under WW conditions which indicates the significant role of these traits in seed yield per plant (Table 4.7). Moreover, the regression model with the all fifteen traits depicts $R^2 = 0.843$ and $R^2 = 0.623$ indicating the model explains the 84.30 and 62.30 *per cent* of phenotypic variation for the seed yield during WW19 and WW20 respectively (Table 4.8). The results indicated that, the selected fifteen traits contribute mainly for seed yield under WW conditions.

In order to identify major independent variables contributing for on seed yield; a improved better fit model was constructed by stepwise multiple regression with backward elimination where regression analysis was carried out by stepwise elimination of an independent variable in each step with non-significant regression co-efficient ($P > 0.05$). Thus out of fifteen traits, seven traits during WW19 and six traits during WW20 significantly predicted seed yield per plant under WW conditions (Table 4.7). The regression model with seven traits in WW19, explained 84.00% ($R^2 = 0.840$, $p < 0.05$) of the phenotypic variation in seed yield and the traits are total biomass, test weight, culm length, total earhead weight per plant, main earhead weight, total number of productive tillers per plant and main earhead length. On the other hand in WW20, six traits *viz.* total biomass, finger length, total number of productive tillers per plant, plant height at reproductive stage, total earhead weight per plant and main earhead weight showed significant contribution of 61.60 *per cent* ($R^2 = 0.616$, $p < 0.05$) to the seed yield per plant (Table 4.8).

Under DS conditions, the analysis of variance indicated highly significant regression for the models studied during. All fifteen traits together significantly predicted role of these traits for seed yield per plant in both the years under DS conditions (Table 4.7). The regression model with the all fifteen traits together depicts $R^2 = 0.813$ and $R^2 = 0.381$ for the year DS19 and DS20 respectively indicating the model explains the 81.30

Table 4.7 Analysis of variance (ANOVA) for prediction models using stepwise regression under well watered (WW) and drought stress (DS) conditions over the two years in finger millet

WW							DS						
Model		Sum of Squares	df	Mean squares	F	Sig	Model		Sum of Squares	df	Mean squares	F	Sig
WW19							DS19						
1a	Regression	5062.79	15	337.52	119.17	0.00a	1a	Regression	2949.35	15	196.62	96.96	0.00a
	Residual	945.97	334	2.83				Residual	677.29	334	2.03		
	Total	6008.76	349					Total	3626.64	349			
2b	Regression	5045.32	7	720.76	255.85	0.00b	2b	Regression	2934.60	7	419.22	207.20	0.00b
	Residual	963.44	342	2.82				Residual	692.09	342	2.02		
	Total	6008.76	349					Total	3626.64	349			
WW20							DS20						
1a	Regression	3646.62	15	243.11	36.73	0.00a	1a	Regression	1676.51	15	111.77	13.73	0.00a
	Residual	2210.53	334	6.62				Residual	2718.33	334	8.14		
	Total	5857.15	349					Total	4394.84	349			
2c	Regression	3609.35	6	601.56	91.79	0.00c	2c	Regression	1579.22	5	315.84	38.59	0.00c
	Residual	2247.80	343	6.55				Residual	2815.62	344	8.19		
	Total	5857.15	349					Total	4394.84	349			
Model 1a. Predictors: (Constant), BM, FNEH, FW, CL, TW, FL, PTLP, PHS, DTF, EHWP, PHH, TLP, EHW, EHP, EHL							Model 1a. Predictors: (Constant), BM, PHS, FNEH, TW, FW, PTLP, FL, EHWP, PHH, DTF, TLP, CL, EHP, EHW, EHL						
Model 2b. Predictors (WW2019): (Constant), BM, TW, CL, EHWP, EHW, PTLP, EHL							Model 2b. Predictors: (Constant), PTLP, FNEH, FL, EHWP, PHH, TLP, EHW						
Model 2c. Predictors (WW 2020): (Constant), BM, FL, PTLP, PHS, EHWP, EHW							Model 2c. Predictors: (Constant), BM, FL, EHWP, PHH, EHW						
Dependent variable: SYP							Dependent variable: SYP						

Days to flowering (DTF), Plant height at reproductive stage (PHS), Plant height at harvest (PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

Table 4.8 Prediction model of multiple stepwise regressions (backward elimination) for traits contributing to seed yield per plant in finger millet under well watered (WW) and drought stress (DS) conditions during 2019 and 2020

WW						DS					
Model	Description	R	R square	Adjusted R square	Standard error of estimate	Model	Description	R	R square	Adjusted R square	Standard error of estimate
WW19						DS19					
1a	All traits	0.918	0.843	0.835	1.68	1a	All traits	0.902	0.813	0.81	1.42
2b	Major traits	0.916	0.840	0.836	1.68	2b	Major traits	0.900	0.809	0.81	1.42
WW20						DS20					
1a	All traits	0.789	0.623	0.606	2.57	1a	All traits	0.618	0.381	0.35	2.85
2c	Major traits	0.785	0.616	0.610	2.56	2c	Major traits	0.599	0.359	0.35	2.86
Model 1a. Predictors: (Constant), BM, FNEH, FW, CL, TW, FL, PTLP, PHS, DTF, EHWP, PHH, TLP, EHW, EHP, EHL						Model 1a. Predictors: (Constant), BM, PHS, FNEH, TW, FW, PTLP, FL, EHWP, PHH, DTF, TLP, CL, EHP, EHW, EHL					
Model 2b. Predictors (WW2019): (Constant), BM , TW, CL, EHWP , EHW , PTLP , EHL						Model 2b. Predictors: (Constant), PTLP, FNEH, FL , EHWP , PHH , TLP, EHW					
Model 2c. Predictors (WW 2020): (Constant), BM , FL, PTLP , PHS, EHWP , EHW						Model 2c. Predictors: (Constant), BM, FL , EHWP , PHH , EHW					
Dependent variable: SYP						Dependent variable: SYP					

Days to flowering (DTF), Plant height at reproductive stage(PHS), Plant height at harvest(PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

per cent and 38.10 *per cent* of phenotypic variation during DS19 and DS20 respectively (Table 4.8). The results indicated that, the selected fifteen traits contribute extensively for seed yield during DS19 conditions, while the contribution of the 15 traits drastically reduced and explain only 38.10 *per cent* of the phenotypic variation for seed yield.

However, many others physiological traits not recorded in the present study reported better plants response against drought such as osmotic adjustment, proline, sugar, glycine betain, leaf succulence, relative water content, chlorophyll content, transpiration efficiency, root traits and cell membrane stability (Naeem *et al.*, 2013; Krishnamurthy, *et al.*, 2016). Thus it can be concluded that by combining agromorphological and physiological traits plays a major role in development of tolerant genotypes under drought stress conditions.

In order to understand the major traits contributing towards seed yield, several model were tested and the best fit model explaining the variation were selected by stepwise regression. Thus out of fifteen traits, seven traits during DS19 and five traits during DS20 significantly predicted seed yield per plant under DS conditions (Table 4.7). The regression model with seven traits in DS19 exhibited 80.90 *per cent* ($R^2=0.809$, $p<0.05$) phenotypic variation in seed yield by the traits *viz.* total number of productive tillers per plant, finger number per earhead, finger length, total earhead weight per plant, plant height at harvest, total number of tillers per plant and main earhead weight predicted significant role in seed yield per plant. On the other hand in DS20, five traits *viz.* total biomass, finger length, total earhead weight per plant, plant height at harvest and main earhead weight showed significant contribution of 35.90 *per cent* ($R^2=0.359$, $p<0.05$) to the seed yield per plant (Table 4.8).

The standardized beta coefficients (b) were compared in order to determine which variable carries the most weight or influence or importance in predicting the dependent variable *i.e.* seed yield per plant in regression model. Four traits *viz.* total earhead weight per plant, main earhead weight, total number of productive tillers per plant and total biomass are found to be common that influencing seed yield under WW conditions in both the years. The results indicated that, total earhead weight per plant ($b=0.791$, $b=0.562$), main earhead weight ($b=0.156$, $b=0.253$), total number of productive tillers per

plant ($b=0.109$, $b=0.109$) and total biomass ($b=0.053$, $b=0.154$) recorded significant and positive effect in increasing seed yield per plant under WW conditions in both the years (Table 4.9). The correlation studies also revealed the significant and positive correlation of total earhead weight per plant, main earhead weight and total biomass with seed yield per plant under WW conditions in both the years. Significant positive association of these traits with seed yield were reported by Ashok *et al.* (2016), Mahanthesha *et al.* (2018), Kandel *et al.* (2019), Sapkal *et al.* (2019) and Chavan *et al.* (2020) in diverse finger millet genotypes under normal environmental conditions.

Under DS conditions, four traits viz. total earhead weight per plant, main earhead weight, plant height at harvest and finger length are found to be common that influencing seed yield in both the years. The results indicated that, total earhead weight per plant ($b=0.835$, $b=0.392$), main earhead weight ($b=0.092$, $b=0.194$) and plant height at harvest ($b=0.063$, 0.120) indicated significant and positive effect on increasing seed yield per plant under DS19 and DS20 conditions respectively (Table 4.9). The correlation studies also revealed the positive association of these traits under DS conditions in both the years. Hence, these traits can be used as an indirect selection criterion for improving seed yield and drought tolerance under DS conditions.

It has been reported in earlier studies, that reduction in mean values for the traits such as plant height, number of earheads per plant, number of tillers per plant, total biomass under DS conditions has huge impact on seed yield (Wondewosen *et al.*, 2012; Fatou, 2016; Kumar *et al.*, 2018, Reddy *et al.*, 2020; Krishna *et al.*, 2021). Therefore it is necessary to identify traits for drought tolerance and seed yield under stress environment. The results from stepwise multiple linear regression under WW and DS conditions indicated that, total earhead weight per plant and main erahead weight are found to be common traits for seed yield under in both the years. The correlation and path analysis studies by Eric *et al.* (2016), Negi *et al.* (2017), Gohel and Chaudhari (2018) and Kandel *et al.* (2019) supported the importance of these two traits for selection of genotypes and improving seed yield. Thus total earhead weight per plant and main erahead weight can be used in selection criteria for improving seed yield and drought tolerance in genotypes.

Table 4.9 Summary of prediction models based on Beta (b) coefficients of major traits (based on table 4.7) contributing to seed yield per plant under well watered (WW) and drought stress (DS) conditions in finger millet

WW							DS						
Model	Traits	Unstandardized Coefficients	Standardized Coefficients		t	Sig.	Model	Traits	Unstandardized Coefficients	Standardized Coefficients		t	Sig.
			Std. Error	Beta						Std. Error	Beta		
2b (2019)	(Constant)	-6.44	1.20		-5.38	0.491	2b (2019)	(Constant)	-0.50	0.83		-0.60	0.548
	PTLP	0.30	0.10	0.10	2.89	0.004		PHH	0.02	0.01	0.06	2.21	0.028
	EHW	0.30	0.08	0.16	3.79	0		EHW	0.17	0.08	0.09	2.27	0.024
	EHWP	0.68	0.03	0.79	23.53	0		EHWP	0.66	0.03	0.84	24.55	0
	BM	0.01	0.01	0.08	3.21	0.001		FL	-0.14	0.06	-0.06	-2.18	0.03
2c (2020)	(Constant)	0.94	1.30		0.72	0.471	2c (2020)	(Constant)	2.01	1.19		1.69	0.092
	PTLP	0.40	0.16	0.11	2.49	0.013		PHH	0.03	0.02	0.12	2.27	0.024
	EHW	0.48	0.10	0.25	4.79	0		EHW	0.38	0.12	0.19	3.32	0.001
	EHWP	0.34	0.03	0.56	11.79	0		EHWP	0.23	0.03	0.39	7.81	0
	BM	0.02	0.03	0.15	4.30	0		FL	-0.26	0.11	-0.11	-2.24	0.026

Days to flowering (DTF), Plant height at reproductive stage (PHS), Plant height at harvest (PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length (CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

The selected major traits identified through stepwise multiple linear regression viz. total number of productive tillers per plant, main earhead weight, total earhead weight per plant, total biomass under WW condition and plant height at harvest, main earhead weight, total earhead weight per plant, finger length under DS conditions were assessed for the degree of association among these traits with seed yield per plant by Pearson's correlation coefficients in each year separately (Table 4.10). The total earhead weight per plant ($r=0.90$, $r=0.73$) followed by main earhead weight ($r=0.54$, $r=0.55$) and total biomass ($r=0.50$, $r=0.29$) recorded highest positive and significant correlation with seed yield per plant under WW conditions in both the years (Table 4.10a). The results indicated that, the selection and improvement for total earhead weight per plant, main earhead weight and total biomass leads to improvement in seed yield under WW environment. The inter-correlation among the other traits suggest that total earhead weight per plant had significant and positive correlation with main earhead weight ($r=0.57$, $r=0.55$) followed by total biomass ($r=0.48$, $r=0.14$). However, total number of productive tillers per plant did not show any significant relationship with seed yield per plant under WW conditions. To the contrary, Eric *et al.* (2016) reported that productive tillers per plant had positive direct effects on seed yield. Whereas, Shinde *et al.* (2014), Kumar *et al.* (2014), Gohel and Chaudhari (2018) reported that biomass, plant height by Mahanthesha *et al.* (2018) had positive direct effects on seed yield.

Under DS conditions, the highest positive and significant correlation of total earhead weight per plant ($r=0.89$, $r=0.53$) followed by main earhead weight ($r=0.58$, $r=0.46$) and plant height at harvest ($r=0.47$, $r=0.36$) observed for seed yield per plant under DS conditions in both the years (Table 4.10b). The results indicated that, the selection and improvement in total earhead weight per plant, main earhead weight and plant height at harvest leads to improvement in seed yield under drought stress environment. The inter-relationship among traits suggest that, plant height at harvest showed a significant positive relationship with main earhead weight ($r=0.47$, $r=0.52$) followed by total earhead

Table 4.10 Estimates of Perason’s corelation coefficients for major traits selected based on regression analysis under WW (a) and DS (b) conditions during 2019 and 2020 in finger millet

(a)	WW20					
WW 19		PTLP	EHW	EHWP	BM	SYP
	PTLP		-0.41**	0.19**	-0.18**	0.08
	EHW	-0.58**		0.55**	0.30**	0.55**
	EHWP	-0.01	0.57**		0.14**	0.73**
	BM	-0.15**	0.37**	0.48**		0.29**
	SYP	-0.01	0.54**	0.90**	0.50**	

(b)	DS20					
DS 19		PHH	EHW	EHWP	FL	SYP
	PHH		0.52**	0.33**	0.35**	0.36**
	EHW	0.46**		0.50**	0.39**	0.46**
	EHWP	0.47**	0.64**		0.23**	0.53**
	FL	0.31**	0.45**	0.26**		0.12*
	SYP	0.47**	0.58**	0.89**	0.19**	



** Significant at the 0.01 level

* Significant at the 0.05 level

Plant height at harvest (PHH), Number of productive tillers per plant (PTLP), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger length (FL), Total biomass (BM) and Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

weight per plant ($r=0.47$, $r=0.33$) and finger length ($r=0.31$, 0.35). Total earhead weight per plant showed significant positive correlation with main earhead weight ($r=0.64$, $r=0.50$) followed by finger length ($r=0.26$, $r=0.23$). Thus selection based on these traits plays important role in improving total earhead weight per plant and main earhead weight thus indirectly seed yield per plant.

Conclusively, under WW conditions four traits viz. total biomass, total earhead weight per plant, main earhead weight and productive tillers per plant are found to be the common and stable traits over the two years contributing majorly to seed yield. Whereas, under DS conditions four traits viz. finger length, total earhead weight per plant, plant height at harvest and main earhead weight exhibited stable contribution to the seed yield over the two years under DS conditions and can be used as an indirect selection criterion for improvement in drought tolerance and seed yield stress conditions. Similarly, Haradari *et al.* (2012) reported that number of productive tillers per plant and earhead weight per plant had direct positive effect on seed yield in finger millet. The present results are in accordance with Wolie and Dessalegn, (2011), Anuradha *et al.* (2013), Jyothsna *et al.* (2016), Negi *et al.* (2017) and Chavan *et al.* (2020) who also reported number of fingers per earhead, number of tillers per plant, plant height had positive and highest effects on improving seed yield per plant in finger millet.

4.1.4 Identification of important traits and genotypes for seed yield and drought tolerance

As the experiment is complex with a large number of genotypes, variables and environments, we have applied the following statistical approaches to reduce the number of variables for sensible interpretations of variables and selection of genotypes for individual environment as well as across environments.

1. Principal component analysis (PCA)
2. Genotype by Yield*Trait (GYT) analysis
3. Genotype plus Genotype-by-Environment (GGE) analysis

4.1.4.1 Principal component analysis (PCA)

Principal component analysis (PCA) is a statistical procedure that used to identify the traits which contribute maximum to the most observed variation within a large group of genotypes and employed to reduce the complexity of the data while minimizing the variation within the data and increasing interpretability (Ringnér, 2008). It is efficient to finding the components useful to group the genotypes that maximize the variation and it is also helpful in reducing the number of variables with a maximum variance but does not estimate the specific effect. The PCA was performed separately for each year for well watered and drought stress environment using data of all sixteen agro-morphological traits evaluated on three hundred fifty genotypes. The resulted PCA from sixteen independent principal components (PCs) explained cumulative variance under WW and DS conditions during 2019 and 2020 is given in table 4.11 and 4.12. The importance of a character towards the PCs could be seen from the corresponding eigenvalues. The results of PCA revealed that, out of the 16 PCs, first five PCs with eigenvalue of greater than 1.00 accounted for 76.25 and 68.27 *per cent* of total variability for WW19 and WW20 respectively (Table 4.11). The contribution of each trait to the respective PCs is given in table 4.13. The PC1 accounted for 33.80 and 29.32 *per cent* of the total variation in the population under WW19 and WW20 condition respectively. The results also showed that main earhead weight (0.36, 0.38) contributed positively and significantly to PC1 followed by plant height at harvest (0.29, 0.32) under WW conditions in both the years (Table 4.13). The other traits viz. main earhead length, days to flowering, finger length, finger numbers per earhead, total biomass, total earhead weight per plant, seed yield per plant, plant height at reproductive stage, finger width, test weight and culm length also contributed positively to the PC1 under WW conditions in both the years. The PC2 accounted for 15.83 and 14.45 *per cent* of the total variation and traits which contributed maximum were total number of productive tillers per plant (0.34, 0.49) followed by number of earheads per plant and total number of tillers per plant.

Table 4.11 Estimate of eigenvalues and percentage of variation contributed by each principal component (PC) to the seed yield and yield related components under well watered (WW) conditions during 2019 and 2020

PC	WW19			WW20		
	Eigenvalue	Variance (%)	Cumulative variance (%)	Eigenvalue	Variance (%)	Cumulative variance (%)
1	5.40	33.80	33.80	5.35	29.32	29.32
2	2.53	15.83	49.63	2.53	14.45	43.77
3	1.72	10.77	60.4	1.52	9.50	53.27
4	1.40	8.78	69.18	1.39	8.14	61.41
5	1.12	7.07	76.25	1.1	6.86	68.27
6	0.91	5.74	81.99	0.82	6.01	74.28
7	0.87	5.44	87.43	0.77	5.65	79.93
8	0.51	3.23	90.66	0.67	5.33	85.26
9	0.40	2.51	93.17	0.46	3.75	89.01
10	0.31	1.93	95.10	0.32	3.02	92.03
11	0.23	1.48	96.58	0.25	2.08	94.11
12	0.15	0.95	97.53	0.20	1.94	96.05
13	0.13	0.87	98.40	0.17	1.54	97.59
14	0.09	0.59	98.99	0.14	1.24	98.83
15	0.08	0.56	99.55	0.10	0.74	99.57
16	0.07	0.45	100.00	0.08	0.43	100.00

Table 4.12 Estimate of eigenvalues and percentage of variation contributed by each principal component (PC) to the seed yield and yield related components under drought stress (DS) conditions during 2019 and 2020

PC	DS19			DS20		
	Eigenvalue	Variance (%)	Cumulative variance (%)	Eigenvalue	Variance (%)	Cumulative variance (%)
1	5.35	33.45	33.45	5.00	31.31	31.31
2	2.53	15.87	49.32	2.08	13.03	44.34
3	1.52	9.53	58.85	1.51	9.48	53.82
4	1.39	8.71	67.56	1.20	7.53	61.35
5	1.10	7.34	74.90	1.10	6.88	68.23
6	0.82	5.13	80.03	1.00	6.28	74.51
7	0.77	4.82	84.85	0.82	5.15	79.66
8	0.67	4.21	89.06	0.72	4.52	84.18
9	0.46	2.88	91.94	0.63	3.96	88.14
10	0.32	2.04	93.98	0.43	2.72	90.86
11	0.25	1.57	95.55	0.41	2.57	93.43
12	0.20	1.27	96.82	0.32	2.02	95.45
13	0.17	1.08	97.90	0.24	1.53	96.98
14	0.14	0.93	98.83	0.21	1.32	98.30
15	0.10	0.65	99.48	0.19	1.24	99.54
16	0.08	0.52	100.00	0.07	0.46	100.00

Table 4.13 Contribution of each trait to first six principle component (PC) under well watered (WW)and drought stress (DS) condition during 2019 and 2020

Traits	WW							DS					
	Year	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
DTF	2019	0.28	-0.13	0.21	0.04	0.44	0.04	0.28	-0.17	0.21	-0.18	0.34	-0.20
	2020	0.27	0.03	0.04	-0.01	-0.35	-0.25	0.27	-0.04	0.03	-0.46	0.01	-0.06
PHS	2019	0.16	0.31	-0.34	-0.16	0.07	-0.30	-0.05	0.27	-0.43	0.23	-0.42	0.32
	2020	0.24	0.06	0.01	0.40	0.36	-0.12	-0.04	-0.05	-0.13	0.66	0.40	0.21
PHH	2019	0.29	0.17	-0.22	-0.08	0.43	-0.17	0.27	0.28	-0.16	0.23	0.07	-0.32
	2020	0.32	0.08	-0.03	0.30	0.15	-0.17	0.30	0.21	-0.01	0.14	0.05	-0.41
TLP	2019	-0.32	0.28	-0.01	0.23	0.08	0.13	-0.29	0.25	0.32	0.08	0.08	0.03
	2020	-0.27	0.36	0.11	0.07	0.12	-0.06	-0.26	0.37	0.23	-0.05	-0.10	0.07
PTLP	2019	-0.30	0.34	0.04	0.24	0.07	0.07	-0.27	0.31	0.36	0.04	0.11	-0.09
	2020	-0.25	0.49	0.08	0.02	-0.02	-0.08	-0.21	0.49	0.17	-0.13	-0.06	0.07
CL	2019	0.03	0.31	-0.36	-0.29	-0.18	-0.24	0.12	0.30	-0.25	0.30	0.10	-0.61
	2020	0.09	0.01	-0.04	0.15	0.64	-0.22	0.29	0.23	0.17	0.16	-0.04	-0.35
EHP	2019	-0.30	0.34	0.04	0.13	0.04	0.06	-0.26	0.36	0.21	0.01	0.07	0.03
	2020	-0.28	0.45	0.06	0.06	-0.02	-0.08	-0.27	0.44	0.06	0.04	0.03	-0.07
EHL	2019	0.28	0.05	-0.34	0.27	-0.20	0.29	0.28	-0.11	0.25	0.48	-0.03	0.16
	2020	0.27	0.12	0.57	-0.08	0.05	0.21	0.30	-0.01	0.51	0.11	0.07	0.09
EHW	2019	0.36	-0.02	0.17	0.07	-0.25	-0.01	0.37	0.04	0.06	-0.17	-0.14	0.05
	2020	0.38	0.06	-0.18	-0.19	0.01	0.04	0.37	0.06	-0.18	-0.05	-0.11	0.10

EHWP	2019	0.22	0.37	0.33	0.18	-0.17	-0.02	0.26	0.37	0.19	-0.20	-0.17	0.12
	2020	0.21	0.45	-0.21	-0.22	-0.02	0.01	0.21	0.39	-0.15	0.14	-0.12	0.30
FNEH	2019	0.21	-0.22	0.12	0.38	0.03	-0.24	0.22	-0.17	0.22	-0.17	-0.32	-0.27
	2020	0.25	-0.01	-0.05	-0.46	0.03	-0.30	0.26	-0.08	0.04	-0.07	-0.38	0.49
FL	2019	0.26	0.03	-0.38	0.28	-0.23	0.32	0.27	-0.08	0.25	0.49	-0.01	0.25
	2020	0.27	0.11	0.57	-0.08	0.04	0.25	0.28	0.01	0.53	0.12	0.11	0.04
FW	2019	0.10	0.12	0.28	-0.43	-0.35	-0.13	0.19	0.17	-0.25	-0.35	0.02	0.10
	2020	0.10	0.07	-0.35	-0.04	0.22	0.71	0.18	0.16	-0.37	-0.03	-0.11	-0.34
TW	2019	0.07	0.04	0.06	-0.45	0.07	0.70	0.10	0.04	-0.25	-0.02	0.65	0.33
	2020	0.08	0.01	-0.08	0.46	-0.28	0.29	0.01	0.13	-0.01	-0.42	0.59	0.25
BM	2019	0.25	0.27	0.08	-0.03	0.46	0.11	0.26	0.19	0.05	0.05	0.24	0.16
	2020	0.22	0.04	0.01	0.41	-0.35	-0.12	0.21	0.078	-0.13	-0.14	0.50	-0.03
SYP	2019	0.21	0.38	0.35	0.09	-0.13	0.01	0.24	0.39	0.17	-0.22	-0.09	0.10
	2020	0.24	0.39	-0.29	-0.04	-0.11	-0.02	0.21	0.31	-0.30	0.11	-0.01	0.30

Days to flowering (DTF), Plant height at reproductive stage(PHS), Plant height at harvest(PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

The first five principle components having eigenvalue greater than 1 accounted for 74.90 and 68.23 *per cent* of the total variability in 350 genotypes under DS environment during 2019 and 2020 respectively (Table 4.12). The PC1 accounted for 33.45 and 31.31 *per cent* of the total variation in the population during DS19 and DS20 respectively. The main earhead weight (0.37, 0.37) had the highest contribution to the PC1 followed by main earhead length in both the years (Table 4.13). The other traits viz. plant height at harvest, day to flowering, finger length, finger numbers per earhead, total earhead weight per plant, total biomass, seed yield per plant, finger width, culm length and test weight contributed positively to the PC1 during DS conditions in both the years. The PC2 accounted for 15.87 and 13.03 *per cent* of the total variation and the traits which contributed maximum were total number of productive tillers per plant (0.31, 0.49) followed by number of earheads per plant (Table 4.13).

In line with this result, Ulaganathan and Nirmalakumari (2015) used 302 finger millet genotypes and observed five PCs explained of 71.8 *per cent* of total variation. Ashok *et al.* (2016) evaluated 18 finger millet genotypes and recorded first four PCs explained 92.16 *per cent* of total variation. They have also observed that the highest contribution to the PC1 was days to maturity followed by days to flowering and plant height. Similarly, Sharma *et al.* (2018) evaluated 113 diverse finger millet accessions for 14 agro-morphological traits and reported five PCs are important. Anteneh *et al.* (2019) used 225 finger millet germplasm accessions and screened for 16 agro-morphological traits. The 65 *per cent* of total variation was explained by the first five PCs. Ladumor *et al.* (2021) evaluated sixty accessions of finger millet evaluated for eight traits and recorded five major PCs are important to explain variation of which PC1 had the highest loading for days to flowering, days to maturity, finger length, fingers per earhead where as PC2 majorly accounted for plant height, grain yield, test weight and days to flowering. In the present study, it has been observed that, the main earhead weight followed plant height at harvest were the major traits contributed to PC1 and total number of productive tillers per plant and number of earheads per plant were the major traits contributed to PC2 under WW conditions in both the years. Whereas, under DS conditions, main earhead weight followed by main earhead length were the major traits contributed to the PC1 and total number of productive tillers per plant and number of earheads per plant were the

major traits contributed to PC2 in both the years. Conclusively, main earhead weight and total number of productive tillers per plant and number of earheads per plant were identified as important traits for both WW and DS conditions which can be exploited in crop improvement.

PCA-Bilpot analysis

PCA based biplot assesses the genotypes based on multiple traits and identifies the genotypes those are superior in the desired variables. The identified genotypes are being used as parental lines or trait donors in the breeding programs. A quick and practical visualization of the genetic correlation between traits is also provided by this analysis. Trait values across of 350 genotypes under WW and DS conditions for each year and the pair-wise correlation among the traits were used to create PCA based biplot (Fig. 4.2 and 4.3). Generally, traits are inter-correlated to varying level and hence all the principal components are not required to summarize the data effectively.

The important consideration of the biplot diagram is the angles of vectors. The angles between the vectors derived from the middle point of biplots exhibit positive or negative interactions of studied traits. The vector length (*i.e.* the distance to the biplot origin) of a trait indicates how well the trait is represented in the biplot; a relatively short vector indicates that the variation of the trait across genotypes is either small or not well presented in the biplot, which is due to its weak or lack of correlation with other traits. This can occur when the goodness of fit of the biplot is relatively poor. The cosine of the angle between the vectors of the two traits indicates the Pearson correlation between them. Therefore, an angle $< 90^\circ$ shows a positive correlation, an angle $> 90^\circ$ shows a negative correlation, and an angle of 90° shows a zero correlation or traits are independent to each other (Yan and Tinker, 2006; Oliveira *et al.*, 2018). However, it cannot exactly tell which genotype is best for which trait.

Based on this, bilpot indicated the seed yield per plant recorded positively and highly correlated with total earhead weight per plant and main earhead weight followed by total biomass, finger width, plant height at harvest, plant height at reproductive stage, main earhead length and finger width consistently in both the years under WW19 and WW20 conditions (Fig. 4.2a and 4.2b). The genotypes were identified that expressing

uniform superiority for these traits under WW19 and WW20 conditions. The genotypes G187, G106, G89, G170, G130 and G3 were found to be superior for total earhead weight per plant, main earhead weight, total biomass, finger width, plant height at harvest, plant height at reproductive stage, main earhead length and finger width under WW19 conditions (Fig. 4.2a). On the other hand, genotype G187, G311, G172, G177, G340, G346, G61 and G325 were found to be superior for the same traits under WW20 conditions (Fig. 4.2b). The genotype G187 found to be suitable genotype under WW conditions over the two years for the traits viz. total earhead weight per plant, main earhead weight, total biomass, finger width, plant height at harvest, plant height at reproductive stage, main earhead length and finger width. Under DS conditions, PCA biplot clearly depicts the seed yield per plant was highly and positively correlated with total earhead weight per plant and main earhead weight followed by plant height at harvest, culm length, total biomass and finger width in both years. The genotypes were identified that expressing uniform superiority for these traits under DS19 and DS20 conditions (Fig. 4.3a and 4.3b). The genotypes G3, G187, G302, G304, G326, G303 and G311 were found to be superior for total earhead weight per plant, main earhead weight, plant height at harvest, culm length, total biomass and finger width under DS19 conditions (Fig. 4.3a). Whereas, genotypes G107, G304 and G106 were found to be superior for these traits under DS20 conditions (Fig. 4.3b). The genotypes G3, G187 and G311 are found to good performing genotypes for total earhead weight per plant, main earhead weight, plant height at harvest, culm length, total biomass and finger width across the WW and DS conditions.

4.1.4.2 Genotype by Yield*Trait (GYT) analysis

The choice of genotypes must be based on multiple traits rather than limiting to single trait. Normally the practice is to select genotype based on single trait by fixing a minimum requirement for any trait, no matter how good genotype is for other traits. However, genotype by yield*trait (GYT) biplot approach provides useful information for genotype evaluation on multiple traits based on the superiority of a genotype measured not only by its levels in individual traits but more importantly by its levels in combining yield with other target traits (Yan and Frégeau-Reid, 2018). For example, the drought

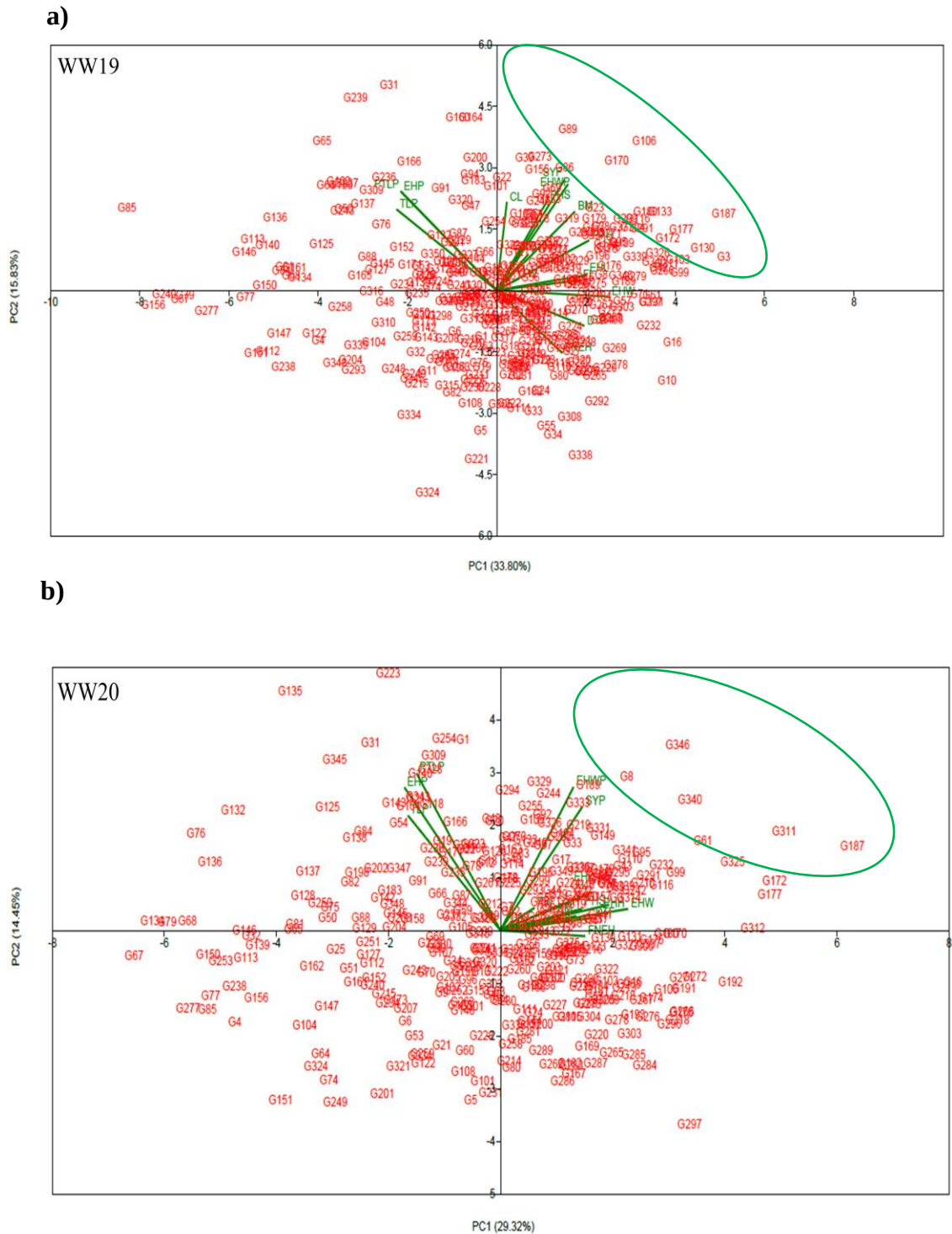


Figure 4.2 PCA-bilpot for the first two principal components in the PCA (Principal component analysis) for sixteen quantitative traits and 350 genotypes (G1-G350) in each year under well watered (WW) environment. a) WW19, b) WW20, Abbreviations for the traits as per table 3.1. Green circle indicated the best performing genotypes for the respective traits

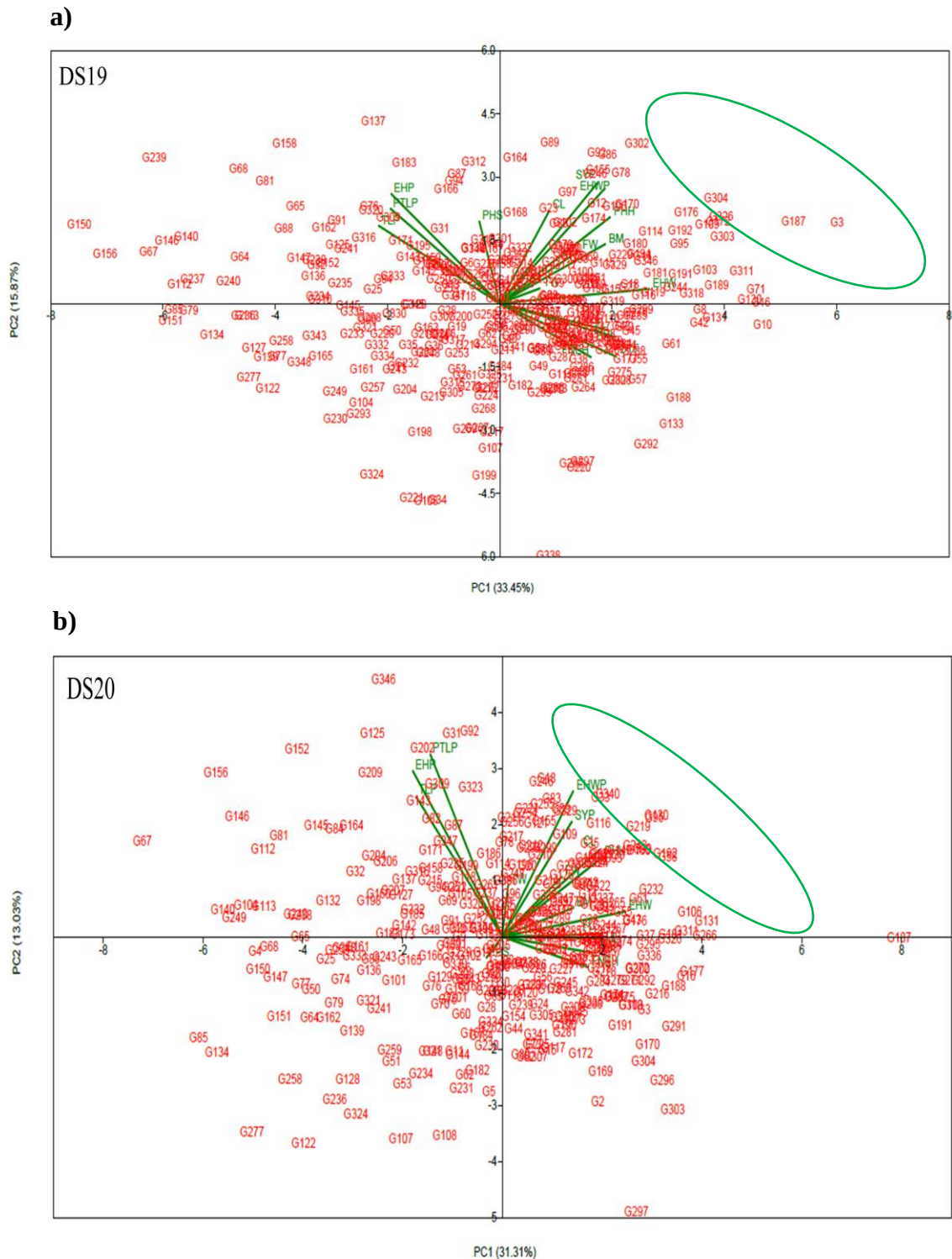


Figure 4.3 PCA-bilpot for the first two principal components in the PCA (Principal component analysis) for sixteen quantitative traits and 350 genotypes (G1-G350) in each year under drought stress (DS) environment. a) DS19, b) DS20, Abbreviations for the traits as per table 3.1 Green circle indicated the best performing genotypes for the respective traits

tolerant genotype is valuable only if it is combined with higher yield. The purpose of evaluation of genotypes for multiple traits is to achieve higher yield. The GYT biplot graphically ranks genotypes based on their superiority in combining yield with other target traits in each of the environment in the present study. The genotype by yield*trait (GYT) biplot approach is graphical, objective, effective, and straightforward which ranks genotypes based on their levels in combining yield with other target traits and at the same time shows their trait profiles (Yan and Frégeau-Reid, 2018). Therefore, in selecting the best genotypes, the combined effects of yield*trait are more meaningful than the effects of individual traits. As a result, the GYT analysis guarantees that selected genotypes and/or managements are superior in combining yield with other target traits and prevents low yielding genotypes and/or managements from being selected and recommended (Yan *et al.*, 2019). In conclusion, the GYT biplot applied in this study offers a new approach to genotyping based on multiple traits in finger millet. This approach is comprehensive and effective since genotypes are ranked based on their level in combination yield with different traits, and simultaneously showing the strengths and weaknesses of the genotypes. This methodology uses GGE (G plus GE) biplot analysis to identify target traits as well as genotype performance and to identify their strengths and weaknesses.

The mean data of sixteen agro morphological traits on 350 genotypes obtained under WW and DS conditions over the two years were used in GYT analysis. The seed yield per plant (Y) with each trait combinations are represented in biplot as Y*DTF, Y*PHS, Y*PHH, Y*TLP, Y*PTLP, Y*CL, Y*EHP, Y*EHL, Y*EHW, Y*EHWP, Y*FNEH, Y*FL, Y*FW, Y*TW and Y*BM. Since all yield-trait combinations had yield as a component, it allowed genotypes to be graphically and meaningfully ranked based on their yield-trait combinations on GYT biplot. The data for each genotype by trait (GT) first transformed into a genotype by seed yield*trait (GYT) and then displayed in a GYT biplot (Fig. 4.4 and 4.5). The different views of GYT biplot has been discussed below.

i) GYT biplot ‘which-won-where’ view

The ‘which-won-where’ polygon view is particularly useful for visualizing the trait profiles of the genotype. The irregular polygon was formed by connecting the genotypes with the longest vectors in all directions. For each polygon side a line was

drawn to start from the biplot origin and to be perpendicular to the polygon side. These lines divided the yield-trait combinations into two sectors; corresponding to each sector there was a polygon vertex. The geometry of the biplot determines that the genotype placed on a vertex has the largest values for the yield-trait combinations placed within the corresponding sector. Thus under WW conditions, the genotypes G31, G55, G136, G239, G223, G58, G135, G158 and G166 had the higher values for Y*EHP, Y*TLP and Y*PTLP meaning that, these genotypes are best in combining seed yield per plant with number of earheads per plant, total number of tillers per plant and total number of productive tillers per plant (Table 4.14; Fig. 4.4a). The study also verified that there is a potential for simultaneous genetic improvement of the number of earheads per plant, total number of tillers per plant and total number of productive tillers per plant reported in earlier studies in finger millet by Jyothsna *et al.* (2016), Mahanthesha *et al.* (2018), Kandel *et al.* (2019), Sapkal *et al.* (2019) and Chavan *et al.* (2020). These genotypes can be used as a donor parents to combine these traits with higher seed yield. The other genotypes G340, G311, G307, G8, G16, G172 and G61 recorded higher values for Y*EHWP, Y*PHS, Y*CL, Y*TW, Y*FW, Y*BM, Y*DTF, Y*PHH, Y*FNEH, Y*EHL and Y*FL (Table 4.14). This means that, these genotypes were the best in combining seed yield per plant with total earhead weight per plant, plant height at reproductive stage, culm length, test weight, finger width, total biomass, days to flowering, plant height at harvest, finger number per earhead, main earhead length and finger length. However, the genotypes G187, G265, G297 and G276 were found to be best in combining seed yield per plant with main earhead weight (Y*EHW) under well watered condition. Earlier reports pointed that, the test weight, finger number per earhead and plant height (Abraham *et al.*, 1989; John, 2007; Shet *et al.*, 2010), number of productive tillers per plant and number of fingers per earhead (Ganapathy *et al.*, 2011), finger width and total biomass (Priyadharshini *et al.*, 2011; Mahanthesha *et al.*, 2018; Kandel *et al.*, 2019), are the important traits for yield improvement. Hence, selection based on these yield*trait combination will be rewarding for yield improvement in finger millet genotypes.

Table 4.14 Genotypes identified based on yield*trait combinations identified through GYT biplot under WW, DS and WWDS conditions over the two years

Treatment	Genotype	Yield (Y)* trait combination
WW	G31, G55, G136, G239, G223, G58, G135, G158, G166	EHP, PTLP, TLP
	G340, G311, G307, G8, G16, G172, G61	EHWP, PHS, CL, TW, FW, BM, DTF, PHH, FNEH, EHL, FL
	G187, G265, G297, G276	EHW
DS	G239, G81, G237, G290, G228, G86, G141, G89	EHP, PTLP, TLP
	G155, G71, G326, G170, G302, G119, G115, G304, G311, G337, G3	EHWP, PHS, CL, TW, FW, BM, DTF, PHH, FNEH, EHL, FL
	G189, G110	EHW
WWDS	G81, G239, G136, G31, G309, G135, G158	EHP, PTLP, TLP
	G170, G155, G340, G326, G339, G307, G336	PHS, CL, TW, FW, EHWP
	G311, G16, G71, G187, G172, G3, G99, G61	EHW, BM, DTF, PHH, FNEH, EHL FL

Days to flowering (DTF), Plant height at reproductive stage (PHS), Plant height at harvest (PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS), across well watered and drought stress (WWDS)

Whereas under DS conditions, the genotype G239, G81, G237, G290, G228, G86, G141 and G89 recorded highest yield trait combination value for Y*EHP, Y*TLP and Y*PTLP meaning that, these genotypes were the best in combining seed yield per plant with number of earheads per plant, total number of tillers per plant and total number of productive tillers per plant (Table 4.14; Fig. 4.4b). The genotypes G155, G71, G326, G170, G302, G119, G115, G304, G311, G337 and G3 recorded higher value for Y*EHWP, Y*PHS, Y*CL, Y*TW, Y*FW, Y*BM, Y*DTF, Y*PHH, Y*FNEH, Y*EHL and Y*FL indicating these genotypes are best in combining seed yield per plant with total earhead weight per plant, plant height at reproductive stage, culm length, test weight, finger width, total biomass, days to flowering, plant height at harvest, finger number per earhead, main earhead length and finger length. However, the genotypes G189 and G110 were found to be best in combining seed yield per plant with main earhead weight (Y*EHW) under well watered condition. Under rainfed conditions, total biomass (Anjum *et al.*, 2011), tiller numbers per plant (Mujahid *et al.*, 2020), mean earhead weight, test weight and number of productive tillers per plant are found to be more important in determining seed yield (Gupta *et al.*, 2011; Reddy, 2020).

Similarly, across both well watered and drought stress (WWDS) condition, the genotype G81, G239, G136, G31, G309, G135 and G158 exhibited highest yield trait combination value for Y*EHP, Y*TLP and Y*PTLP meaning that, these genotypes were the best in combining seed yield per plant with number of earheads per plant, total number of tillers per plant and total number of productive tillers per plant (Table 4.14; Fig. 4.4c). Whereas, the genotypes G170, G155, G340, G326, G339, G307 and G336 recorded highest value for Y*PHS, Y*CL, Y*TW, Y*FW and Y*EHWP indicating that these genotypes are best in combining seed yield per plant with plant height at reproductive stage, culm length, test weight, finger width and total earhead weight per plant. However, the genotypes G311, G16, G71, G187, G172, G3, G99, G61 recorded higher values for Y*EHW, Y*BM, Y*DTF, Y*PHH, Y*FNEH, Y*EHL and Y*FL indicating these genotypes are suitable for combining seed yield per plant with main earhead weight, total biomass, days to flowering, plant height at harvest, finger number per earhead, main earhead length, and finger length. The results indicated that, though

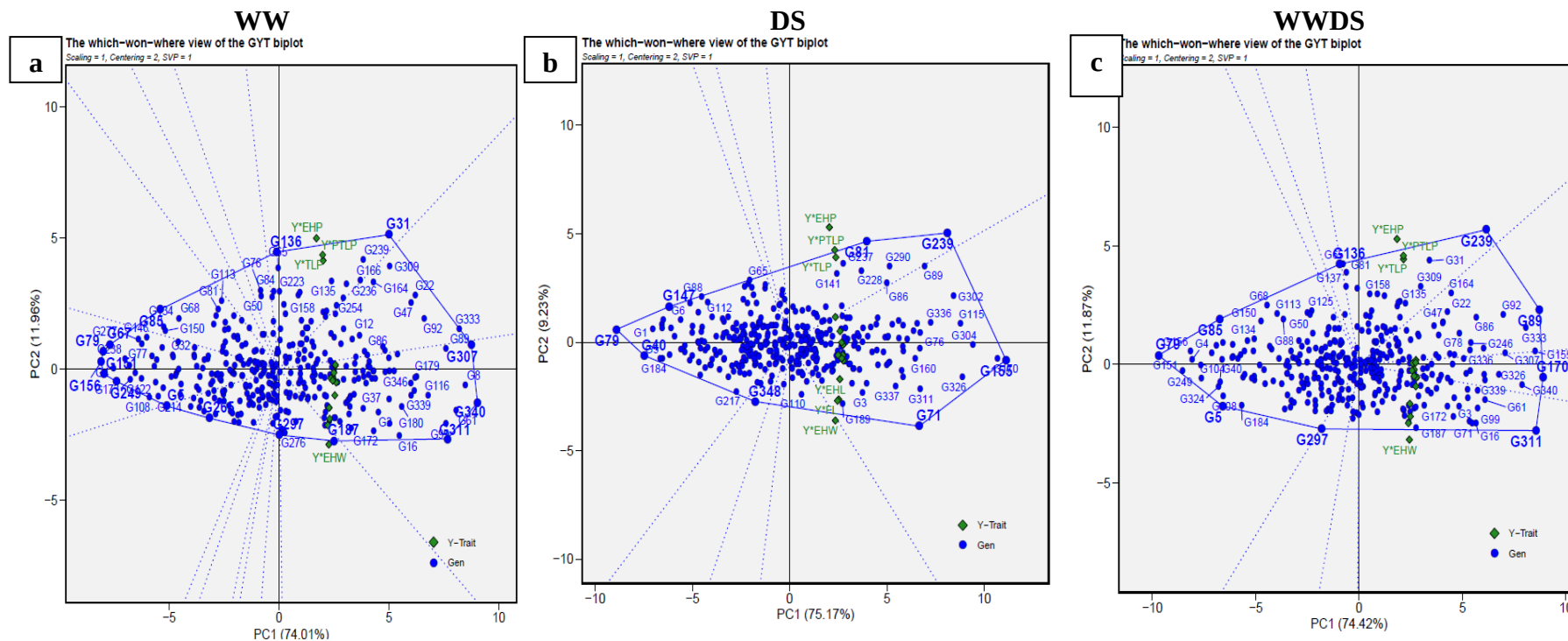


Fig. 4.4 The ‘which-won-where’ view of Genotype by Yield*Trait (GYT) biplot describing the 350 genotypes (G1-G350) with one or more trait combination for yield under (a) WW, (b) DS and (c) across WWDS conditions³

the yield*trait combinations are similar, the genotypes were performing differentially for yield*trait combinations under both well watered and drought stress condition.

It has been also observed that, the genotype which performed better in WW environment has been performed poorly in DS environment and *vice versa*. Thus it has been inferred that, the selection of donor genotype for yield improvement must be chosen on yield*trait combinations and environment. Hence, selection based on multiple traits can produce balanced outcomes for yield under DS environment. The use of the GYT biplot in selecting multiple traits became a promising strategy as compared to the traditional drought tolerance indices. The differential of this approach allowed the selection of the most yielding genotypes associated with the other evaluated traits in oat (Yan *et al.*, 2019). The genotype by yield*trait (GYT) biplot for genotype selection and trait profiles was used for exploring the combination of yield with traits and trait profiles of the genotypes to obtain high genetic gains in crops like cow pea (De Oliveira *et al.*, 2019; Da Cruz *et al.*, 2020; Araújo *et al.*, 2021), durum wheat (Mohammadi, 2019; Kendal, 2019), sesame (Boureima and Abdoua, 2019), soybean (Woyann *et al.*, 2020), oat (Yan *et al.*, 2019) and barley (Kendal, 2020) has been reported.

ii) GYT biplot- Average Tester Coordination (ATC) view

The Average Tester Coordination (ATC) view of the Genotype by Yield*Trait (GYT) biplot model to rank the genotypes based on their overall superiority for yield*trait combination under WW, DS and WWDS conditions represented in figure 4.5.

The small arrow in the biplot represents the placement of the “average yield-trait combination,” which is determined by the coordinates of all yield-trait combinations included in the biplot. The line with a single arrow passes through the biplot origin and the average yield-trait combination and is called the average tester axis (ATA). The arrow points to higher mean values for the genotypes, across all yield-trait combinations. The ATA serves the purpose of ranking the genotypes based on their overall superiority or usefulness. The vertical line passes through the biplot origin and is perpendicular to the ATA line serves to separate genotypes better than average (placed on its right, on the same side as the ATA arrow) from those poorer than average (placed on the left side). This separation intuitively suggests the researcher to focus on the genotypes ranked better

than average. The vertical line also helps indicate whether a genotype had an all-rounded or balanced trait profile or had obvious strengths and/or weaknesses; the latter determines how a “useful” genotype should be used in terms of environmental adaptation and/or end use. Genotypes placed close to ATA (*i.e.* with short projections to the vertical line) tend to have balanced trait profiles whereas those placed away from the ATA in either direction tend to have obvious strengths and/or weaknesses (Yan and Frégeau-Reid, 2018).

In accordance with this, the genotype G307 exhibited highest balanced trait profile for overall yield*trait combinations followed by G333, G8, G89, G340, G61, G99, G311, G92, G47 and G22 under WW conditions over the two years (Table 4.15; Fig. 4.5a). The poor performance for yield*trait combinations was recorded in G151, G56, G79 and G67. Whereas under DS conditions, the genotype G155 recorded highest balanced trait profile for overall yield*trait combinations followed by G170, G304, G115, G326, G302 and G239 (Table 4.15; Fig. 4.5b). However, genotype G79 identified for poor performance for yield*trait combinations under DS conditions. Interestingly it was observed that, the genotype G155 showed best overall performance for all the traits under DS condition where as it was poor under WW condition. The performance of genotypes for yield related traits combinations were different in WW and DS conditions. Hence, different genotypes for different situations should be selected as trait donor parents. The overall performance of the genotypes for yield*trait combinations across WWDS conditions were assessed. The results indicated that, G170, G89, G155, G33G, G311, G340, G333, G307 and G92 found to be best balance trait profile genotypes for overall yield*trait combinations across WWDS conditions (Table 4.15; Fig. 4.5c).

Table 4.15 Genotypes with overall balanced of yield*trait combinations identified through GYT biplot under WW, DS and WWDS conditions over the two years

Treatment	Genotypes
WW	G307, G333, G8, G89, G340, G61, G99, G311, G92, G47, G22
DS	G155, G170, G304, G115, G326, G302, G239
WWDS	G170, G89, G155, G33G, G311, G340, G333, G307, G92

Well watered (WW), Drought stress (DS), across well watered and drought stress (WWDS)

However, genotype G76, G151 and G156 found to be poor performing for yield*trait combination across WWDS conditions. The results indicated that, pool of genotypes with balanced performance with respect to all yield*trait combinations. These genotypes can be selected for yield improvement program rather than only few combinations to improve yield under drought stress conditions.

4.1.4.3 Genotype plus Genotype-by-Environment (GGE) analysis

The genotype main effect plus genotype $\times$ environment interaction (GGE) biplot used to understand interrelationship between genotypes, environment and the interaction and thus widely used to identify the stable and high yielding genotypes based on two principal components under and across the multiple environments (Yan, 2001; Yan and Tinker, 2006). In GGE biplot, the main effect of the genotype and G $\times$ E interaction are evaluated simultaneously. GGE biplots show which genotype(s) has (have) the highest yield and stability performance in which environment(s) (Yan and Kang 2002). The present experiment involved evaluation of genotypes in two environment viz. DS and WW in each year over the two years thus totally four environments. In order to understand and to explain genotype $\times$ environment interaction different statistical methods have been developed including biplots derived from additive main effects and multiplicative interaction (AMMI; Gauch, 1988), best linear unbiased prediction (BLUP) and genotype plus genotype $\times$ environment interaction (GGE; Yan *et al.*, 2000; Yan and Kang, 2002) are being used. Among them, GGE-biplot method is more popular useful in identifying stable and high yielding genotypes in multi environmental interaction. GGE biplot used to visual analysis of genotype $\times$ environment interaction estimate variance compound and predict genetic value and select genotypes that serve a stable and adapted to tolerant environment.

In the present study, the high yielding and stable genotypes under WW, DS and across WWDS conditions were identified using GGE biplot method based on the common traits viz. total earhead weight per plant, main earhead weight and seed yield per plant which was identified through multiple stepwise regression analysis and other statistical methods mentioned earlier. Three major components can be elucidated using the biplot such as (i) ‘which-won-where’ pattern, proposed by Yan *et al.* (2000), is an

effective approach to visualize the pattern of genotype $\times$ environment interaction (GEI) based on the correlation between G and E; (ii) stability vs mean performance over the environment for genotype evaluation; (iii) ranking genotypes relative to the ideal genotype (average-environment coordination or AEC view of the graph).

i) GGE biplot ‘which-won-where’ pattern

The which-won-where pattern of multi-environment data is necessary to analyze the possible existence of different environments in a region (Yan, 2001). The polygon view of the GGE biplot pattern for seed yield per plant has been illustrated in Fig. 4.6a, 4.6b and 4.6c for WW, DS and across well watered and drought stress (WWDS) environments separately. Similarly, it has been illustrated in Fig. 4.6d, 4.6e and 4.6f for total earhead weight per plant and in Fig. 4.6g 4.6h and 4.6i main earhead weight WW, DS and across WWDS conditions respectively. The environmental indicators positioned into different segments or sections of biplot with different genotypes winning in each segment. The genotype that attached with a vertex of the polygon in a sector of environment markers suggested, such genotype provided greater yield and performed best in that environment. Based on the 350 genotypes and two year well watered conditions (WW19) and (WW20), the generated GGE biplot for seed yield divided into 10 fan-shaped sections (Fig. 4.6a). The genotype G307 recorded the highest seed yield per plant followed by G22 and both were highly stable in WW19 (E1). Whereas, genotype G8 performed was the best for seed yield in WW20 (E2) condition (Fig. 4.6a). Under drought stress conditions, the GGE biplot divided into 12 fan-shaped sections (Fig. 4.6b). The genotype G229 exhibited highest seed yield per plant followed by G92 in DS19 (E1), whereas G267 followed by G293 performed better in DS20 (E2) (Fig. 4.6b). The GGE biplot generated to identify the genotype with higher seed yield per plant across the four environments viz. WW19 (E1), WW20 (E2), DS19 (E3) and DS20 (E4) showed 16 fan-shaped sections (Fig. 4.6c). The positioning of all environmental indicators into one section of biplot directed that a unique genotype performs best under all tested environments. However, E1, E2, E3 and E4 positioned into different sections of biplot indicating that there were no unique genotype under WW and DS conditions over the two years. The genotype G89, G92 and G267 performed better across the WW and DS

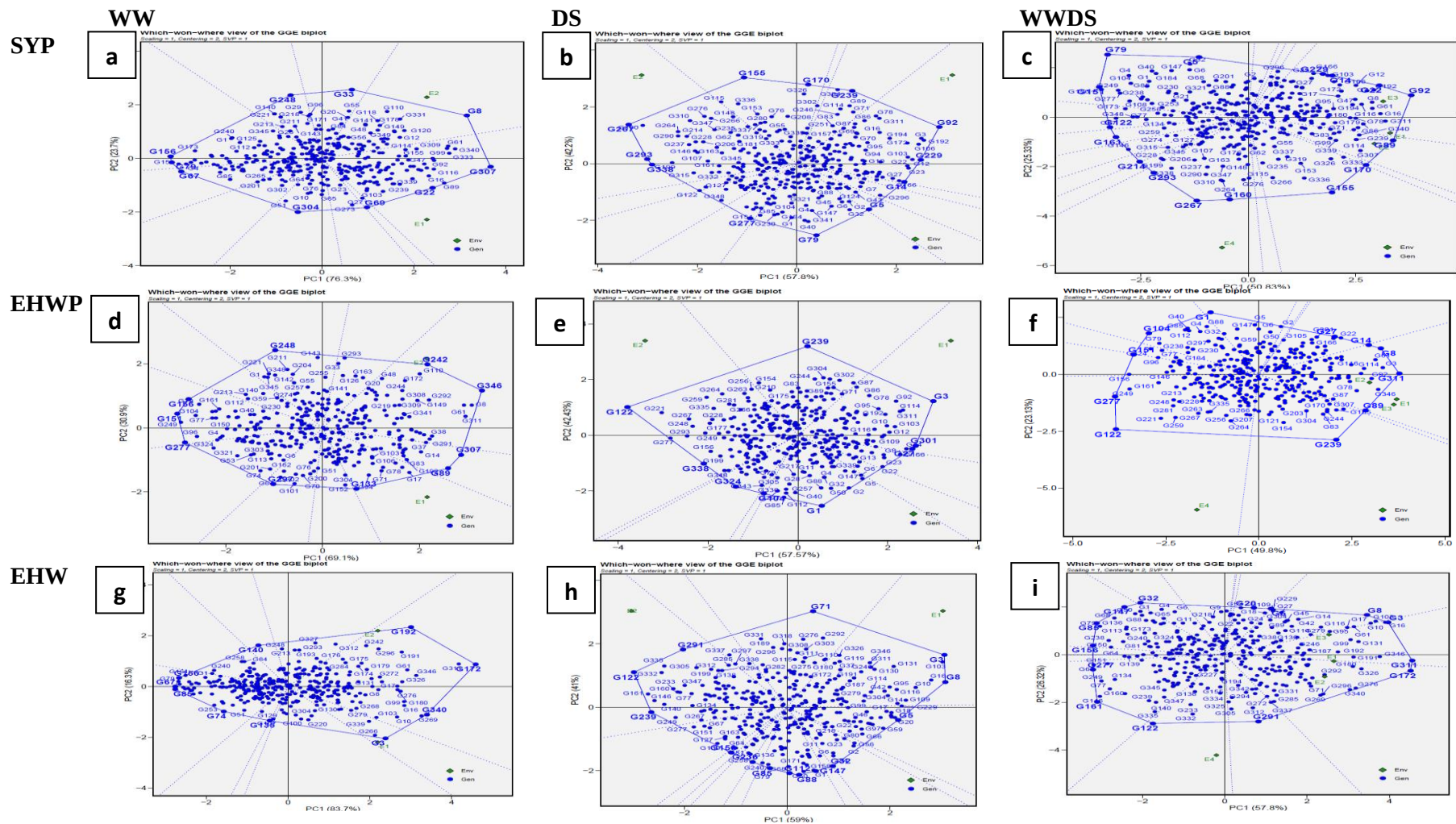


Fig. 4.6 The which-won-where view of the genotype plus genotype by environment (GGE) model to highlight genotypes with their performance under WW, DS and across both WWDS conditions based on the selected traits viz. SYP.

conditions in WW20 (E2), DS19 (E3) and DS20 (E4) conditions respectively (Fig. 4.6c). The genotypes placed within the polygon are less responsive to the environment than the corner genotypes. Besides, the genotypes placed at the polygon vertex in a section of biplot where there is no environmental indicator are treated as poorly performing genotypes under all tested environments (Oladosu *et al.*, 2017).

The traits viz. total earhead weight per plant and main earhead weight were predicted as important traits associated with seed yield per plant in terms of higher contribution to the total variation and high correlation under WW and DS conditions. Hence, GGE plot generated to identify superior and stable genotypes for total earhead weight per plant and main earhead under both WW and DS conditions. The which-won-where GGE biplot for total earhead weight per plant was divided into 10, 9 and 12 fan-shaped sections for WW, DS and WWDS environments (Fig. 4.6d, 4.6e and 4.6f). The genotype G307 and G346 recorded higher total earhead weight per plant under WW19 (E1) and WW20 (E2) respectively (Fig. 4.6d). The genotype G301 and G277 performed better under DS19 (E1) and DS20 (E2) conditions respectively (Fig. 4.6e). The two genotypes G311 and G8 performed better for total earhead weight per plant across WW and DS conditions in the three tested environment viz. WW19 (E1), WW20 (E2) and DS19 (E3) (Fig. 4.6f). The GGE biplot for main earhead weight was divided into 9, 13 and 12 fan-shaped section sections for WW, DS and WWDS environment (Fig. 4.6g 4.6h and 4.6i). The genotype G3 recorded high main earhead weight under E1(WW19) (Fig. 4.6g). Whereas, genotypes G172, G340 and G192 recorded better performance for main earhead weight under WW20 (E2). The genotype G3 in DS19 (E1) and the genotypes G8, G239, G122 and G291 performed better under DS20 (E2) conditions (Fig. 4.6h). The genotype G311 performed better across two different environments WW19 (E1) and DS19 (E3) (Fig. 4.6i).

ii) GGE biplot pattern of ‘mean vs. stability’

The average environment coordinate (AEC) line crosses through the biplot’ s origin if genotype focused SVP = 1 (single value portioning into the genotype eigenvectors) were used to assess the genotype performance in terms of their stability for the trait over and across the environment. The ‘mean vs. stability’ view frequently stating

to as AEC and SVP that helps to simplify the genotype assessment based on the mean performance and stability under a wide range of environment (Fig. 4.7). The single arrowed line is AEC abscissa (or AEA) line consists of a single arrow that pointed towards higher mean performance for each trait across environments. The arrow sign on the AEC abscissa line directed the ranking of genotypes in increasing order with a greater value of traits evaluated. The genotype G307 and G170 recorded higher mean performance and with high stability for seed yield per under WW, DS and across the WWDS environment over the two years (Fig. 4.7a 4.7b and 4.7c). The dotted projection line in the ‘mean vs. stability’ biplot is the AEC ordinate; it points to greater variability (poorer stability) of the genotype in either direction with respect to the trait. In accordance with this, the genotypes viz. G8, G155, G89 recorded high seed yield per plant but high variability observed under WW, DS and WWDS environments thus were considered as poorly stable genotypes across the environment. The higher mean performance for total earhead weight per plant was recorded in G311 and G239 under WW and DS conditions respectively (Fig. 4.7d and 4.7e). However, genotype G239 recorded overall high mean performance and stability across the tested WWDS environments (Fig. 4.7f) for total earhead weight per plant. Similarly, the higher mean performance and stability for main earhead weight were recorded in the genotype G172 and G71 under WW and DS conditions respectively (Fig. 4.7g and 4.7h) over the two years. The genotype G340 found to be highly stable with high value of main earhead weight across WWDS environment (Fig. 4.7i).

iii) Genotype ranking: best and ideal genotype assessment

An ideal genotype should have both high mean performance and high stability across environments. The genotype positioned near to the concentric rings, determining the best performing genotype and the less projection from average environment coordinate (AEC) abscissa indicate the genotype stability (Yan and Tinker, 2006). Therefore, genotypes located closer to the ‘ideal genotype’ are more desirable than others. The genotype G307 found to be the ideal followed by G333 and G340 under well watered conditions for seed yield per plant (Fig. 4.8a). Under drought stress, genotype G170 followed by G326 were recorded as ideal for higher mean and high stability (Fig.

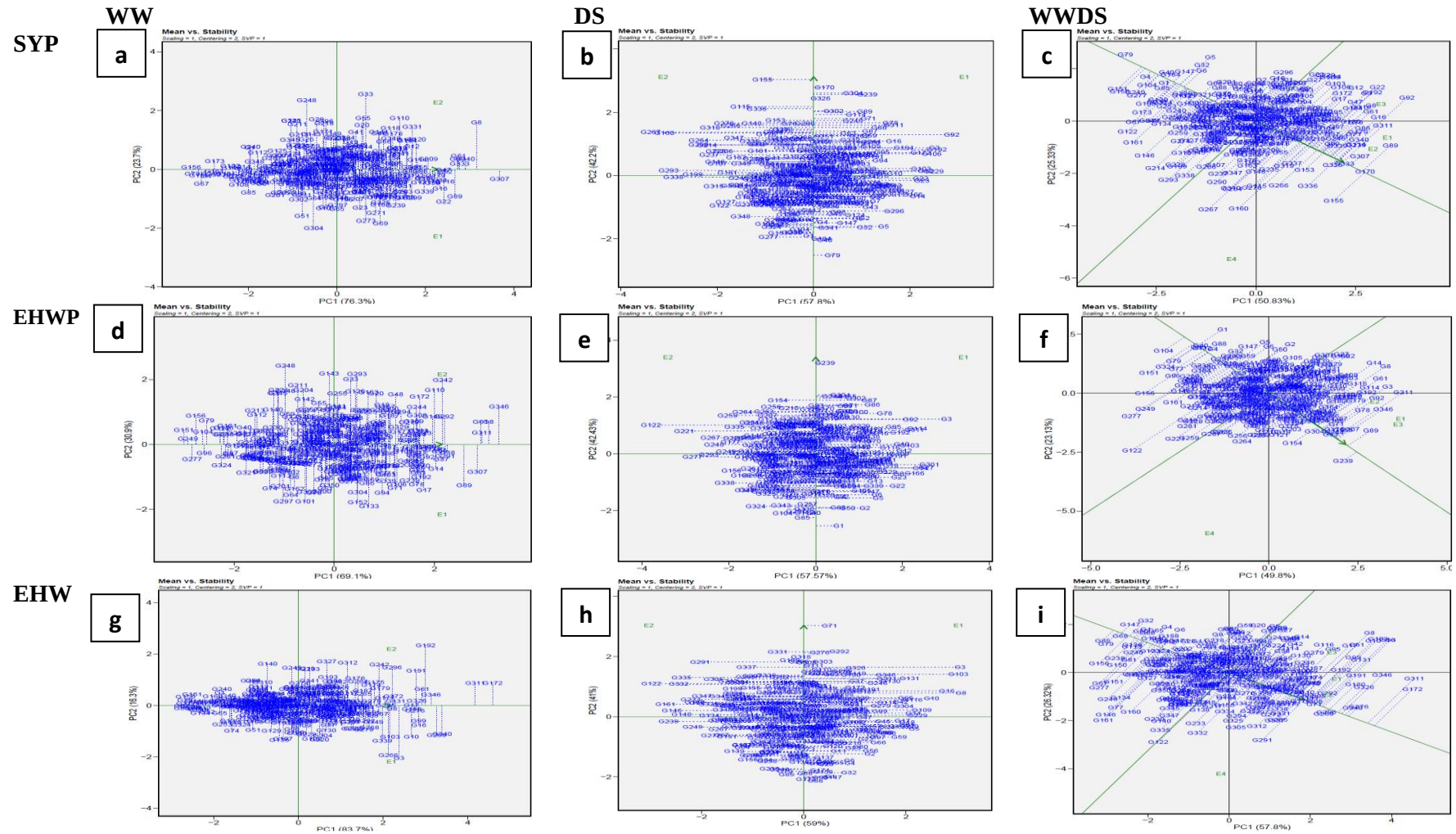


Fig. 4.7 The Mean vs Stability view of the genotype plus genotype by environment (GGE) model to highlight genotypes with their performance under WW, DS and across both WWDS conditions based on the selected traits viz. SYP, (a,b,c) EHWP(d,e,f)

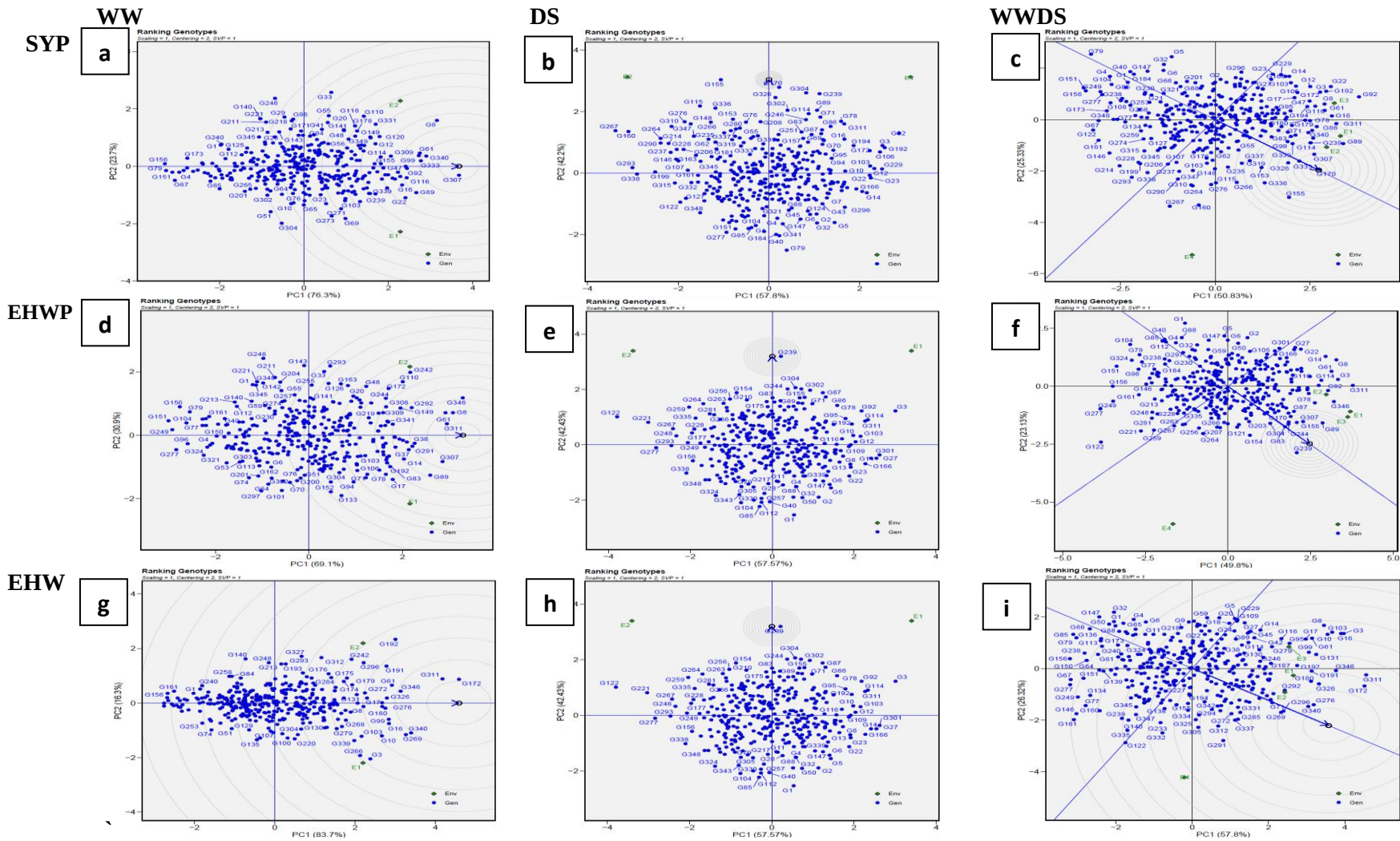


Fig. 4.8 The genotype ranking view of the genotype plus genotype by environment (GGE) model to highlight genotypes with their performance under WW, DS and across both WWDS conditions based on the selected traits *viz.* SYP, (a,b,c) EHWP(d,e,f) and EHW (g,h,i).

4.8b) for seed yield per plant. However, genotype G170 also exhibited ideal performance for seed yield per plant across the entire tested WWDS environments followed by G333 and G326 (Fig. 4.8c). The genotypes G311 and G239 were recorded high and stable performing genotypes under WW and DS conditions for total earhead weight per plant (Fig. 4.8d and 4.8e). The genotype G239 followed by G244 and G83 were recorded as high and stable performance for total earhead weight per plant across the tested WWDS environments (Fig. 4.8f). The genotype G172 and G71 were found to be the ideal genotypes for main earhead weight under WW and DS environments respectively (Fig. 4.8g and 4.8h). However, genotype G340 followed by G276 and G296 recorded as ideal genotypes for main earhead weight for WWDS environment (Fig. 4.8i).

The identification of suitable genotype/s for certain environment is difficult task due to high genotype $\times$ environment interaction (GEI). The majority of genotypes tested in multiple environments are examined based multiple traits. In the crop performance, GEI is important when different genotypes respond to different environments (Farshadfar *et al.*, 2012). The GGE biplot provides a graphical representation to explore the interactions between genotype by environment, genotype by traits or environment by traits (Akcura *et al.*, 2016; Karimizadeh *et al.*, 2016). It is evident that, a genotype exhibiting good stability for one trait can be less stable for another trait and *vice-versa* because different set of genes govern different traits and environment has varying influence on their performance. Hence, the suitable genotypes for seed yield per plant, total earhead weight per plant and main earhead weight under well watered and drought stress conditions are identified separately based on their performance in each year under WW and DS conditions. The GGE biplot was used to identify superior genotypes in each environment or across multiple environments in many crops such as sorghum (Rakshit *et al.* 2012), maize (Kuchanur *et al.*, 2015; Choudhary *et al.*, 2019), watermelon (Dia *et al.* 2016), wheat (Kumar *et al.* 2018), peanut (Lal *et al.* 2019) and finger millet (Anuradha *et al.*, 2022). Conclusively, the genotypes G307, G333, G326, G340 and G170 recorded higher mean performance with high stability for seed yield per plant under WW, DS and across both the environments. Under drought stress, genotype G92, G170, G229 and G326 were found to be superior for seed yield as per GGE biplot. The genotype G311 and G239 recorded high and stable performance under WW and DS conditions for total

earhead weight per plant. The genotype G340 was identified as an ideal genotype for main earhead weight and seed yield per plant under WW and DS conditions.

4.1.5 Determination of drought tolerance indices (DTI) and selection of genotypes for drought tolerance

Drought tolerance is a complex trait and the lack of fast reproducible screening technique based on a reliable inheritable trait linked to drought tolerance remains a challenge to identify tolerant genotype under unpredictable drought stress conditions. The indirect selection indicators such as morphological and physiological responses (Kumar *et al.*, 2015c; Purbajanti *et al.*, 2017) are frequently used, and such indirect selection methods relay on many characters that need to be evaluated and the selected tolerant genotypes may not necessarily be stable for yield *per se* under stress conditions. So there is no reliable trait linked to drought tolerance not only in finger millet but also in other crops. For this reason, selection for drought tolerance based on indices developed from grain yield is considered a more rapid and effective approach for selecting drought tolerant genotypes.

The average seed yield per plant under WW condition was 14.26 ± 0.22 and 16.16 during 2019 and 2020 respectively. The mean seed yield under DS condition greatly reduced to 8.93 and 10.31 g per plant during 2019 and 2020 respectively (Table 4.3 and 4.4). The seed yield reduction under DS compared to WW conditions in both the seasons indicated that drought stress at initial reproductive stage affected performance of the genotypes. At reproductive stage, drought stress alters the physiological mechanism which ultimately affects the yield and related traits of finger millet (Krishna *et al.*, 2021). Earlier findings reported that the drought stress reduce the seed yield in finger millet up to 25 *per cent* (Anon, 2008; Reddy *et al.*, 2020). The seed yield reduction also depends on the severity, duration and the stage of the crop. In the present study, reduction in grain yield due to reproductive stage drought stress was 41.45 *per cent* and 35.62 *per cent* during DS19 and DS20 respectively. The genotypes studied recorded significant variability for seed yield under both WW and DS conditions in both the years. The significant variability among germplasm lines for seed yield under WW and DS conditions has been reported in finger millet (Patil *et al.*, 2021). The seed yield per plant

under WW condition was varied from 4.38 to 25.96 g and from 5.98 to 30.00g in 2019 and 2020 respectively. Whereas, under DS the seed yield per plant ranged from 1.66 to 17.86 g and from 2.64 to 22.26 g during 2019 and 2020 respectively (Table 4.3 and 4.4). The reduction in seed yield due to moisture stress was also significant among the genotypes suggesting genotypic tolerance to drought stress. A few genotypes produced high seed yield with a small reduction compared to WW condition under stress environment. However it is difficult to conclude the tolerance of a genotype by *per se* performance under stress environment (Edmeades, 2013). The plants cope with drought stress by a mixture of strategies that vary with genotypes and growth stage of the crop. In this regard, several drought tolerance indices (DTI) have been proposed to select genotypes based on their productivity in an environment under drought stress or without stress conditions (Naghavi *et al.*, 2013).

Stable yield performance of genotypes under both favorable and drought stress conditions is vital for plant breeders to identify drought tolerant genotypes. The high-yielding genotypes under optimum conditions may not be drought tolerant (Mardeh *et al.*, 2006). Therefore, many studies preferred the selection based on drought tolerant indices that provide a measure of drought based loss in yield in comparison to well watered condition for screening the genotypes (Ali and El-Sadek, 2016). Different drought tolerance indices have been proposed as selection criteria for selection of drought tolerant genotypes. The use of drought tolerance indices such as geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HM), drought resistance index (DRI), yield index (YI), yield stability index (YSI) has been widely studied to identify drought tolerant genotypes in different crops (Naghavi *et al.*, 2013; Jalilvandy and Rozrokh, 2013; Menezes *et al.*, 2014; Darkwa *et al.*, 2016).

In the present study, seed yield under stress and well watered conditions were primarily used to derive the different drought tolerance indices of the genotypes. The drought tolerance indices viz. GMP, MP, HM, DRI, YI and YSI were calculated for each genotype for both the years separately. High variability was observed among genotypes for all the indices studied (Table 4.16). The genotypes were classified into five groups viz. tolerant, moderately tolerant, moderately susceptible, susceptible and highly

susceptible based on their DTI values (Table 4.17). In both the years, majority of the genotypes were grouped under moderately susceptible followed by susceptible group for all the indices and a very few genotypes recorded high susceptibility. During 2019, in GMP 12 genotypes, MP 11 genotypes, HM 13 genotypes, DRI 15 genotypes, YI 13 genotypes and YSI only one genotype and in 2020, 13 genotypes in GMP, 11 genotypes in MP, 12 genotypes in HM, 17 genotypes in DRI, 15 genotypes in YI and 05 genotypes in YSI were found in drought tolerant group (Table 4.18). In both the years, the number of genotypes in tolerant group was least for YSI compared to other DTI. However some studies pointed out that the genotypes showing low fluctuation of yield under various levels of drought stress can be considered as drought tolerant along with drought tolerance indices for the stability of tolerance in the genotype (Ali and El-Sadek, 2016). Thus, by combining the performance of genotypes in both the years and across all the DTI, three genotypes viz. GE-1234, GE-1286 and GE-3003 were considered as drought tolerant genotypes (Table 4.19). Among the three tolerant genotypes, the genotype GE-3003 found consistently in tolerant group for all the DTI and in both the years. Similarly, GE-1234 was also found in tolerant group for all the DTI in both the years except for YSI in 2019. Across all the DTI in both the years the genotypes GE-1641, GE-3722 and GE-4719 were considered as highly drought susceptible genotypes. Out of the three susceptible genotypes, the genotype GE-1641 found consistently in susceptible group for all the DTI except for MP in 2020. Similarly, GE-4719 was also found in susceptible group for all the DTI except MP in 2019. The highest seed yield reduction under stress environment among tolerant genotype was observed in GE-1286 (26.42%) during 2020 followed by GE-1234 (25.25%) in 2019. The least reduction in seed yield among tolerant genotypes under stress was observed in GE-1286 (17.46%) during 2019 followed by GE-3003 (3.86%) in 2020 (Table 4.19). These genotypes recorded high values for drought tolerance indices viz. GMP, MP, HM, DRI, YI and YSI in both the years. The lowest seed yield per plant under drought stress was recorded in genotype GE-1641 (1.76 g) during 2019 and in GE-4719 (1.34 g) during 2020. The highest reduction in seed yield per plant over the two years was recorded in GE-3722 (83.68% and 83.50%) followed by GE-4719

Table 4.16 Mean and range of different drought tolerance indices over the two years

DTI	Year	Mean $\pm$ SE	Range (min-max)
GMP	2019	10.79 $\pm$ 0.18	3.47-19.65
	2020	11.49 $\pm$ 0.19	3.16-22.54
MP	2019	11.32 $\pm$ 0.17	4.11-20.09
	2020	11.99 $\pm$ 0.18	4.01-22.84
HM	2019	10.31 $\pm$ 0.18	2.80-19.56
	2020	11.05 $\pm$ 0.19	2.27-22.48
DRI	2019	0.37 $\pm$ 0.01	0.01-1.03
	2020	0.38 $\pm$ 0.01	0.01-1.24
YI	2019	1.00 $\pm$ 0.02	0.19-2.12
	2020	0.99 $\pm$ 0.02	0.14-0.32
YSI	2019	0.60 $\pm$ 0.01	0.11-0.98
	2020	0.59 $\pm$ 0.01	0.14-0.98

Table 4.17 Number of genotypes into different drought tolerance categories based on drought tolerance indices over the two years

Category	Year	GMP	MP	HM	DRI	YI	YSI
Tolerant	2019	12	11	13	15	13	01
	2020	13	11	12	17	15	05
Moderately tolerant	2019	35	45	42	38	36	58
	2020	41	43	40	33	38	48
Moderately susceptible	2019	241	236	230	231	235	233
	2020	239	242	241	252	238	239
Susceptible	2019	57	54	60	54	60	50
	2020	53	48	52	38	57	46
Highly susceptible	2019	05	04	05	12	04	08
	2020	04	06	05	10	02	12

Where, Geometric mean productivity (GMP), Mean productivity (MP), Harmonic mean (HM), Drought resistance index (DRI), Yield index (YI), Yield stability index (YSI)

Table 4.18 Drought tolerant and highly susceptible genotypes based on drought tolerance indices over the two years

Year	DTI	Tolerant genotypes	Highly susceptible genotypes
2019	GMP	GE-1286, GE-1260, GE-3758, GE-5118, GE-156, GE-2644, GE-1077, GE-1026, GE-290, GE-3003, GE-1234, GE-1013	GE-2272, VL-146, GE-4719, GE-2215, GE-1641
	MP	GE-1260, GE-1286, GE-3758, GE-5118, GE-290, GE-156, GE-2644, GE-1026, GE-1077, GE-1234, GE-3003	GE-1080, GE-1641, GE-2215, GE-2272
	HM	GE-1286, GE-1260, GE-3758, GE-5118, GE-260, GE-156, GE-2644, GE-1077, GE-3003, GE-1026, GE-1234, GE-5004, GE-290, GE-1013	VL-146, GE-4839, GE-1743, GE-1641, KJNS-52
	DRI	GE-1286, GE-5004, GE-3090, GE-1381, GE-4995, GE-4013, GE-3003, GE-3758, GE-2664, GE-6595, GE-1077, GE-1531, GE-1234, GE-3638, GE-5118	GE-1417, GE-4601, GE-6336, GE-3101, GE-4951, GE-3722, VL-146, GE-1641, GE-4719, GE-1684, GE-4839, KJNS-52
	YI	GE-1286, GE-3758, GE-5004, GE-69, GE-3003, GE-1077, GE-5118, GE-1381, GE-1260, GE-2644, GE-1026, GE-2664, GE-1234	GE-4839, GE-4719, GE-1641, KJNS-52
	YSI	GE-3764	GE-4719, GE-4601, GE-4839, GE-4690, GE-1684, GE-4951, GE-3722, KJNS-52
2020	GMP	GE-489, GE-106, GE-1424, GE-832, GE-127, GE-3003, GPU-28, GE-3454, GE-909, GE-2056, KOPN-330, GE-1286, GE-1234	GE-1641, VL-146, GE-997, GE-4719
	MP	GE-106, GE-489, GE-1424, GE-832, GE-127, GPU-28, GE-909, GE-3003, KOPN-330, GE-3454, GE-2056	GE-4172, GE-1080, GE-70, GE-2215, GE-4719, GE-997
	HM	GE-489, GE-106, GE-1424, GE-832, GE-127, GE-3003, GE-3454, GE-2056, GPU-28, GE-909, GE-1234, GE-1286	GE-6336, GE-4601, GE-1641, GE-4719, VL-146
	DRI	GE-3003, GE-489, GE-469, GE-832, GE-1102, GE-3454, GE-127, GE-1234, GE-1260, GE-1298, GE-2056, GE-4149, GE-1309, GE-1424, GE-1640, GE-2275, GE-1381	GE-597, GE-1933, GE-6336, GE-2398, GE-4951, GE-3722, GE-1641, GE-4601, GE-4719, VL-146
	YI	GE-489, GE-3003, GE-832, GE-127, GE-1424, GE-3454, GE-469, GE-2056, GE-1234, GE-1260, GE-106, GE-4149, GE-1298, GE-1309, GE-1286	VL-146, GE-4719
	YSI	GE-469, GE-1102, GE-3003, GE-4995, GE-1234	GE-1026, GE-6336, GE-2770, GE-1641, GE-1399, GE-4951, GE-597, GE-2398, GE-4719, GE-3722, VL-146, GE-4601

Where, Geometric mean productivity (GMP), Mean productivity (MP), Harmonic mean (HM), Drought resistance index (DRI), Yield index (YI), Yield stability index (YSI)

Table 4.19 Stable drought tolerant and highly susceptible genotypes selected based on the drought tolerance indices over the two years

	Genotypes	DTIs (summer 2019)						DTIs (summer 2020)						GYP (summer 2019)		GYP (summer 2020)	
		GMP	MP	HM	DRI	YI	YSI	GMP	MP	HM	DRI	YI	YSI	Yp	Ys	Yp	Ys
DT	GE-1234	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	19.96	14.92 (25.25)	20.22	17.28 (14.54)
	GE-1286	✓	✓	✓	✓	✓		✓		✓				21.64	17.86 (17.46)	21.80	16.04 (26.42)
	GE-3003	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	19.96	16.08 (19.43)	20.18	19.40 (3.86)
DS	GE-1641	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	6.86	1.76 (74.34)	8.30	1.98 (76.14)
	GE-3722				✓		✓				✓		✓	18.76	3.06 (83.68)	17.46	2.88 (83.50)
	GE-4719	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	8.04	1.84 (77.11)	7.48	1.34 (82.08)
Mean seed yield per plant														14.26	8.39	14.99	8.99

Where, Drought tolerant (DT), Drought susceptible (DS), ✓ : indicates the genotype selected across the DTI, GYP: Grain yield per plant (g), Geometric mean productivity (GMP), Mean productivity (MP), Harmonic mean (HM), Drought resistance index (DRI), Yield index (YI), Yield stability index (YSI), Yp and Ys are the seed yield per plant (g) under well watered (WW) and drought stress (DS) respectively. Values in parenthesis indicates the *per cent* reduction in seed yield per plant under the DS compared to the WW conditions

(77.11% and 82.08%). The results indicate that all the three tolerant genotypes consistently showed less than 26% yield reduction under stress environment while the three susceptible genotypes recorded more than 74% yield reduction in both the years. Mousavi *et al.*, (2008) reported the yield stability is more important than high yield for the selection of drought tolerant genotypes. Ghasemi and Farshandfar (2015) in wheat, Yousefi (2015) in barely, and Mohammadi (2016) in durum wheat confirmed that high values of STI, MP, GMP, YSI, and YI are the best indices for identification of superior genotypes under drought and non-stress conditions. Naghavi *et al.*, (2013) in maize reported that GMP, MP, YI, YSI, and DRI were positively correlated with seed yield under drought stress conditions. Similarly the mean grain yield under drought stress was significantly correlated with GMP, MP, YI, YSI, DRI, and HM in tef (Ferede *et al.*, 2020). In view of consistent performance of the genotypes for different DTI over the two years, the genotype GE-1234, GE-3003 and GE-1286 were considered as stable drought tolerant genotypes and the genotypes GE-1641, GE-4719 and GE-3722 were considered as drought susceptible genotypes for future studies.

The results indicated significant variability among the genotypes for seed yield under stress and well watered conditions. The yield reduction under stress was also not uniform across genotypes over the two years suggesting genotypes respond differentially for reproductive stage drought stress in finger millet. Similarly, there was a variation in the response of genotypes for drought stress across two years. However, by using a combination of drought tolerance indices which mainly depended on the effect of drought stress on grain yield three stable drought tolerant genotypes viz. GE-1234, GE-1286 and GE-3003 and highly susceptible genotypes viz. GE-1641, GE-3722 and GE-4719 were identified. The selected drought tolerant genotypes showed least reduction in seed yield under stress. Similarly, the susceptible genotypes recorded highest reduction in seed yield under stress confirming the usefulness of drought tolerance indices in the identification of stable contrasting genotypes for drought tolerance. Six drought tolerant genotypes viz. GE-1234, GE-1260, GE-1531, GE-5118, GE-2268, GE-909 and six susceptible genotypes viz. GE-1080, GE-1417, GE-1641, GE-2439, GE-4172 and GE-4719 were

selected to study the effect of early reproductive growth stage stress on flowering, quality and quantity of pollen grains under field conditions.

The identification of stable genotypes for drought tolerance was carried out using DTI as well as GGE biplot approach. These two approaches use different statistical methods to assess the performance of genotypes under WW and DS conditions. The DTI method is based on drought indices which uses the relative reduction in seed yield under stress conditions as compared to well water conditions. This helps in identifying genotypes with higher yield under DS conditions. The limitation of this method is that, it won't consider effect of environmental interaction with genotypes and their seed yield related traits. On the other hand, in GGE biplot method measures the value of each genotype in a particular test environment (either WW or DS) by considering individual genotype plus genotype $\times$ environment interaction simultaneously for seed yield and yield related traits (Yan and Kang 2002). The GGE biplot method identifies the superior genotype/s based on their *per se* performance and stability for the yield and yield trait combinations under WW and DS conditions separately. This provides an opportunity to breeder for selection of donor genotypes with desired traits for improving cultivars for yield and stress tolerance. It is interesting to note that, in the present study both the approaches identify the same set of genotypes as drought tolerant and drought susceptible. The results support the hypothesis that, the identified tolerant genotypes not only stable in the performance in drought stress environment but also high yielding and least influenced by environmental conditions.

4.2 Genome wide association mapping and identification of QTLs for seed yield and yield related traits for well watered and drought stress environment

4.2.1 Genetic diversity and population structure analysis using SSR markers

4.2.1.1 Genetic diversity analysis in 350 genotypes using SSR markers

Assessment of genetic diversity among the plant genetic resources provides an excellent opportunity to develop and improve cultivars with desirable traits. In the pre-

genomic era, the two techniques were routinely performed for assessment of genetic diversity within and between plant populations such as (i) morphological characterization (ii) biochemical characterization (allozyme), whereas in the post genomic era molecular marker (DNA marker) analysis especially SSRs and SNPs are widely used for assessment of genetic diversity (Bhandari *et al.*, 2017; El-Esawi, 2019) in several millets including finger millet (Ramakrishna *et al.*, 2016a; Lee *et al.*, 2017); foxtail millet (Kim *et al.*, 2012; Zhang *et al.*, 2012a); proso millet (Cho *et al.*, 2010) and sorghum (Ramu *et al.*, 2013).

Genetic diversity among the 350 finger millet genotypes was assessed by fifteen genomic SSR markers developed using genome sequence of finger millet in our laboratory. Genetic diversity measured through analysis of data on fifteen genomic SSR markers revealed varying degrees of genetic relatedness among the different genotypes of finger millet. Totally 27 amplicons (alleles) were produced from randomly selected 15 SSR primers across 350 genotypes. The number of scorable alleles produced per primer varied from one to three with an average of 1.80 alleles per SSR. The SSR primers, UASBFM26 and UASBFM257 generated highest number of 3 alleles. The major allele frequency of SSR markers tested was ranged from 0.70 to 1.00 with an average of 0.89 (Table 4.20 and Plate 4.2a to 4.2c). Among 15 genomic SSR markers evaluated, 10 showed polymorphism across 350 diverse finger millet genotypes. To measure the informativeness of these genomic SSR markers, the genetic diversity and polymorphic information content (PIC) were estimated (Table 4.20). The gene diversity was in the range of 0.00-0.44 with an average value of 0.17. The highest gene diversity was found in the primer UASBFM19 (0.44) followed by UASBFM252 (0.44), UASBFM32 (0.42) and UASBFM251 (0.40). Similarly, the PIC ranged from 0.00 to 0.39 with an average of 0.17. The SSR marker UASBFM251 produced the highest PIC value of 0.40 followed by UASBFM19 (0.44), UASBFM252 (0.44) and UASBFM32 (0.42).

Table 4.20 Number of alleles generated, gene diversity, polymorphic information content (PIC) and major allele frequency of genomic SSR markers used to assess genetic diversity of 350 finger millet genotype

Sl. No	Genomic SSR marker	Allele number	Gene diversity	PIC	Major allele frequency
1.	UASBFM24	1.00	0.00	0.00	1.00
2.	UASBFM33	2.00	0.10	0.09	0.95
3.	UASBFM31	2.00	0.20	0.19	0.89
4.	UASBFM22	1.00	0.00	0.00	1.00
5.	UASBFM20	1.00	0.00	0.00	1.00
6.	UASBFM261	1.00	0.00	0.00	1.00
7.	UASBFM256	1.00	0.00	0.00	1.00
8.	UASBFM35	2.00	0.02	0.02	0.99
9.	UASBFM251	3.00	0.40	0.39	0.79
10.	UASBFM253	2.00	0.02	0.02	0.99
11.	UASBFM25	2.00	0.32	0.28	0.81
12.	UASBFM26	3.00	0.30	0.29	0.83
13.	UASBFM32	2.00	0.42	0.38	0.74
14.	UASBFM19	2.00	0.44	0.38	0.71
15.	UASBFM252	2.00	0.44	0.37	0.70
	Average	1.80	0.17	0.17	0.89
	Minimum	1.00	0.44	0.39	0.70
	Maximum	3.00	0.00	0.00	1.00

Among the SSR markers tested, high percentage of markers (66.67%) showed polymorphism across 350 diverse finger millet genotypes. The high *per cent* polymorphism of SSR markers observed in the present study is comparable to other studies in finger millet. Ramakrishnan *et al.* (2016a) reported 72 out of 87 (82.73 %) genomic SSR markers were polymorphic in a study using 128 Indian and non-Indian originated finger millet genotypes. Similarly, Pandian *et al.* (2018b), also reported 77 *per*

cent polymorphism in 90 exotic germplasm of finger millet using 56 markers; Panwar *et al.* (2010a) reported 70.19 *per cent* polymorphism in 10 genomic SSR evaluating 83 genotypes and Naga *et al.* (2012) reported 66 *per cent* polymorphism of 30 EST-SSR primers evaluated across 15 morphological diverse accession of finger millet.

The PIC of a marker is a good index to evaluate genetic diversity and estimate the level of genetic variation expressed by a particular marker. The estimation of PIC values also depends upon many factors such as genetic diversity existing in the collection, breeding behavior of the species, size of the collection, sensitivity of genotyping method and nature of the marker system (genic and genomic) used for study (Singh *et al.*, 2013; Chen *et al.*, 2017). The average PIC value obtained from our study was 0.17 which was high as compared with those obtained (0.15) for 59 cultivated accessions of finger millet, using SNP markers (Gimode *et al.*, 2016), while the same study found a higher PIC value of 0.30 for wild finger millet. Backiyalakshmi *et al.* (2021) obtained PIC value 0.011 for 306 finger millet accessions. However, finger millet diversity studies have reported moderate to high informative PIC values (0.25 to 0.79), using SSR markers (Panwar *et al.*, 2010a; Bheema Lingewara Reddy *et al.*, 2011; Kumar *et al.*, 2012; Babu *et al.*, 2014a; Nirgude *et al.*, 2014; Lee *et al.*, 2017; Pandian *et al.*, 2018b). The inherent disadvantage of low polymorphism observed in finger millet may be because of narrow genetic base existing among the cultivated genotypes (Salimath *et al.*, 1995; Gimode *et al.*, 2016; Ramakrishnan *et al.*, 2016a; Pandian *et al.*, 2018a).

The dendrogram was constructed on neighbor joining tree based on unweighted pair-group method of mathematical averages (UPGMA) as implemented in DARWIN software with 350 genotypes with 15 SSR markers. The dendrogram revealed two major clusters (Fig. 4.9). The cluster I had 18 genotypes while cluster II comprised of 332 genotypes. Each cluster was sub divided into two sub clusters. The main cluster I and II consisted of the genotypes from both Asian and African regions. It was observed that, grouping of genotypes in each cluster were irrespective of their geographical region which is contrasting to earlier reports (Kumar *et al.*, 2016b; Puranik *et al.*, 2020). Channaveeriah and Hiremath (1974) and Hilu *et al.* (1979) provided the evidence

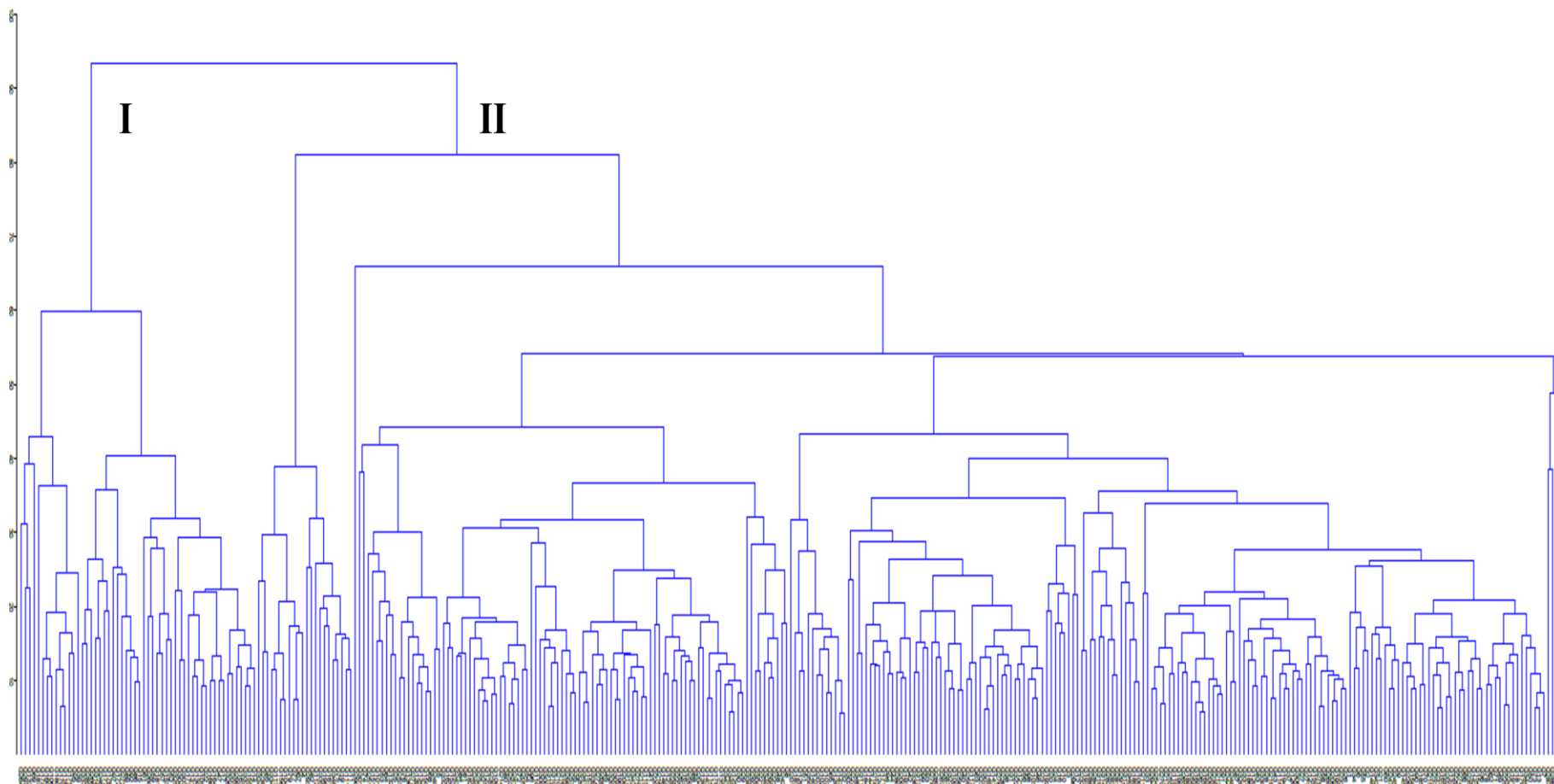


Fig. 4.9 Hierarchical cluster based dendrogram displaying the genetic relationship among 350 finger millet genotypes using SSR markers. I and II indicates the major clusters

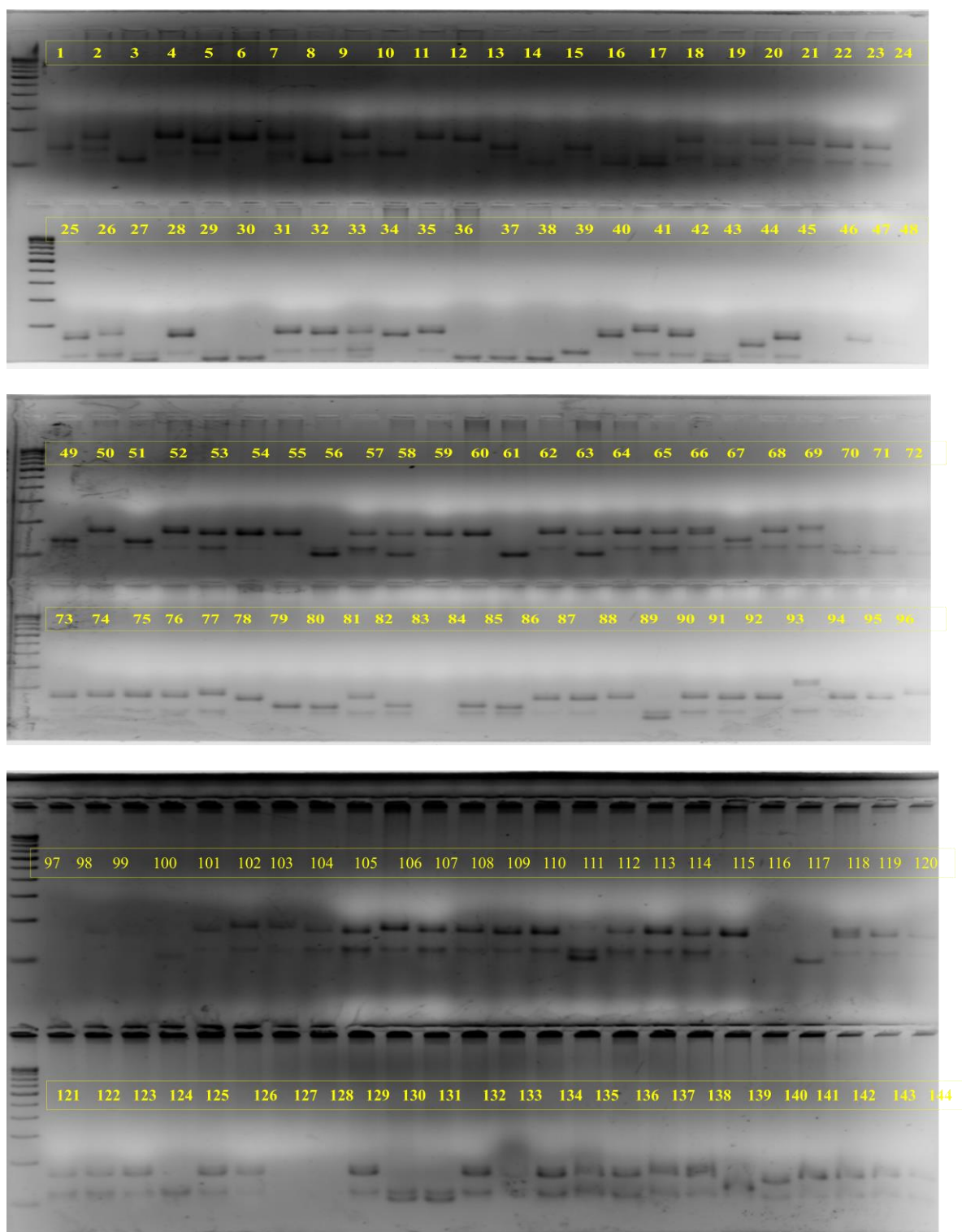


Plate 4.2a Agarose gel picture depicting banding pattern of SSR primer UASBFM251 among the genotype 1-144 of finger millet (First lane from left:100 bp ladder, each lane with number depicting the genotype)

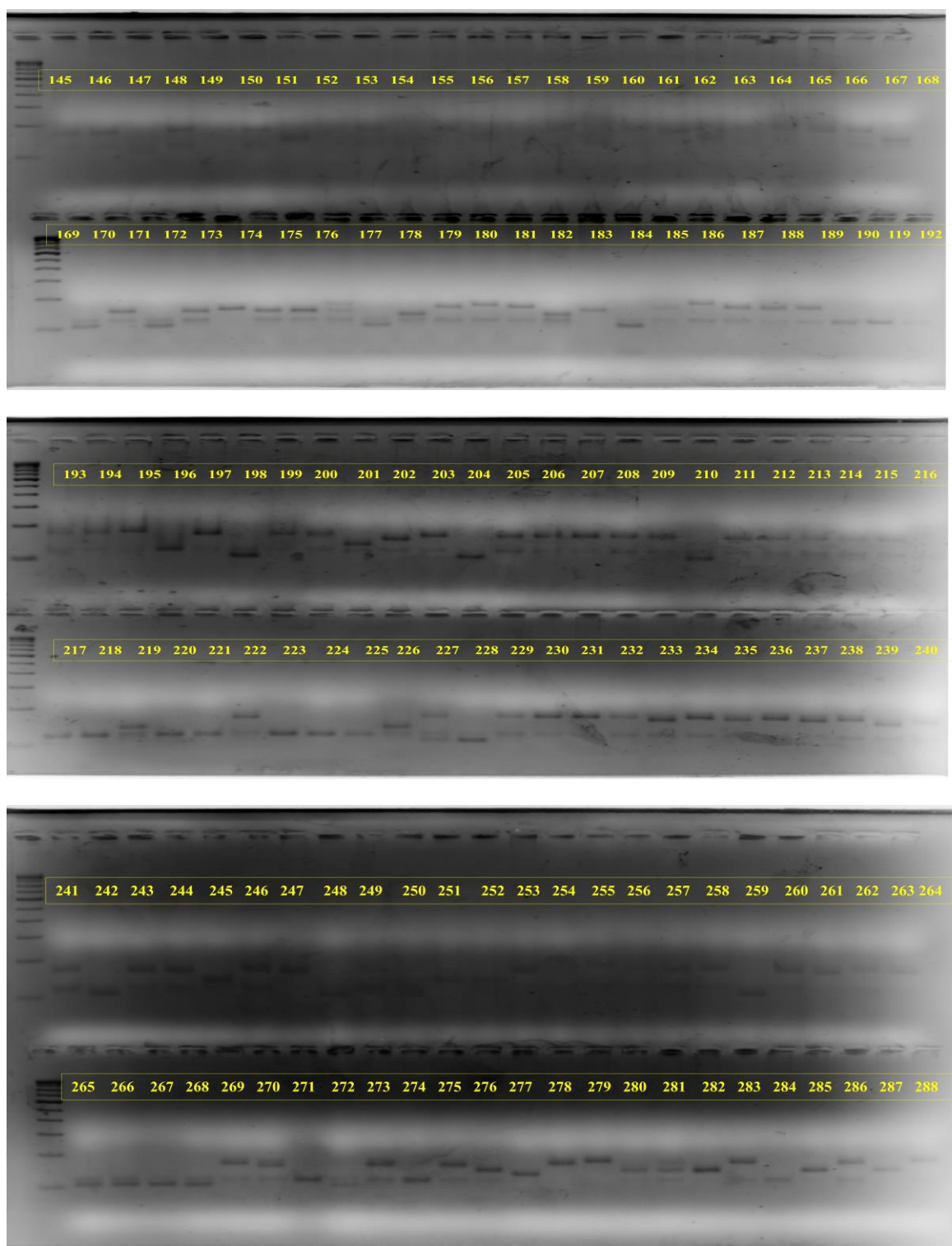


Plate 4.2b Agarose gel picture depicting banding pattern of SSR primer UASBFM251 among the genotype 145-288 of finger millet (First lane from left:100 bp ladder, each lane with number depicting the genotype)

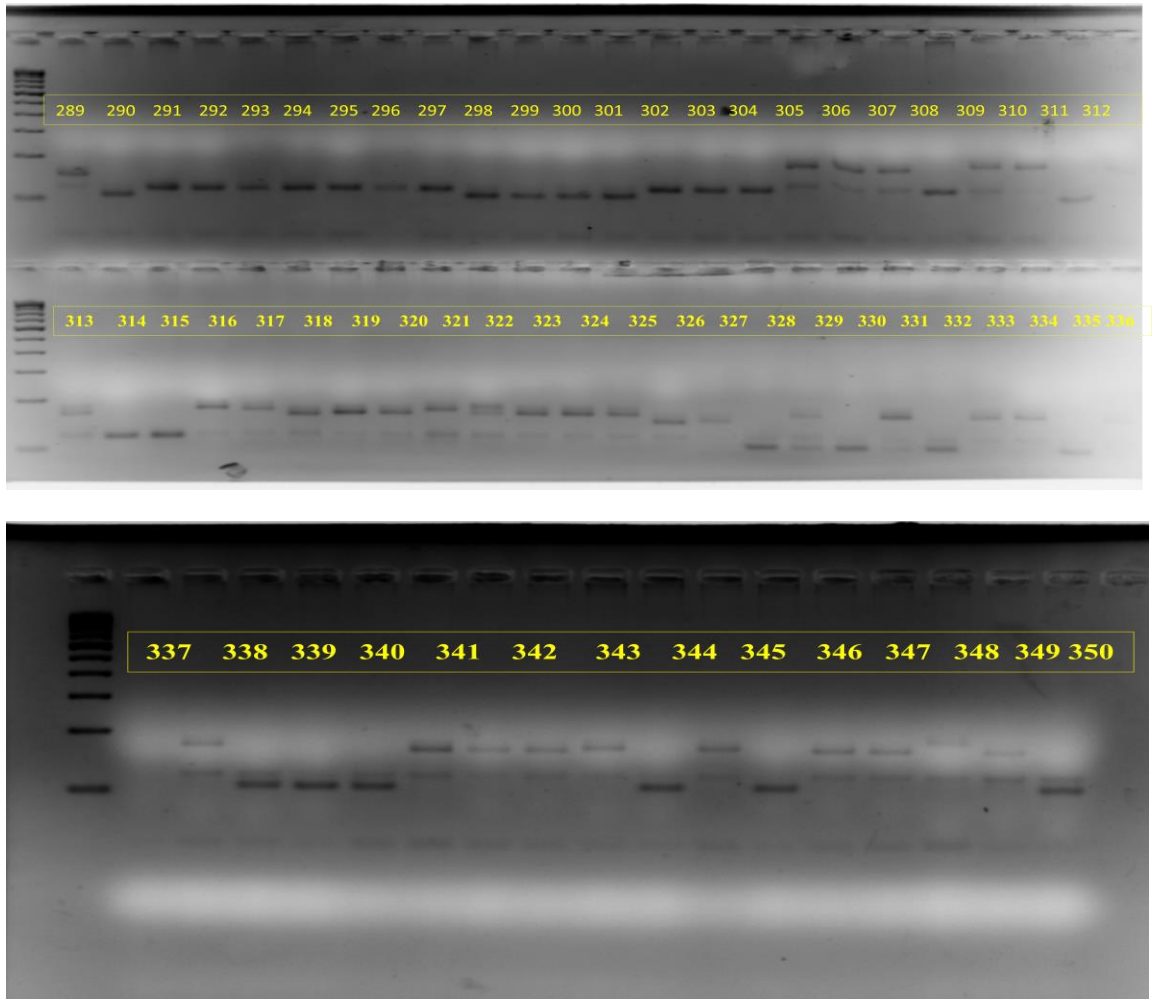


Plate 4.2c Agarose gel picture depicting banding pattern of SSR primer UASBFM251 among the genotype 289-350 of finger millet (First lane from left:100 bp ladder, each lane with number depicting the genotype)

cytological and morphological in favour of African origin of finger millet and subsequently brought to Southern parts of India from Africa (Hilu and Dewet, 1976b) led to interaction and mixing of genome and germplasm. Evidence of admixture between Indian and African genotypes was found in the Indian accessions and *vice versa* probably as a result of intercrossing between Indian and African germplasm as a part of breeding strategies to improve seed yield and tolerance against biotic and abiotic stresses.

4.2.1.2 Population structure analysis using SSR markers

Utilization of SSRs is an impeccable strategy for characterization of a large number of landraces/germplasm in a comparatively short span of time with low cost (Zhu *et al.*, 2016). Nowadays, SSR markers are widely hired for analyzing the genetic diversity, phylogenetic and population genetic studies (Lee *et al.*, 2017; Singh *et al.*, 2020b). In the present study, population structure analysis among 350 genotypes with polymorphic SSRs primers by assuming 10 sub-populations were performed (Pritchard *et al.*, 2000). The parameters of the STRUCTURE (v.2.3.4) software were set to default, specifying K value 1 to 10, with each K repeated for 5 times. Bayesian model, groups the genotypes based on the pattern of inferred ancestry of each individual to the assumed number of populations and the optimum number was selected based on the maximum likelihood method (Pritchard *et al.*, 2000). The results obtained from the STRUCTURE analysis were subjected to Evanno “Delta-K” method (Evanno *et al.* 2005) in the STRUCTURE Harvester software (Earl and Von Holdt, 2012) to establish an optimum value of K. Once the optimum K value was established, the final run was made with initial burning period of 100,000 and Markov Chain Monte Carlo (MCMC) iteration to 1000,000 specifying K = 3 to generate a STRUCTURE ‘Q’ matrix. Analysis of molecular variance (AMOVA) was performed using program GenAlEx (v. 6.5) (Peakall and Smouse, 2012) to estimate the genetic variance components within populations and between populations.

The maximum likelihood based ad-hoc method gave highest value of ΔK at K=3 explaining that 350 genotypes can be grouped in to three distinct sub-populations (Fig. 4.10) consisting of 104, 150 and 96 genotypes each in sub-population (Fig. 4.11). The sub-population 1 consisted of 73 genotypes from Asian region, 23 genotypes from

African region and 8 genotypes from unknown region. The sub-population 2 was completely dominated by Asian genotypes with 109 genotypes followed by 35 genotypes from African region and 6 from unknown region. The sub-population 3 was found to be an admixture of Asian and African region genotypes with 46 and 39 genotypes respectively. The remaining 11 genotypes were from unknown region.

It is well documented that, geographical origin is a key determinant of population structure in finger millet germplasm (Dida *et al.*, 2008; Kumar *et al.*, 2016b). Population structure and clustering patterns of finger millet germplasms in the present study were not consistent with previous classifications (Dida *et al.*, 2008; Kumar *et al.*, 2016b; Puranik *et al.*, 2020), which are based on geographical origin. Backiyalakshmi *et al.* (2021) performed the population structure among the 306 finger millet accessions the results revealed the existence of two major populations (pop1 and pop2) according to a geographical region. The accessions in pop1 were mostly from Africa (87%) and those in pop2 mostly from Asia (88%). This demonstrated the genetic differentiation between finger millet accessions originating in Africa and Asia. A strong genetic structure existed in finger millet; thus the selection of accessions based on origin would be more diverse and effective in a finger millet crop improvement program. The genotypes grouped into present study are not largely based on a geographical region. For instance, a few Asian accessions were assembled into the African population and *vice versa*, in concurrence with previous reports (Dida *et al.*, 2008; Ramakrishnan *et al.*, 2016a; Sood *et al.*, 2016). Evidence of admixture between Indian and African germplasm was found in the Indian accessions and *vice versa* probably as a result of intercrossing between Indian and African germplasm, a breeding strategy that was adapted in India in the 1970s (Bharathi, 2011). In another study, 43 polymorphic markers were used to evaluate polymorphism between 90 diverse finger millet genotypes, revealed a range of PIC value 0.41 to 0.79. Also population based cluster analyses pointed out a strong pattern of ‘isolation by distance’ in between the genotypes. A Bayesian model-based cluster analysis evidenced a high extent of admixture between the gene pools from the different geographical origins in finger millet (Pandian *et al.*, 2018b).

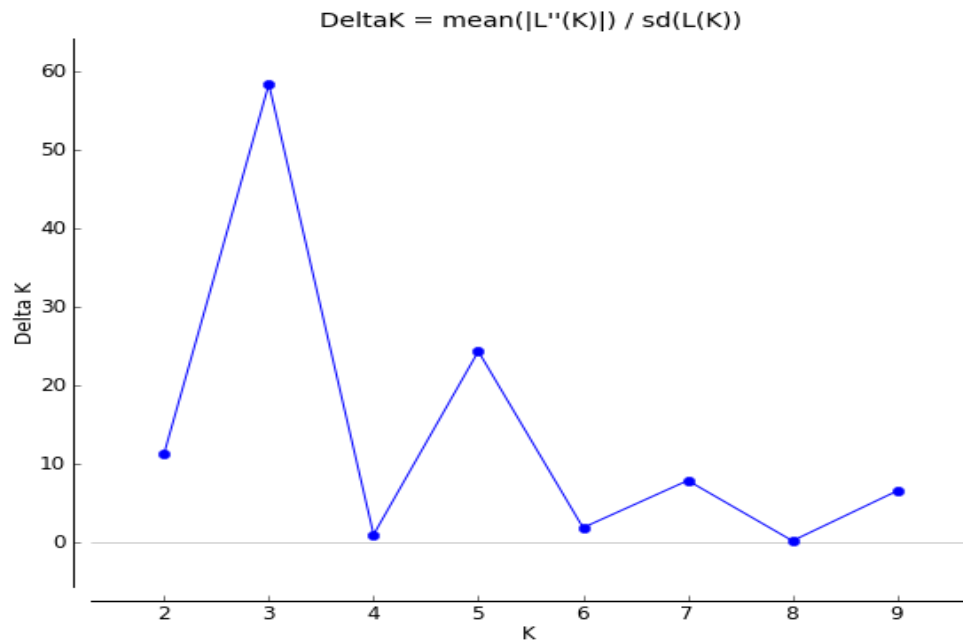


Fig. 4.10 Optimum subpopulation determined by ad-hoc method from result of population structure analysis implemented through STRUCTURE software

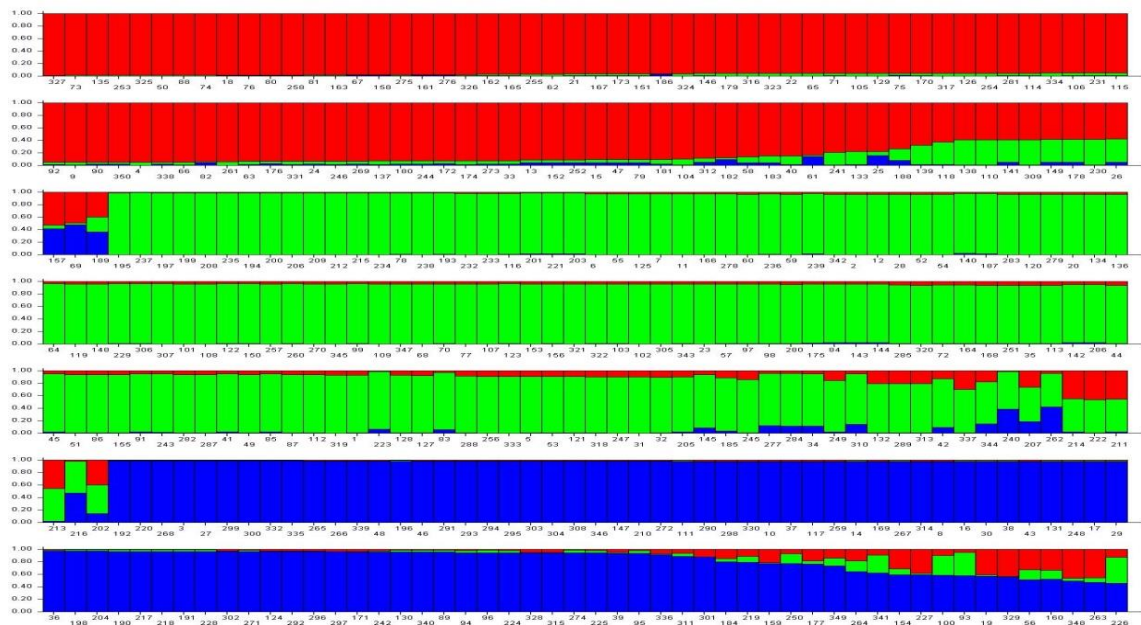


Fig. 4.11 Bar plot depicting genomic contribution of individual genotypes of the association panel towards prevailing three subpopulations in the panel derived from polymorphic SSR markers, implemented through STRUCUTRE software. X-axis in the figure represents genotypes in the association mapping panel (Appendix I)

4.2.1.2 .1 Genetic differentiation and diversity among sub-populations

Various genetic diversity parameters were calculated for analyzing the genetic diversity in 3 sub-populations of 350 finger millet genotypes. The AMOVA analysis was carried out between and within populations in a hierarchical analysis of genetic variation. The higher genetic variation was detected among genotypes within sub-populations (51%) followed by within genotypes in a among population (45%) (Table 4.21). These results suggested that the genetic variation predominantly occurred within populations, accounting for 96 *per cent* of the total variation. The significantly higher within sub-population variation than the among-population variation in finger millet could be partly explained by the fact that selection of finger millet genotypes based on different criteria, such as good performance in grain yield and tolerance to various biotic and abiotic stresses. Also selected individuals contribute seeds to the next generation, and hence, the selection of genetically diverse genotypes leads to high genetic diversity within populations (Brhane *et al.*, 2021).

Table 4.21 Analysis of molecular variance (AMOVA) among three sub-populations based on genomic SSR loci in finger millet

Source of variation	Degrees of freedom (df)	Sum of squares	Mean of squares	Estimated variance	Percentage of variation
Among sub-populations	2	33.988	16.994	0.064	4%
Among genotypes within sub-populations	347	849.993	2.450	0.850	51%
Within genotypes in a sub-population	350	262.500	0.750	0.750	45%
Total	699	1146.481		1.663	100%

The average number of alleles per SSR locus (N_a) varied from 1.46 to 1.60 in 3 sub-populations. The effective number of alleles (N_e) *i.e.* the number of alleles that would be expected at an SSR locus in each population ranged from 1.31 to 1.36. The mean for N_a and N_e values are 1.53 and 1.34 respectively for overall populations (Table 4.22). Values of Shannon's information index (I) ranged between 0.26 and 0.28 per locus, with an average of 0.27 and the overall percentage of polymorphic loci (P_p) was 44.45 *per cent*. Expected heterozygosity is the estimate of proportion of heterozygotes under random mating and the values were ranged from 0.09 to 0.12 with the mean of 0.10.

However, observed heterozygosity is the estimate of proportion of observed heterozygotes at a given locus and the values ranged from 0.16 to 0.18 with the mean value of 0.17 (Table 4.22). The intensity of genetic diversity can be estimated by different parameters, including the percentage of polymorphic loci (Pp), Nei's gene diversity (He) and Shannon's information index (I). The Nei's gene diversity (h) values were in the range of 0.00–0.44 with an average value of 0.17. These results were highly agreed with previous reports by Babu *et al.* (2014a) and Ramakrishnan *et al.* (2016a) (0.02–0.35) by using genomic SSR markers and Nirgude *et al.* (2014) (0.02–0.32) based on genic SSRs in finger millet genotypes. It was also reported that, the African germplasm is more diverse compared with Asian germplasm (Dida *et al.*, 2008; Panwar *et al.*, 2010b; Arya *et al.*, 2013; Babu *et al.*, 2014a; Kumar *et al.*, 2016b; Ramakrishnan *et al.*, 2016a; Babu *et al.*, 2017). This could be due to the introduction and extensive utilization of African germplasm into the Asian finger millet breeding program, especially for introgression of blast resistance and for new source of diversity, may result in equal or slightly higher diversity in Asian germplasm than in African germplasm.

Table 4.22 Genetic diversity and differentiation estimated within 3 sub-populations of finger millet using SSR markers

Sub population	N	Na	Ne	I	Ho	He	Pp
1	101.86±0.52	1.60±0.19	1.31±0.13	0.26±0.09	0.09±0.06	0.16±0.05	46.67
2	145.26±1.43	1.53±0.16	1.35±0.13	0.28±0.09	0.12±0.07	0.18±0.05	46.67
3	93.53±0.81	1.46±0.16	1.36±0.14	0.27±0.09	0.09±0.07	0.18±0.06	40.00
Grand mean	113.56±3.46	1.53±0.09	1.34±0.07	0.27±0.05	0.10±0.03	0.17±0.03	44.45

N= Number of genotypes in each subpopulation, Na = Average number of different alleles, Ne = No. of effective alleles, I = Shannon's information index, Ho = Observed heterozygosity, He = Expected heterozygosity, Pp=Per cent polymorphism

4.2.2 Identification of polymorphic SNP markers by resequencing of 350 genotypes

The panel of 350 finger millet genotypes was resequenced by assembly with Illumina and new Bionano Saphyr optical genome/methylation mapping system (Bionano

ver3.0) with 3-5x Illumina coverage. The genome resequencing and SNP identification were carried at Functional Genomics Center Zurich, Zurich, Switzerland. The details SNP mining as provided by Functional Genomics Center Zurich, Zurich, Switzerland are discussed. The genome was separated as A and B sub-genome and aligned with the help of high coverage of reference genome sequence of PR-202. The SNP calling was performed with Genome Analysis ToolKit (GATK) haplotype caller and GATK joint genotyping. The genome wide comparison revealed a total of 80,82,179 SNPs (516Mb) in sub-genome A and 90,71,360 SNPs (650Mb) in sub-genome B. Further SNPs were subjected to hard filtering with custom script for discarding false positives. The SNP calling was imputed by BEAGLE application by using high coverage variant call format (vcf) as a reference to get high quality SNPs. SNPs were filtered out based on missing percentage (<50% missing), homozygosity (inbreeding $F \geq 0.5$) and minor allele frequency (MAF $\geq 5\%$). Thus after PLINK filtering and quality check (QC) a total of 32,04,543 SNPs from sub-genome A and 22,71,900 SNP's from sub-genome B were used to study association with sixteen agro-morphological traits recorded under WW and DS conditions over the two years.

4.2.2.1 Population structure analysis using SNP markers

The availability of SSR markers in finger millet is limited (Dida *et al.*, 2008; Gimode *et al.*, 2016) and only a few reports are available on association mapping using SSR markers (Babu *et al.*, 2014a; Babu *et al.*, 2014b; Sood *et al.*, 2016). The SNPs are considered to be the ideal markers in plant genetic studies and genome analyses not only because of their excellent genetic attributes, such as ubiquitous distribution, co-dominant nature of inheritance, chromosome-specific location and high reproducibility (Cho *et al.*, 1999; Gupta *et al.*, 2001; Sachidanandam *et al.*, 2001) but also because of early, fast and cost effective high throughput analysis.

The population structure analysis was performed using 54,76,443 SNPs based on Bayesian model to determine the genetic relatedness among the genotypes using STRUCTURE (v2.3.4) software. The graphical output of STRUCTURE shows that genotypes from Indian source and African source are mixed together in the population (Fig.4.14a). This is due to several grasses and wild species were domesticated and

hybridized to India and African origin. The same is true for *Eleusine* species also. The cross-validation errors depicts that $K=5$ i.e. five sub-populations were inferred according to the admixture analysis (Fig. 4.14b). From the admixture, the covariance matrix obtained from the STRUCTURE analysis was further used for the GWAS analysis. Bayesian model has been extensively used to study the optimum population number in various crop species to study the population structure analysis. The population structure analysis showed the overlap of accessions of Indian and exotic origin, which might be due to gene flow among both groups resulting in overlapping of accessions (Tiwari *et al.*, 2020). The population structure analysis was carried out at Functional Genomics Center Zurich, Zurich, Switzerland and further details are not presently obtained.

4.2.3 Marker trait association (MTA) using genome wide association studies (GWAS)

Improvement to a crop generally involves exploiting the existing genetic variability in desired traits, and nature as well as degree of their association among them (Mnyenyembe and Gupta, 1998). Most of the agro-morphological characters are controlled by multiple genes showing complex inheritance. Grain yield is a complex trait and is considered to be a definitive product of its component traits. Therefore, it is necessary to have knowledge on the extent of association between grain yield and its contributing characters. Association mapping is considered to be one of the most promising tools for plant breeders. GWAS has become an important method for identification of alleles associated with the traits for productivity and biotic and abiotic stress tolerance in plants. It is based on linkage disequilibrium (LD) and overcomes the drawbacks of classical linkage analysis which is based on biparental mapping populations. Moreover, this approach helps in the detection of a large number of alleles and enhances the mapping resolution (Mott *et al.*, 2000; Yu and Buckler, 2006). The association mapping for sixteen seed yield and yield related traits were carried out using SNP data of 350 genotypes evaluated under well watered and drought stress conditions during 2019 and 2020. The details of the genome wide association studies are given below.

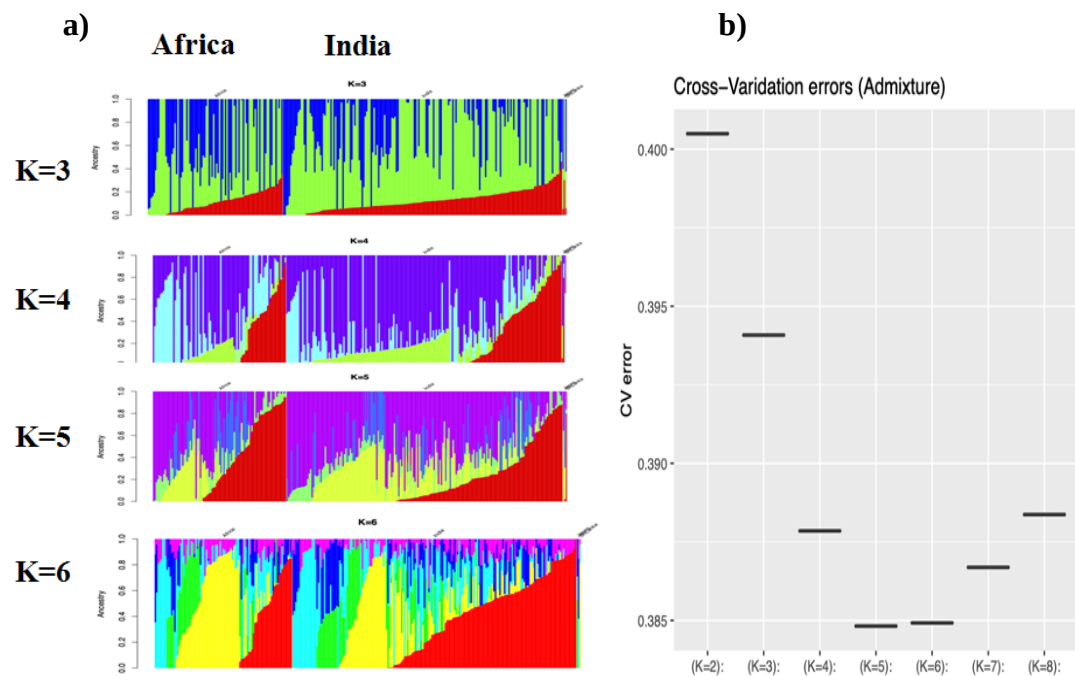


Fig. 4.14 a) Genetic relationships of 350 finger millet genotypes with the K=3 to K=6 sub-populations estimated using STRUCTURE software based on SNP data b) The cross-validation errors for K=3 to K=5 depicts that K=5 *i.e.*, five sub-populations were inferred according to the admixture analysis

4.2.3.1 Kinship matrix of SNP markers

The kinship matrix (K model) was generated in TASSEL by converting the distance matrix obtained from TASSEL's cladogram function into similarity matrix. A kinship matrix obtained from large number of SNP markers of A and B sub-genome did not show strong or clear clustering between related germplasm and but showed formation of three different hierarchical groups. The color histogram shows the distribution of coefficient of co-ancestry and the strong red color indicates more relatedness among individuals (Fig. 4.13).

4.2.3.2 Linkage disequilibrium (LD) and LD decay

Linkage disequilibrium (LD) is the nonrandom association of alleles of different loci in the genome. Recombination interacts in a complex way with selection, mutation and genetic drift to determine levels of LD. As a consequence, local and genome-wide patterns of LD can provide insight into patterns of natural selection and the past history of population growth and dispersal (Slatkin, 2008). Linkage disequilibrium analyses are the pre-requisites for natural population based genomic analysis. The LD plot displays the LD between pairs of polymorphic sites. 'R square' represents the correlation between alleles at two polymorphic SNP loci, which is informative for evaluating the resolution of association approaches. One simple way to estimate LD between polymorphisms, at which r^2 drops below 0.1 is a rough estimate of the resolution within specific population. The rate of LD decay in most cases needs to be determined empirically for any given population. The distribution of data points in the plot of LD (r^2) decay against distance (kb) within the scaffolds showed that LD was not a simple monotonic function of the distance between markers.

In the present study, LD decay of A sub-genome observed was quite complicated because admixture happened over domestication and introduction compared to B sub-genome (Fig. 4.12). The B sub-genome was stable because of constant population structure. When we compared LD decay of A and B sub-genome, LD decay observed in A sub-genome was 1500 kb at 0.2 r^2 , whereas in B sub-genome was around 1000 kb at 0.2 r^2 . The results indicated that the LD decay was faster in B sub-genome than the A

sub-genome. This may be due to introgression of the wild species genome recombination or due to admixtures in A sub-genome but not in B sub-genome. Moreover, the donor of A sub-genome is still unknown and may contain admixtures from wild and indica genotypes from Asian region. The results are contradictory to earlier studies by Bančič *et al.* (2021) who reported that, r^2 decayed to 0.2 at a distance of 106 kb for the whole genome when correcting for genetic structure (compared to a distance of 1.77 Mb for the naïve model). They have also reported that the decay was slower for the B sub-genome ($r^2 = 0.2$ at 168 kb, with correction) compared to A sub-genome ($r^2 = 0.2$ at 88 kb), and varied markedly across chromosomes within sub-genomes. Overall, the relatively slow rate of LD decay was consistent with other self-pollinating crops (Flint-Garcia *et al.*, 2003), including other millets (Jaiswal *et al.*, 2019).

4.2.3.4 Marker trait association (MTA)

In the present study, the GWAS approach was followed to unravel the genetic loci linked to seed yield and yield related traits using the SNP genotypic data and four environment (WW19, WW20, DS19 and DS20) phenotypic data on 350 finger millet genotypes. The genotypic data consisted of 54,76,443 quality SNPs and the phenotypic data consisted of 16 quantitative traits viz. days to flowering, plant height at reproductive stage, plant height at harvest, total number of tillers per plant, number of productive tillers per plant, culm length, number of earheads per plant, main earhead length, main earhead weigh, total earhead weight per plant, finger numbers per earhead, finger length, finger width, test weight, total biomass, seed yield per plant recorded over the two years (2019 and 2020) under well watered (WW) and drought stress (DS) conditions. The Mixed Linear Model (MLM) in Genome Association and Prediction Integrated Tool (GAPIT) package available in R studio was used for association mapping. The Bonferroni threshold for significant associations between SNPs and trait was set as $p < 1.826 \times 10^{-7}$ ($P = 1/n$, where n = the number of SNP markers used; $-\log_{10}(1/54,76,443) = 6.73$) which also has been widely adopted in literature (Liu *et al.*, 2016; Huang *et al.*, 2018) used on the False Discovery Rate (FDR) corrected p-values for eliminating false positives. The trait-wise significantly associated SNPs under WW and DS conditions in each year were identified separately. Mixed linear model (MLM)

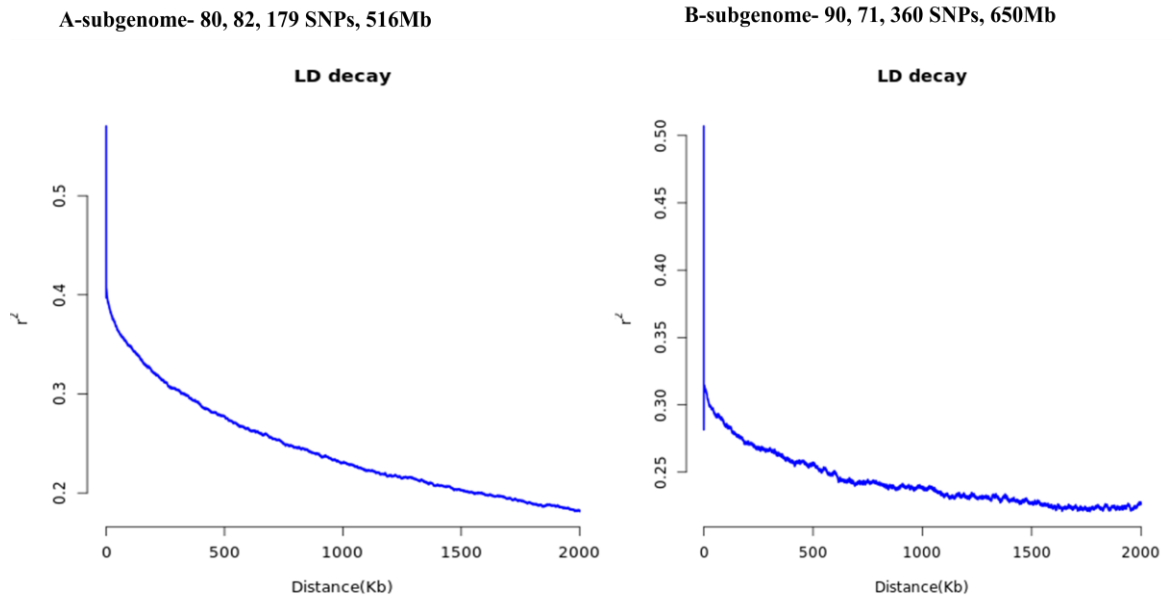


Fig. 4.12 Linkage disequilibrium (LD) decay after imputation of SNPs identified in A-sub genome and B-sub genome in 350 finger millet genotypes

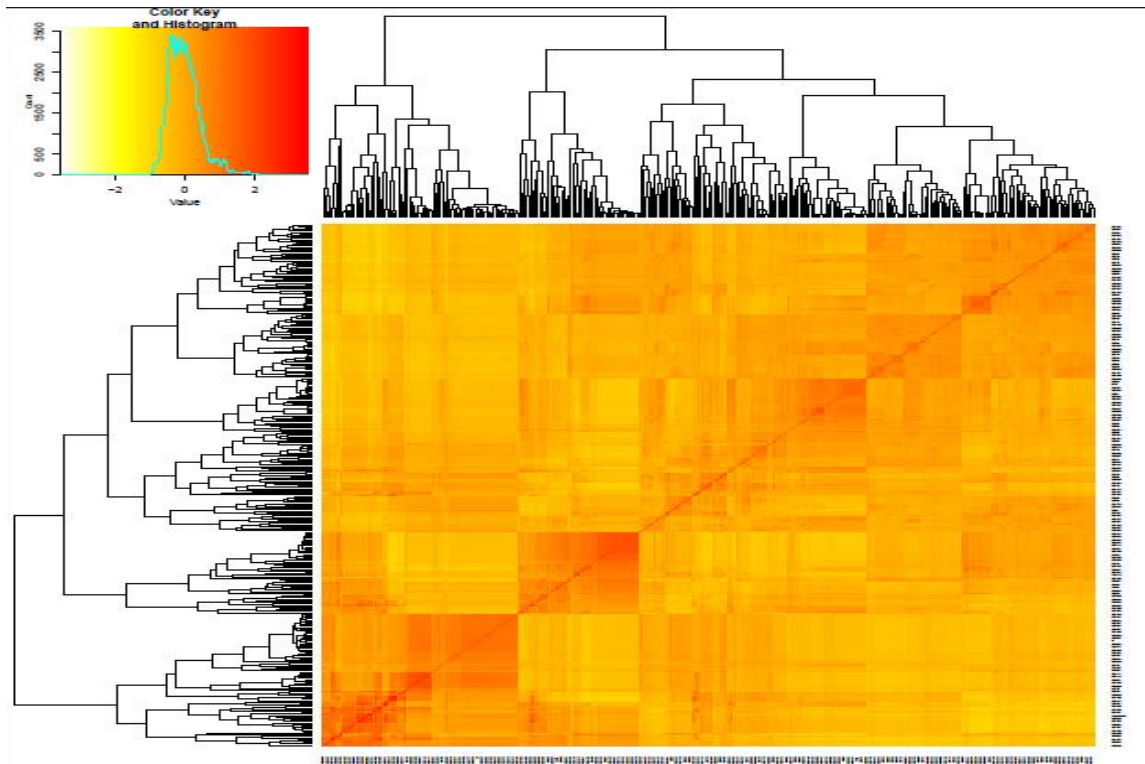


Fig. 4.13 Kinship matrix based on SNP data and 350 diverse finger millet genotypes. The color histogram shows the distribution of coefficient of co-ancestry and the strong red color indicates more relatedness among individuals

methods have proven useful in controlling for population structure and relatedness within genome-wide association studies. In the MLM-based methods, population structure (Pritchard *et al.*, 2000; Zhao *et al.*, 2007) is fit as a fixed effect, whereas kinship among individuals is incorporated as the variance-covariance structure of the random effect for the individuals.

4.2.3.4.1 Manhattan plot and Q-Q plot

The Manhattan plot depicts the p-value of the entire SNPs used in GWAS analysis on genomic scale with the chromosomes in order and their positions in the chromosome, on the X-axis and $-\log_{10}(p)$ value of SNPs on Y-axis. Because of the linkage disequilibrium arising from infrequent genetic recombination; p-value of annotated SNPs tend to shoot up high on the Manhattan plot from rest of the SNPs resembling a Manhattan skyline (Ehret, 2010). In Manhattan plot, a longer Y-coordinate value or large peaks in the Manhattan plot (*i.e.* “skyscrapers”) suggest that the surrounding genomic region has a strong association with the trait. The x-axis shows the scaffolds *i.e.* SA is sub-genome A and SB is sub-genome B, but the scaffold in the plot doesn’t show which chromosome of the finger millet since the physical map of the finger millet is not available. The association between each trait and the SNPs were calculated based on MLM model and represented in Manhattan plot. The significantly associated SNPs with a respective trait above the Bonferroni threshold $p < 1.826 \times 10^{-7}$ or $p < 6.73$ used on the False Discovery Rate (FDR) corrected p-values for eliminating false positives are represented in Manhattan plot under WW (Fig. 4.15a to 4.15d) and DS conditions (Fig. 4.16a to 4.16c) along with corresponding Quantile-Quantile plot (Q-Q plot). The Q-Q plot is the graphical display of the deviation of the observed p-values ($-\log_{10}(p)$) from the expected p-values ($-\log_{10}(p)$) of SNPs under null hypothesis (Korte and Farlow, 2013). Q-Q plot is routinely followed to find out if the GWAS models control false positives and false negatives (Kristensen *et al.*, 2018). The Q-Q plot shows deviation from straight line close to the 1:1 line *i.e.* with a sharp upward or downward deviated tail indicating the association analysis with control of false negative and false positives with causal polymorphism or significant association detected. Deviations from this line suggest the presence of spurious associations due to population structure and

familial relatedness, and that the GWAS model does not sufficiently account for these spurious associations. It is expected that the SNPs on the upper right section of the graph deviate from the diagonal. These SNPs are most likely associated with the trait under study. The solid blue line in the Manhattan plot is the $-\log_{10}(p)$ value threshold using Bonferroni correction. Hence, SNPs above 6.43 on Y-axis are considered as significant marker trait associations (MTAs).

4.2.3.4.2 Marker trait association (MTA) analysis under WW conditions

The significant marker trait associations (MTAs) were found for different traits under WW conditions. Totally 18 and 31 SNPs recorded significant MTA under WW19 and WW20 conditions respectively. The highest number of significant MTAs (12) was observed for finger width followed by culm length (10), finger length (05), main earhead length (01), main earhead weight (01), total number of tillers per plant (01) and days to flowering (01) under WW20 condition (Table 4.23). On the other hand, under WW19 condition, eight significant MTAs were observed for number of earheads per plant followed by main earhead weight (03), finger length (03), total earhead weight per plant (01), number of productive tillers per plant (01), main earhead length (01) and seed yield per plant (01).

Under WW19 conditions, among the eight significant MTAs for number of earheads per plant, the highest phenotypic variance was explained by SSA0004_6476006 (11%) and the lowest was by SSA0004_7802930 (10.00%) with the marker effect ranges from -0.73 to 0.79 (Table 4.23). For finger length three MTAs viz. SSB0007_17154962, SSA0009_2114820 and SSB0003_26394833 explained the phenotypic variance of 8.00 *per cent* each. Similarly, for main earhead weight, three significant MTAs viz. SSB0007_14678607, SSB0007_14678627 and SSB0007_14678598 recorded with the phenotypic variance of 10.00 *per cent* each. Only one MTA was found for total number of productive tillers per plant (SSA0001_35610702) which explained the phenotypic variance of 12.00 *per cent*. The SNP associated with total earhead weight per plant was SSA0012_6750700 (11.00%), seed yield per plant was SSA0012_6750700 (11.00%) and earhead length was SSB0007_17154962 (8.00%).

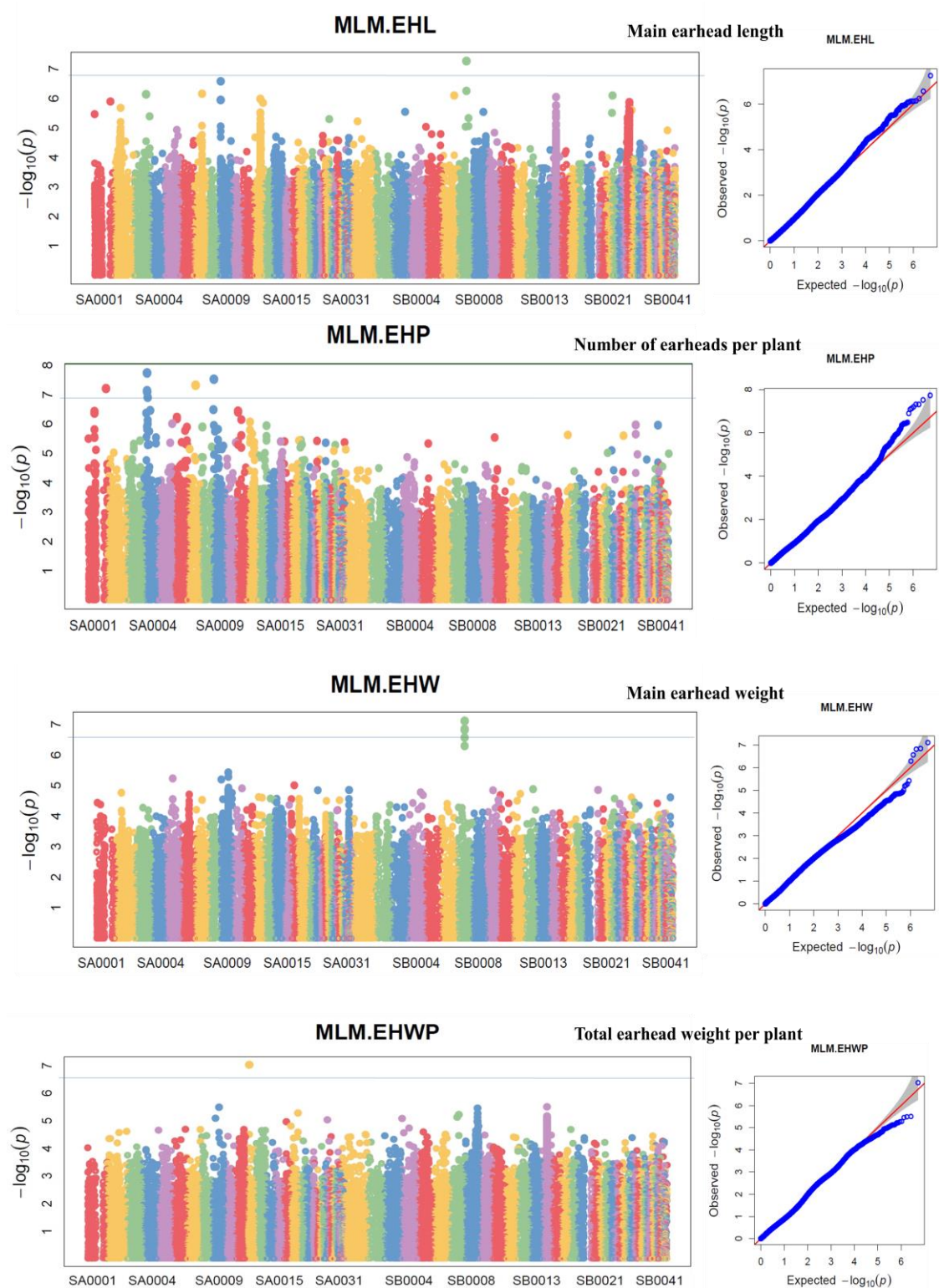


Fig. 4.15a Manhattan plot (left side) with corresponding Q-Q plot (right side) depicting putatively associated SNPs to main earhead length (EHL), number of earheads per plant (EHP), main earhead weight (EHW) and total earhead weight per plant (EHWP) under well watered 2019 (WW19) condition. The solid line in blue colour in Manhattan plot indicates the Bonferroni threshold value 6.73 for

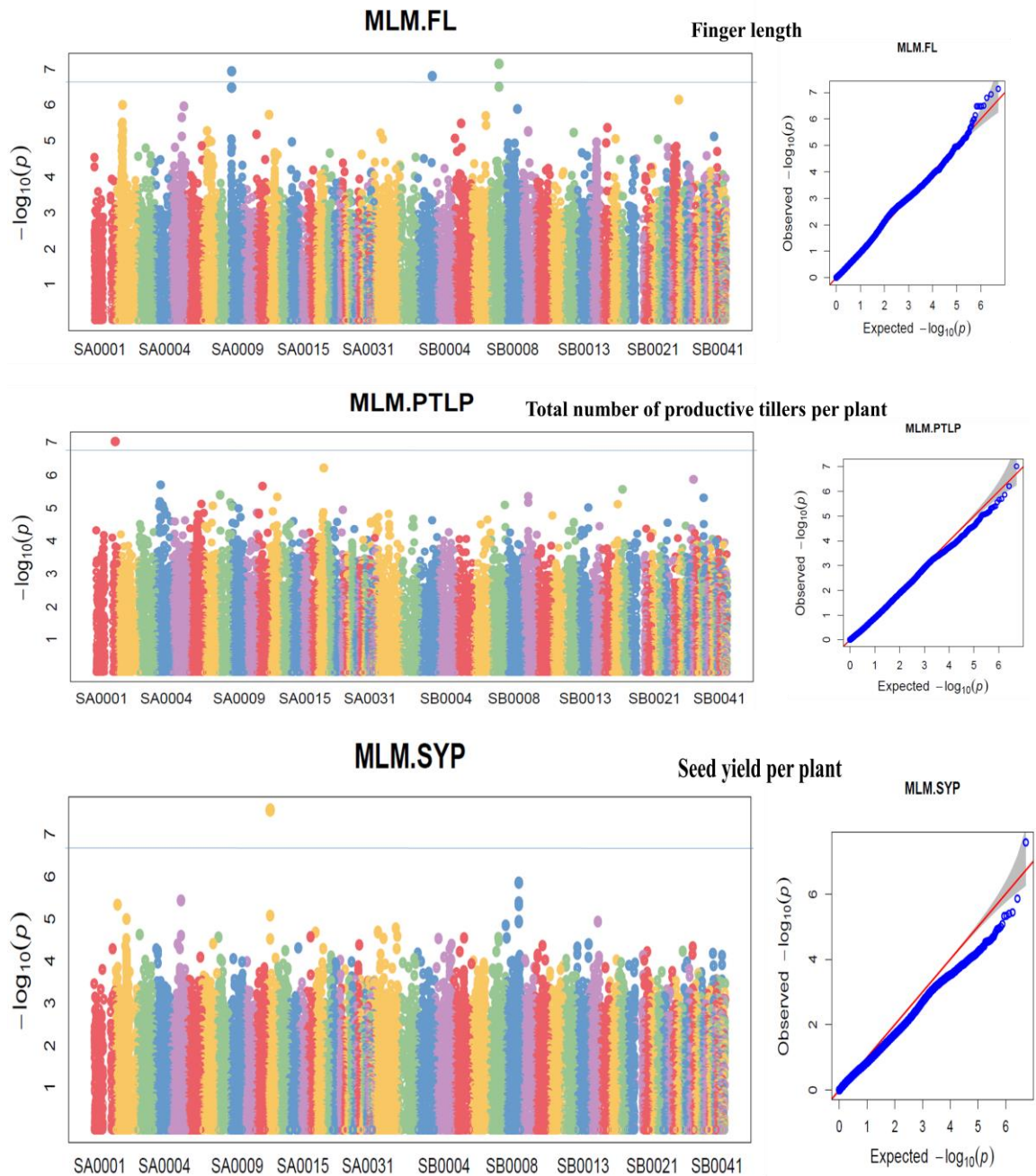


Fig. 4.15b Manhattan plot (left side) with corresponding Q-Q plot (right side) depicting putatively associated SNPs to finger length (FL), total number of productive tillers per plant (PTLP) and seed yield per plant (SYP) under well watered 2019 (WW19) condition. The solid line in blue colour in Manhattan plot indicates the Bonferroni threshold value 6.73 for significant marker trait association

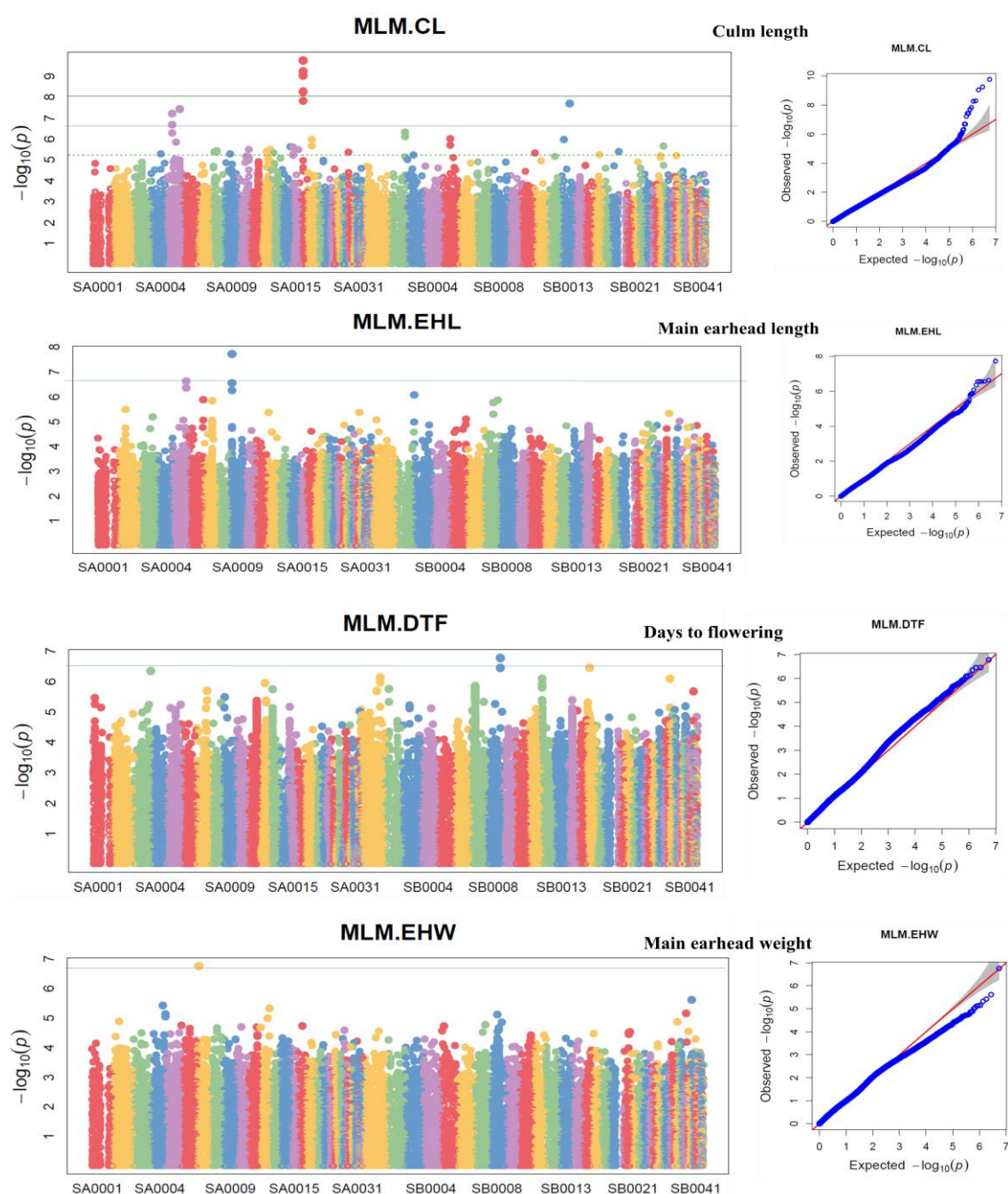


Fig. 4.15c Manhattan plot (left side) with corresponding Q-Q plot (right side) depicting putatively associated SNPs to culm length (CL), main earhead length (EHL), days to flowering (DTF) and main earhead weight (EHW) under well watered 2020 (WW20) condition. The solid line in blue colour in Manhattan plot indicates the Bonferroni threshold value 6.73 for significant marker trait association

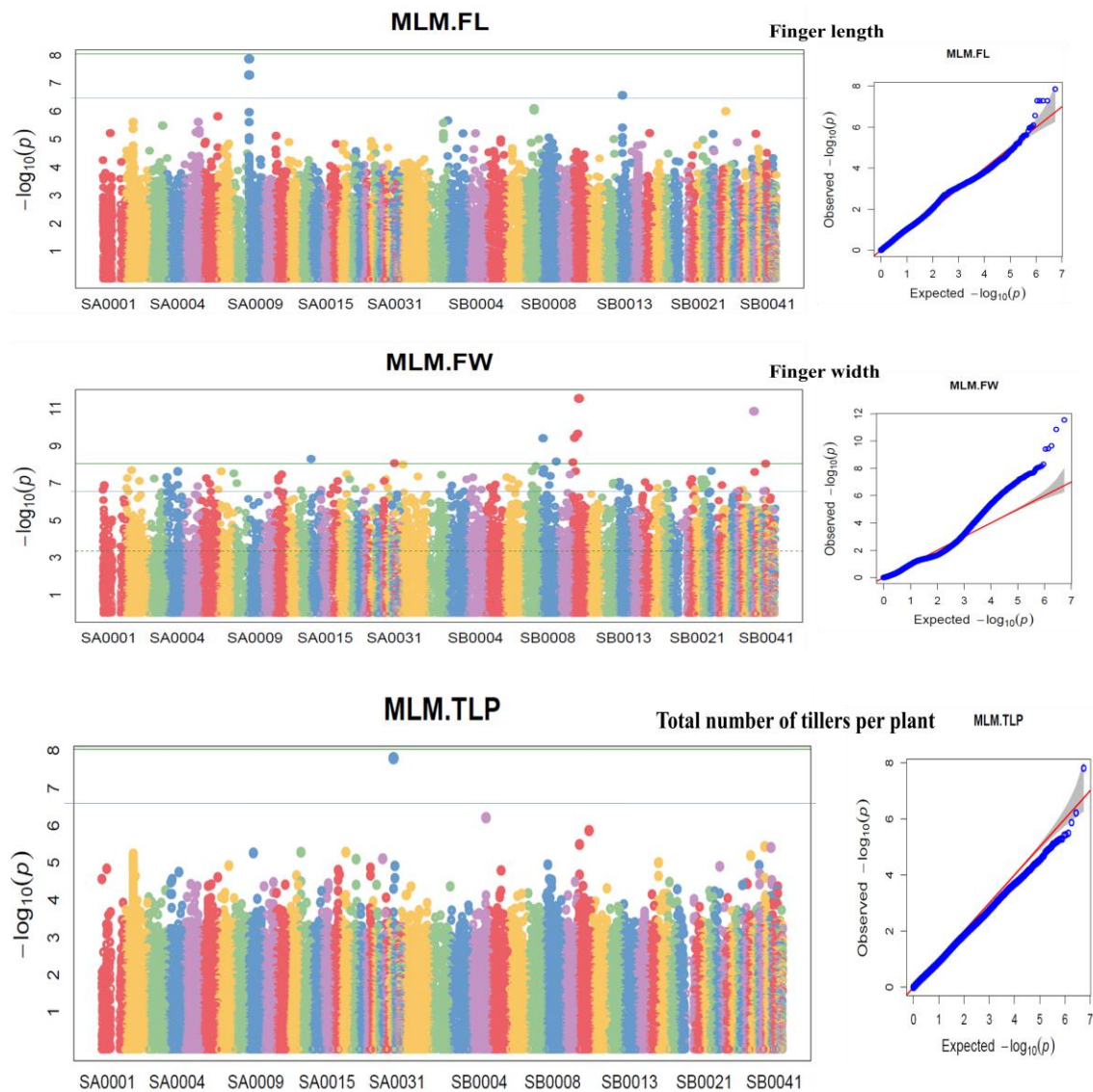


Fig. 4.15d Manhattan plot (left side) with corresponding Q-Q plot (right side) depicting putatively associated SNPs to finger length (FL), finger width (FW) and total number of tillers per plant (TLP) under well watered 2020 (WW20) condition. The solid line in blue colour in Manhattan plot indicates the Bonferroni threshold value 6.73 for significant marker trait association

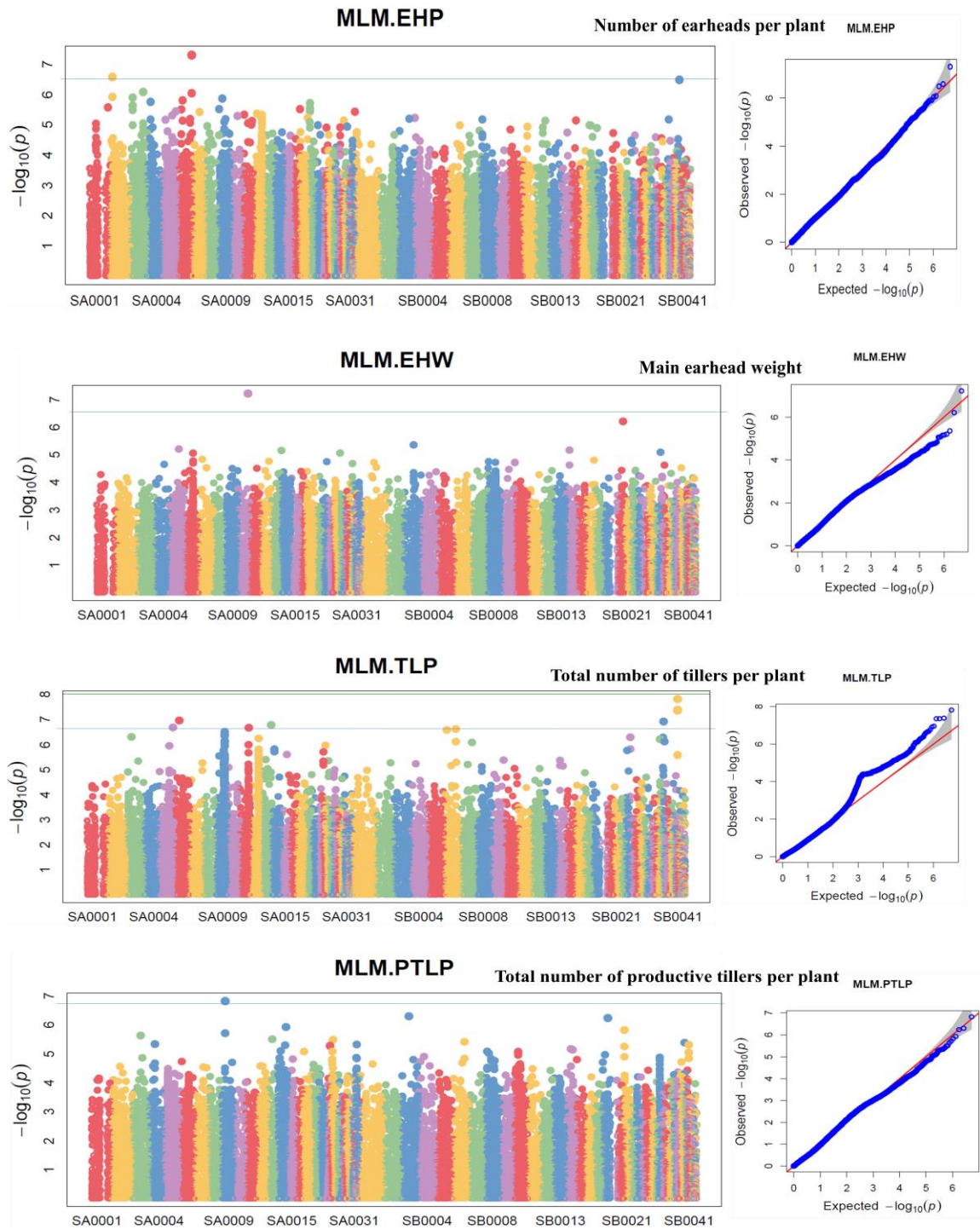


Fig. 4.16a Manhattan plot (left side) with corresponding Q-Q plot (right side) depicting putatively associated SNPs to number of earheads per plant (EHP), main earhead weight (EHW), total number of tillers per plant (TLP) and total number of productive tillers per plant (PTLP) under drought stress 2019 (DS19) condition. The solid line in blue colour in Manhattan plot indicates the Bonferroni threshold value 6.73 for significant marker trait association

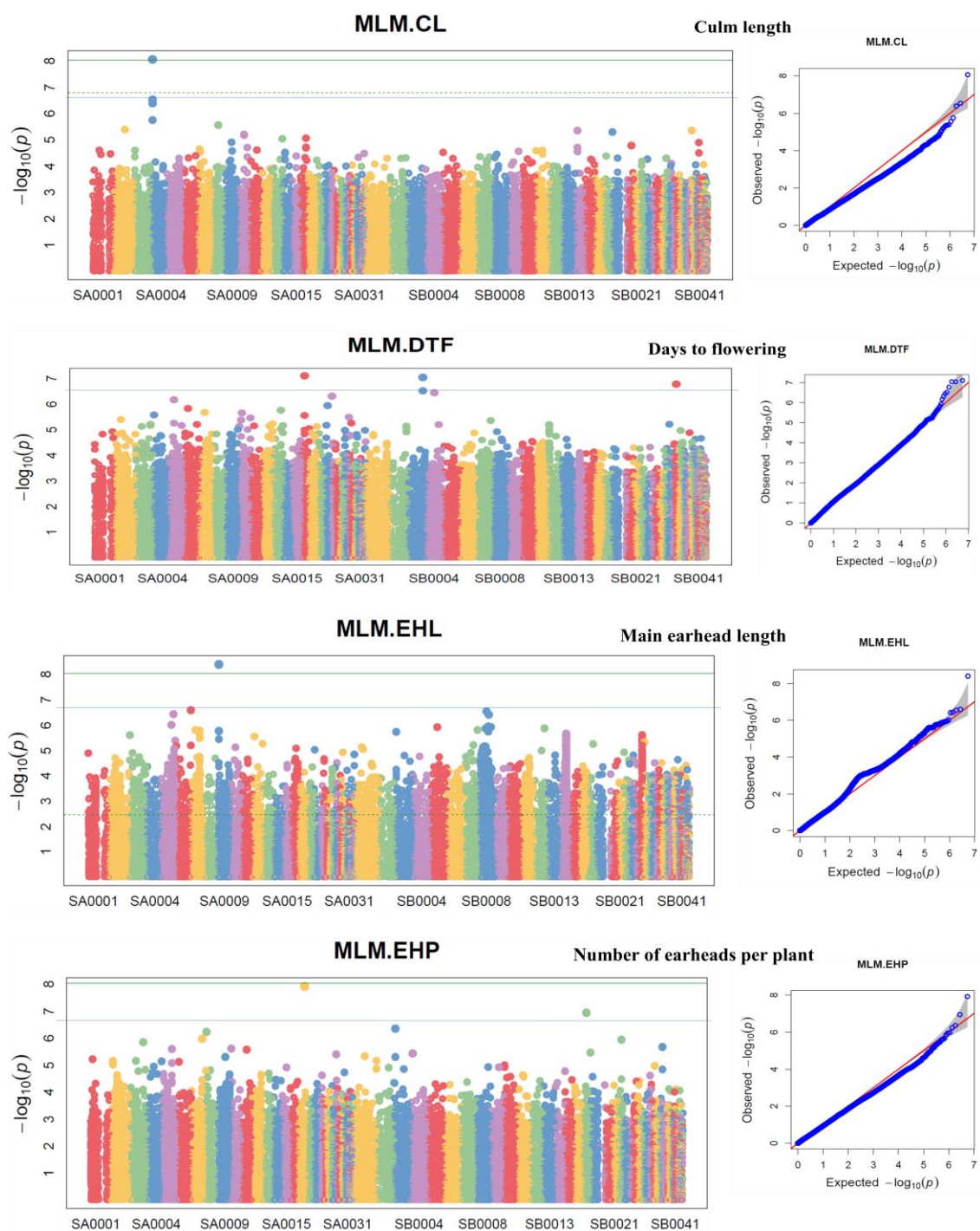


Fig. 4.16b Manhattan plot (left side) with corresponding Q-Q plot (right side) depicting putatively associated SNPs to culm length (CL), days to flowering (DTF), main earhead length (EHL) and number of earheads per plant (EHP) under drought stress 2020 (DS20) condition. The solid line in blue colour in Manhattan plot indicates the Bonferroni threshold value 6.73 for significant marker trait

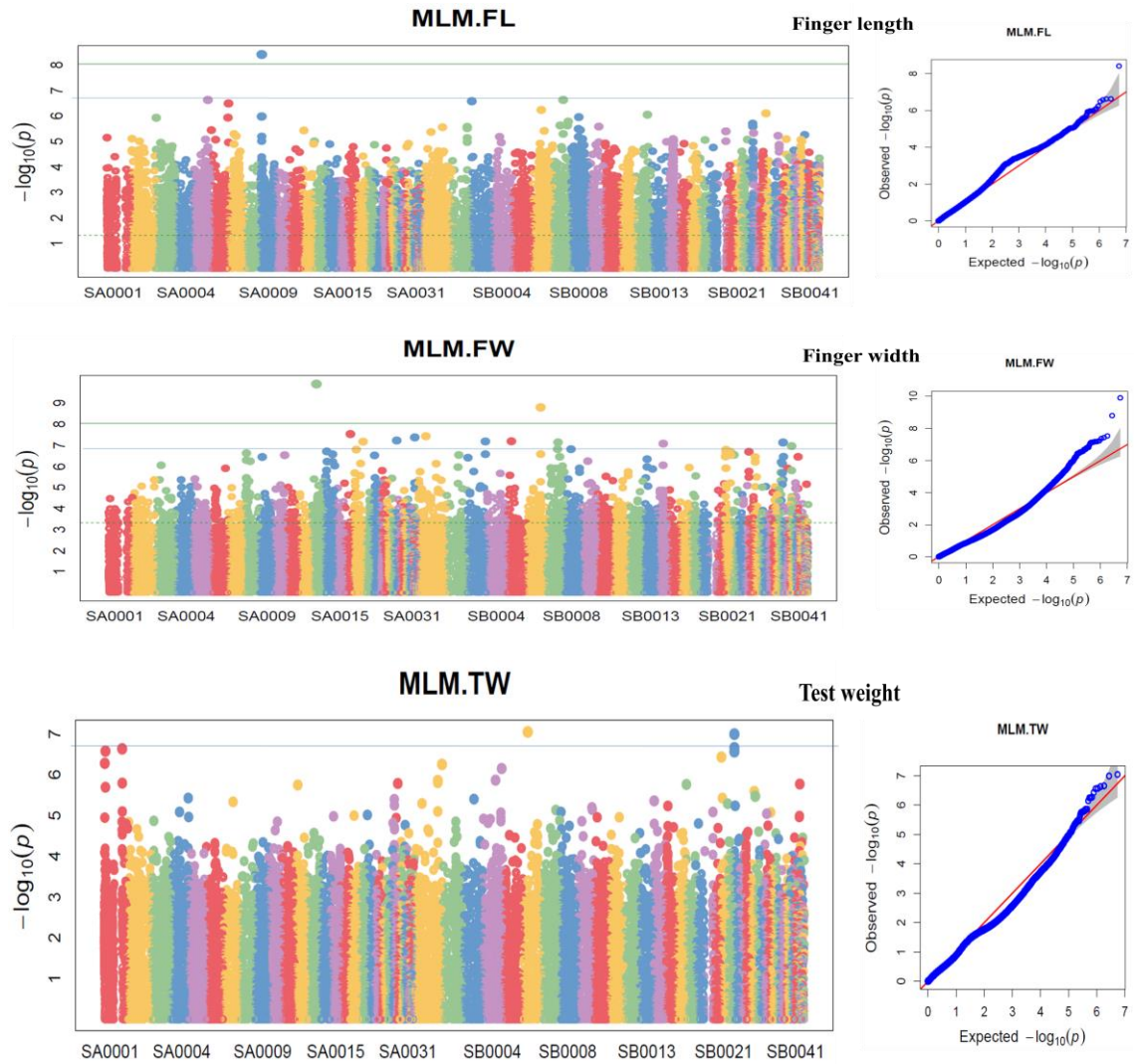


Fig. 4.16c Manhattan plot (left side) with corresponding Q-Q plot (right side) depicting putatively associated SNPs to finger length (FL), finger width (FW) and test weight (TW) under drought stress 2020 (DS20) condition. The solid line in blue colour in Manhattan plot indicates the Bonferroni threshold value 6.73 for

Among the 12 significant MTAs for finger width, the highest phenotypic variance (r^2 of model with SNP) was explained by SSB0010_10948960 (18.00%) and the lowest was by SSB0007_21895928 (13.00%) under WW20 condition with the marker effect ranges from 0.45 to 0.27 (Table 4.23). For culm length ten significant MTAs were recorded, where the highest phenotypic variance was explained by SSA0016_6626688 (16.00%) and the lowest by SSA0005_10053094 (13.00%) under WW20 with the marker effect ranges from -2.38 to -2.06. Similarly among the five MTAs observed for finger length, the higher phenotypic variance explained by SSA0009_2114820 (10.00%) and the lowest was by SSA0009_2076536 (9.00%). Only one MTA was found for main earhead length (SSA0009_2114820) which explained the 10.00 *per cent* phenotypic variance. The SNP associated with days to flowering was SSB0008_25409968 (10.00%), main earhead weight was SSA0007_4887629 (12.00%) and the total number of tillers per plant was SSA0029_1196206 (12.00%) under WW20 condition. Three SNPs with pleiotropic loci (*i.e.* SNPs that controls more than one traits) were identified viz. SSA0001_35610702 identified on chromosome SSA0001 for number of earheads per plant and total number of productive tillers per plant and it recorded a phenotypic variance of 11.00 and 12.00 *per cent* respectively (Table 4.25). Similarly, another loci with common SNP SSA0012_6750700 on chromosome SSA0012 was identified for total earhead weight per plant and seed yield per plant with the phenotypic variance of 10.00 *per cent* and 11.00 *per cent* respectively. One more loci with common SNP SSA0009_2114820 on chromosome SSA0009 found to be associated with main earhead length and finger length with the phenotypic variance ranging from 8.00 to 10.00 *per cent*. These multi-trait association of indicates the shared genetic SNPs the traits under WW conditions.

The QTLs are considered as major when they explained >10% of the phenotypic variation in the population (Tanksley, 1993). Under WW19 conditions, totally 12 significant MTAs recorded as a major QTLs. Out of which, number of earheads per plant recorded 8 major QTLs followed by main earhead weight (2), total number of productive tillers per plant (01) and seed yield per plant (01). On the other hand, totally 26 significant MTAs recorded as a major QTLs under WW20 conditions (Table 4.23). The highest major QTLs were recorded in finger width (12), followed by culm length (10),

Table 4.23 SNP markers putatively associated with seed yield and yield related traits among 350 genotypes of association panel in finger millet under well watered conditions in WW19 and WW20.

Trait		SNP	Chromosome	Position	P value	maf	R square. of Model without SNP	R square of Model with SNP	FDR Adjusted P-values	Effect
WW19										
EHL	01	SSB0007_17154962	SB0007	17154962	5.52E-08	0.09	-0.01	0.08	0.29	1.32
EHP	08	SSA0004_6476006	SA0004	6476006	1.84E-08	0.12	0.02	0.11	0.06	-0.73
		SSA0009_1049320	SA0009	1049320	3.01E-08	0.08	0.02	0.11	0.06	0.90
		SSA0007_16530639	SA0007	16530639	4.83E-08	0.05	0.02	0.11	0.06	-1.23
		SSA0007_16530640	SA0007	16530640	4.83E-08	0.05	0.02	0.11	0.06	-1.23
		SSA0001_35610702	SA0001	35610702	6.24E-08	0.05	0.02	0.11	0.06	-1.19
		SSA0004_6476024	SA0004	6476024	7.35E-08	0.10	0.02	0.11	0.06	-0.73
		SSA0004_6695339	SA0004	6695339	8.21E-08	0.12	0.02	0.10	0.06	-0.72
		SSA0004_7802930	SA0004	7802930	1.25E-07	0.09	0.02	0.10	0.08	0.79
EHW	03	SSB0007_14678607	SB0007	14678607	7.88E-08	0.07	0.01	0.10	0.27	1.89
		SSB0007_14678627	SB0007	14678627	1.44E-07	0.07	0.01	0.10	0.27	-1.83
		SSB0007_14678598	SB0007	14678598	1.54E-07	0.07	0.01	0.10	0.27	-1.80
EHWP	01	SSA0012_6750700	SA0012	6750700	9.44E-08	0.42	0.02	0.11	0.50	-1.75
FL	03	SSB0007_17154962	SB0007	17154962	7.29E-08	0.09	-0.01	0.08	0.22	1.10
		SSA0009_2114820	SA0009	2114820	1.17E-07	0.07	-0.01	0.08	0.22	-1.01
		SSB0003_26394833	SB0003	26394833	1.60E-07	0.12	-0.01	0.08	0.22	-1.08
PTLP	01	SSA0001_35610702	SA0001	35610702	9.67E-08	0.05	0.04	0.12	0.51	-0.81
SYP	01	SSA0012_6750700	SA0012	6750700	2.66E-08	0.42	0.02	0.11	0.14	-1.57
WW20										
CL	10	SSA0016_6626688	SA0016	6626688	1.74E-10	0.12	0.04	0.16	0.00	-2.38
		SSA0016_6626629	SA0016	6626629	5.78E-10	0.18	0.04	0.16	0.00	-2.02
		SSA0016_6626653	SA0016	6626653	9.23E-10	0.17	0.04	0.15	0.00	-2.19
		SSA0016_6626642	SA0016	6626642	5.20E-09	0.21	0.04	0.14	0.01	-1.83
		SSA0016_6626614	SA0016	6626614	5.64E-09	0.20	0.04	0.14	0.01	-1.75

		SSA0016_6626648	SA0016	6626648	1.49E-08	0.22	0.04	0.14	0.01	-1.73
		SSB0013_15455704	SB0013	15455704	2.00E-08	0.06	0.04	0.13	0.02	-2.97
		SSA0005_24583784	SA0005	24583784	3.67E-08	0.14	0.04	0.13	0.02	-1.98
		SSA0005_24583775	SA0005	24583775	3.72E-08	0.14	0.04	0.13	0.02	-1.94
		SSA0005_10053094	SA0005	10053094	6.08E-08	0.12	0.04	0.13	0.03	-2.06
EHL	01	SSA0009_2114820	SA0009	2114820	1.90E-08	0.07	0.01	0.10	0.10	1.28
DTF	01	SSB0008_25409968	SB0008	25409968	1.69E-07	0.06	0.02	0.10	0.44	3.98
EHW	01	SSA0007_4887629	SA0007	4887629	1.76E-07	0.14	0.04	0.12	0.90	1.40
FL	05	SSA0009_2114820	SA0009	2114820	1.37E-08	0.07	0.00	0.10	0.06	1.11
		SSA0009_2057353	SA0009	2057353	5.15E-08	0.07	0.00	0.09	0.06	1.04
		SSA0009_2066519	SA0009	2066519	5.15E-08	0.07	0.00	0.09	0.06	1.04
		SSA0009_2066811	SA0009	2066811	5.15E-08	0.07	0.00	0.09	0.06	1.04
		SSA0009_2076536	SA0009	2076536	5.15E-08	0.07	0.00	0.09	0.06	1.04
FW	12	SSB0010_10948960	SB0010	10948960	2.88E-12	0.24	0.03	0.18	0.00	0.45
		SSB0034_3696785	SB0034	3696785	1.40E-11	0.25	0.03	0.17	0.00	0.47
		SSB0010_8855876	SB0010	8855876	2.26E-10	0.25	0.03	0.16	0.00	0.43
		SSB0010_3409351	SB0010	3409351	3.66E-10	0.25	0.03	0.15	0.00	0.42
		SSB0008_3400058	SB0008	3400058	3.88E-10	0.24	0.03	0.15	0.00	0.37
		SSA0014_1551876	SA0014	1551876	5.00E-09	0.04	0.03	0.14	0.00	0.38
		SSB0008_26220187	SB0008	26220187	6.89E-09	0.05	0.03	0.13	0.00	0.30
		SSB0010_488172	SB0010	488172	7.62E-09	0.05	0.03	0.13	0.00	0.29
		SSA0031_1158071	SA0031	1158071	8.47E-09	0.05	0.03	0.13	0.00	0.29
		SSB0040_1878205	SB0040	1878205	8.99E-09	0.05	0.03	0.13	0.00	0.31
		SSB0001_4243148	SB0001	4243148	1.00E-08	0.06	0.03	0.13	0.00	0.28
		SSB0007_21895928	SB0007	21895928	1.23E-08	0.05	0.03	0.13	0.01	0.27
TLP	01	SSA0029_1196206	SA0029	1196206	1.59E-08	0.08	0.03	0.12	0.09	0.82

Days to flowering (DTF), Plant height at reproductive stage(PHS), Plant height at harvest(PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

main earhead length (01), days to flowering (01), main earhead weight (01) and total number of tillers per plant (01). The major QTLs for main earhead weight recorded the in both the years. It is also interesting to note that, the another two major QTLs *i.e.* >10% of the phenotypic variation by SNP markers with pleiotropic effects on multiple traits were recorded by SSA0001_35610702 on chromosome SSA0001 for number of earheads per plant and total number of productive tillers per plant and by SSA0012_6750700 on chromosome SSA0012 was identified for total earhead weight per plant and seed yield per plant under WW condition.

4.2.3.4.3 Marker trait association (MTA) analysis under DS condition

The significant marker trait associations (MTAs) were found for different traits under DS conditions. Totally 10 and 24 SNPs recorded significant MTAs under DS19 and DS20 conditions respectively (Table 4.24). The highest number of significant MTAs (13) were recorded for finger width followed by days to flowering (04), test weight (02), number of earheads per plant (02), culm length (01), main earhead length (01), and finger length (01) under DS 20 conditions. Whereas, seven significant MTAs observed for total number of tillers per plant and only one MTAs each for main earhead weight, number of earheads per plant and total number of productive tillers per plant under DS19.

Among the 07 significant MTAs recorded for total number of tillers per plant, the highest phenotypic variance was explained by SSB0041_365099 (10.00%) and the lowest was by SSA0013_19151605 (9.00%) under DS19 with the marker effect ranges from 0.89 to 0.52 (Table 4.24). Only one MTA was recorded for total number of productive tillers per plant (SSA0009_6177990) which explained the phenotypic variance of 11.00 *per cent* followed by number of earheads per plant was SSA0006_27528229 (10.00%) and main earhead weight by SSA0010_19849699 (9.00%).

Under DS20, among the 13 significant MTAs for finger width, the highest phenotypic variance was explained by SSA0013_6980927 (14.00%) and the lowest was by SSB0014_9466771 (10.00%) with the marker effect ranges from 0.22 to 0.16 (Table 4.24). For days to flowering, four significant MTAs were recorded with the highest

Table 4.24 SNP markers putatively associated with seed yield and yield related traits among 350 genotypes of association panel in finger millet under drought stress conditions in DS19 and DS20

Trait		SNP	Chromosome	Position	P value	maf	R square. of Model without SNP	R square of Model with SNP	FDR Adjusted P-values	Effect
DS19										
EHP	01	SSA0006_27528229	SA0006	27528229	4.93E-08	0.05	0.01	0.10	0.26	-0.92
EHW	01	SSA0010_19849699	SA0010	19849699	6.02E-08	0.23	0.01	0.09	0.32	0.96
TLP	07	SSB0041_365099	SB0041	365099	1.52E-08	0.06	0.00	0.10	0.06	0.90
		SSB0041_365130	SB0041	365130	4.09E-08	0.06	0.00	0.10	0.06	0.88
		SSB0041_365163	SB0041	365163	4.37E-08	0.06	0.00	0.10	0.06	-0.88
		SSB0041_365164	SB0041	365164	4.37E-08	0.06	0.00	0.10	0.06	-0.88
		SSA0006_9022849	SA0006	9022849	1.10E-07	0.05	0.00	0.09	0.06	-0.87
		SSB0033_2009605	SB0033	2009605	1.21E-07	0.09	0.00	0.09	0.06	0.58
		SSA0013_19151605	SA0013	19151605	1.66E-07	0.14	0.00	0.09	0.06	0.52
PTLP	01	SSA0009_6177990	SA0009	6177990	1.51E-07	0.18	0.03	0.11	0.71	0.29
DS20										
CL	01	SSA0004_1706406	SA0004	1706406	8.65E-09	0.29	0.01	0.11	0.05	-2.15
DTF	04	SSA0016_6626492	SA0016	6626492	7.98E-08	0.17	0.05	0.14	0.17	-4.57
		SSB0003_28233276	SB0003	28233276	9.21E-08	0.20	0.05	0.14	0.17	-4.33
		SSB0003_28233277	SB0003	28233277	9.21E-08	0.20	0.05	0.14	0.17	-4.33
		SSB0030_4969655	SB0030	4969655	1.69E-07	0.48	0.05	0.13	0.23	-2.81
EHL	01	SSA0009_2114820	SA0009	2114820	4.00E-09	0.07	0.00	0.11	0.02	1.22
EHP	02	SSA0017_12236508	SA0017	12236508	1.20E-08	0.05	0.03	0.13	0.07	1.34

		SSB0017_6712142	SB0017	6712142	1.14E-07	0.06	0.03	0.11	0.31	1.70
FL	01	SSA0009_2114820	SA0009	2114820	3.91E-09	0.07	0.00	0.10	0.02	1.05
FW	13	SSA0013_6980927	SA0013	6980927	1.23E-10	0.06	0.01	0.14	0.00	0.22
		SSB0006_23152644	SB0006	23152644	1.58E-09	0.05	0.01	0.12	0.00	0.21
		SSA0016_9752519	SA0016	9752519	2.92E-08	0.05	0.01	0.11	0.03	0.20
		SSB0001_12358020	SB0001	12358020	3.68E-08	0.11	0.01	0.10	0.03	0.13
		SSA0034_384028	SA0034	384028	4.20E-08	0.07	0.01	0.10	0.03	0.13
		SSA0024_4909691	SA0024	4909691	5.88E-08	0.05	0.01	0.10	0.03	0.20
		SSB0005_7484568	SB0005	7484568	6.40E-08	0.05	0.01	0.10	0.03	0.16
		SSB0003_30947229	SB0003	30947229	6.55E-08	0.06	0.01	0.10	0.03	0.13
		SSA0017_15418520	SA0017	15418520	6.66E-08	0.06	0.01	0.10	0.03	0.14
		SSB0007_19674056	SB0007	19674056	7.21E-08	0.05	0.01	0.10	0.03	0.15
		SSB0033_3064060	SB0033	3064060	7.31E-08	0.05	0.01	0.10	0.03	0.15
		SSB0033_3064064	SB0033	3064064	7.35E-08	0.05	0.01	0.10	0.03	0.16
		SSB0014_9466771	SB0014	9466771	8.33E-08	0.05	0.01	0.10	0.03	0.16
TW	02	SSB0006_8350137	SB0006	8350137	9.05E-08	0.06	0.01	0.10	0.25	0.51
		SSB0023_416374	SB0023	416374	1.02E-07	0.07	0.01	0.10	0.25	0.50

Days to flowering (DTF), Plant height at reproductive stage (PHS), Plant height at harvest (PHH), Total number of tillers per plant (TLP), Number of productive tillers per plant (PTLP), Culm length(CL), Number of earheads per plant (EHP), Main earhead length (EHL), Main earhead weight (EHW), Total earhead weight per plant (EHWP), Finger numbers per earhead (FNEH), Finger length (FL), Finger width (FW), Test weight (TW), Total biomass (BM), Seed yield per plant (SYP), Well watered (WW), Drought stress (DS)

phenotypic variance explained by SSA0016_6626492 (14%) and the lowest was by SSB0030_4969655 (13.00%) with the marker effect ranges from -4.56 to -2.81. Two MTAs recorded for number of earheads per plant with the phenotypic variance 13.00 *per cent* (SSA0017_12236508) and 11.00 *per cent* (SSB0017_6712142). Similarly, two significant MTAs recorded for test weight DS20 by SSB0006_8350137 (10.00 %) and SSB0023_416374 (10.00%). Only one MTA is recorded for culm length by SSA0004_1706406 (11.00%), main earhead length by SSA0009_2114820 (11.00%) and finger length by SSA0009_2114820 (10.00%). Only one SNP with pleiotropic loci (*i.e.* SNP that control more than one trait) were identified for main earhead length and finger length by SSA0009_2114820 on chromosome SA0009 was found to be significantly associated with the phenotypic variance range from 11.00 to 10.00 *per cent* under DS20 conditions (Table 4.25).

The major QTLs were recorded for MTAs under DS environment in both the years. Two major QTL for main earhead weight and total number of productive tillers per plant were recorded under DS19 conditions. On the other hand, totally 16 MTAs recorded as a major QTLs were under DS20 conditions (Table 4.24). Out of which, finger width recorded 9 major QTLs followed by days to flowering (04), culm length (01), earhead length (01) and finger length (01). It is also interesting to note that, the another two major QTLs *i.e.* >10% of the phenotypic variation by SNP markers with pleiotropic effects on multiple traits were recorded by SSA0009_2114820 on chromosome SA0009 for main earhead length and finger length was found to be significantly associated with the phenotypic variance range from 11.00 to 10.00 *per cent* under DS20. It is interesting to note that, a common SNP SSA0009_2114820 on chromosome SSA0009 found to be associated with main earhead length and finger length across WW and DS conditions with the phenotypic variance ranging from 8.00 to 11.00 *per cent* (Table 4.25).

Genome wide association studies (GWAS) has become an important method for identification of alleles associated with important traits for productivity and drought tolerance in crop plants (Ahmed *et al.*, 2022.). The current study was under taken to identify SNP marker associated with sixteen quantitative agro-morphological traits *viz.* days to flowering, plant height at reproductive stage, plant height at harvest, total number

of tillers per plant, number of productive tillers per plant, culm length, number of earheads per plant, main earhead length, main earhead weight, total earhead weight per plant, finger numbers per earhead, finger length, finger width, test weight, total biomass, seed yield per plant which are important for productivity in finger millet under normal well watered conditions which could be potentially used for increased productivity under well watered conditions. Similarly, drought tolerance is an important objective in finger millet and identification of SNPs associated with productivity traits under drought environment is also important for drought tolerance breeding to improve the productivity under drought stress. An association mapping panel (AMP) of finger millet consisting of 350 genotypes representing the geographical and agro-morphological diversity of the world germplasm has been assembled and used in the present study which served as potential source of variability for the traits. Further, the experiment was repeated twice over the two years, therefore, the current results are expected to identify the reliable SNPs for the traits and are valuable for finger millet breeding for high yield as well as drought tolerance. The population structure of the panel, the variance components, heritability and genetic correlations for the phenotypic traits under WW and DS environment confirmed that the panel of genotypes was suitable for use in a genome wide association study involving yield related traits.

Marker trait association (MTA) analysis resulted in total of 83 SNPs associated through mixed linear model (MLM) at the significant Bonferroni threshold level of $p < 1.826 \times 10^{-7}$ with sixteen agro morphological traits recorded under WW and DS conditions over the two years. The MLM model, which accounts for both structure and relatedness (Q+K), was used for the marker–trait association study. The 49 SNPs, out of 83 under WW conditions and 34 SNPs under DS conditions were identified for significant MTAs over the two years. Drought tolerance is highly influenced by genotype by environment interaction (Yagdi and Sozen, 2009) which is explained by the lower number of significant MTAs observed under drought-stressed than non-stressed condition. Association analysis revealed that one or more SNPs significantly associated with one or more traits under both WW and DS conditions. The chromosomes from A sub-genome and B sub-genome exhibited 46 and 37 significant MTAs respectively for

Table 4.25 The common SNP markers putatively associated with seed yield and yield related traits among 350 genotypes of association panel in finger millet either under well watered and/ drought stress conditions during 2019 and 2020

Year	Trait	SNP	Chromosome	Position	P value	maf	R square. of Model without SNP	R square of model with SNP	FDR Adjusted P-values	Effect
WW19	EHP	SSA0001_35610702	SA0001	35610702	6.24E-08	0.05	0.02	0.11	0.06	-1.19
WW19	PTLP	SSA0001_35610702	SA0001	35610702	9.67E-08	0.05	0.03	0.12	0.51	-0.80
WW19	EHWP	SSA0012_6750700	SA0012	6750700	9.44E-08	0.42	0.02	0.10	0.49	-1.75
WW19	SYP	SSA0012_6750700	SA0012	6750700	2.66E-08	0.42	0.02	0.11	0.14	-1.56
WW19	FL	SSA0009_2114820	SA0009	2114820	1.17E-07	0.07	-0.01	0.08	0.22	-1.00
WW20	EHL	SSA0009_2114820	SA0009	2114820	1.90E-08	0.07	0.01	0.10	0.10	1.28
WW20	FL	SSA0009_2114820	SA0009	2114820	1.37E-08	0.07	-0.01	0.09	0.06	1.11
DS20	EHL	SSA0009_2114820	SA0009	2114820	4.00E-09	0.07	0.01	0.11	0.02	1.22
DS20	FL	SSA0009_2114820	SA0009	2114820	3.91E-09	0.07	-0.01	0.10	0.02	1.04

Number of productive tillers per plant (PTLP), Number of earheads per plant (EHP), Main earhead length (EHL), Total earhead weight per plant (EHWP), Finger length (FL), Seed yield per plant (SYP), minor allele frequency (maf), Well watered (WW), Drought stress (DS)

different agro morphological traits under WW and DS conditions. The details of significant MTAs identified for various traits under WW and DS conditions in each year with SNP id, chromosome number in sub-genome, position on chromosome, p-value, minor allele frequency, r^2 of model without SNP, r^2 of model with SNP, FDR adjusted p-values and marker effect is given in Table 4.23 and 4.24. Out of total 83 significant SNPs, 47 SNPs were identified on the chromosomes of A sub-genome and 36 SNPs on the chromosomes of B sub-genome. In A sub-genome, 11 SNPs were observed on chromosome SA0009 followed by 08 SNPs on chromosome SA0016. On the other hand, 06 SNPs were recorded on chromosome SB0007, followed by 04 SNPs each on chromosome SB0003, SB0010 and SB0041 of sub-genome B.

Conclusively, under WW and DS conditions together from both the years, total 83 significant MTAs were identified for twelve agro-morphological traits. Across the environment, the highest significant MTAs recorded for finger width (25) followed by number of earheads per plant (11) and culm length (11). The other important traits viz. finger length (9), total number of tillers per plant (8), main earhead weight (5), days to flowering (5), main earhead length (2), total number of productive tillers per plant (2), test weight (2), main earhead length (2), total earhead weight per plant (1) and seed yield per plant (1). On the other hand, significant SNPs were not found for plant height at reproductive stage, plant height at harvest, finger numbers on main earhead and total biomass.

Totally 38 major QTLs under WW and 18 major QTLs under DS environments were identified. The major QTLs were identified for number of earheads per plant, main earhead weight, total number of productive tillers per plant, finger width, seed yield per plant, culm length, main earhead length, days to flowering, and total number of tillers per plant under WW conditions. It is interesting to observe that the major QTLs identified to be associated with agronomic traits viz. number of earheads per plant, total number of productive tillers per plant, main earhead length, finger length, finger width, main earhead weight recorded high heritability ($h^2 > 60\%$) under WW conditions in both years. The correlation coefficient results from our study revealed that the total earhead weight per plant followed by main earhead weight exhibited highest positive and significant

correlation with seed yield per plant under WW conditions. The other traits viz. total biomass, plant height at harvest, plant height at reproductive stage, days to flowering, finger width, main earhead length, finger number per earhead, finger and test weight recorded positive correlation with seed yield per plant under WW conditions in both the years. This was supported by earlier studies by Wolie and Dessalegn (2011), Eric *et al.* (2016) and Singh *et al.* (2018) in finger millet. However, the correlation between seed yield traits and their interaction with other traits can be partially explained by the presence of pleiotropic genes and was observed by Liu *et al.* (2017), Roncallo *et al.*, (2017) and Schulthess *et al.* (2017). The regression analysis revealed the four traits viz. total earhead weight per plant, main earhead weight, total number of productive tillers per plant and total biomass are found to be common that influencing seed yield under WW conditions in both the years.

On the other hand, totally 18 major QTLs under DS environments observed for the traits viz. main earhead weight, total number of productive tillers per plant, finger width, days to flowering, culm length, main earhead length and finger length. It has been observed that QTLs identified for main earhead weight, days to flowering, main earhead length and finger length recorded high heritability ($h^2 > 60\%$) under DS conditions in both the years. The correlation coefficient results also revealed that the highest positive and significant correlation of seed yield observed with total earhead weight per plant followed by main earhead weight under DS environment. The plant height at harvest followed by total biomass, finger width, culm length, days to flowering, finger number per earhead, finger length and main earhead length also exhibited positive correlation with seed yield in both years. The regression analysis revealed the four traits viz. total earhead weight per plant, main earhead weight, plant height at harvest and finger length are found to be common that influencing seed yield in both the years. The high heritability estimates for these traits under both WW and DS situation across year indicate the stable inheritance of these traits and the least influence of environmental conditions on these traits suggest that these traits respond to selection. It is interesting to note that, a common SNP loci or QTL region on chromosome SSA0009 found to be associated with main earhead length and finger length across WW and DS conditions. Ideally, the effects of such loci may not be influenced by the change in external environment (Mathews *et al.*, 2008).

High phenotypic trait correlations were explained in terms of direct or indirect contribution of one trait to another under WW and DS environment. Looking into the genome, loci controlling such traits could be similar. This is evidenced by the existence of several multi-trait associations where one gene will have pleiotropic effects on highly correlated traits. Remarkably, three SNP markers under WW conditions for the traits viz. number of earheads per plant, total number of productive tillers per plant, total earhead weight per plant, seed yield per plant, main earhead length and finger length and one SNP marker for main earhead length and finger length under DS conditions was detected in this study. These markers have pleiotropic effects at one locus. Dholakia *et al.* (2003) and Mwadzingeni *et al.* (2017) reported that highly correlated traits are often controlled by a common QTL. The common markers were located on the same chromosome which indicated that these genomic regions represented one QTL provides an opportunity for effective simultaneous improvement in seed yield and drought tolerance using the overlapping markers. SNP markers with pleiotropic effects on salt tolerance across growth stages were reported in wheat by Oyiga *et al.* (2018) and Hasseb *et al.* (2022).

The expression of any morphological trait is influenced by the underlying genetic make-up (G), the surrounding environment (E), and their interactions (G×E). Yield and its related traits are quantitative and polygenic, or complex traits. They are continuously distributed, controlled by quantitative trait loci (QTL), genomic regions that contain the associated genes, and heavily influenced by the environment. As a result, QTL or genes affecting physiological traits, yield and its components are environmentally sensitive, or have different effects in different environments and therefore exhibit QTL-environment interaction (QTL×E) (Said *et al.*, 2022). Such QTL×E has been observed in the present study for the traits viz. number of earheads per plant, main earhead weight, total number of productive tillers per plant, total number of tillers per plant and culm length under WW and DS conditions. The overall pattern of QTL×E effects can constrain or facilitate responses to artificial selection (Falconer, 1952). Therefore, these effects are critical and should be carefully addressed in breeding programs (Des Marais *et al.*, 2013; El-Soda *et al.*, 2014). The marker-trait association with agro-morphological traits clearly showed the importance of SNP markers in deciphering QTLs for various yield component traits in the present study. Since, SNPs occur in the genomes in much higher frequency than

SSRs, they provide high density of markers near a locus of interest, evolutionary stable and do not change significantly from generation to generation (Sharma *et al.*, 2018). The Mixed Linear Model (MLM) based strategy adopted for GWAS in our study is a powerful mapping tool for plant breeding and genomics programs to identify true functional associations that condition genetic diversity (Kumar *et al.*, 2016b; Lipka *et al.*, 2012). The results from our study are in accordance with Tiwari *et al.* (2020) who also used MLM to identify MTAs between seed yield related traits in finger millet and found common MTA for seed protein content, seed yield and days to maturity. Their study revealed 4 significant MTAs for seed protein content, 7 MTAs for grain yield and 6 MTAs for days to maturity at two different locations. It is interesting to observe that the SNP markers identified to be associated with agronomic traits viz. total number of tillers plant, total number of productive tillers per plant, main earhead length, finger length, finger width, main earhead weight recorded high heritability under WW conditions in both years. On the other hand, SNPs identified for main earhead weight, test weight, total number of tillers per plant, number of earheads per plant recorded high heritability under DS conditions. The MTAs recorded for main earhead weight and total earhead weight per plant are identified as a major yield contributing traits for drought tolerance and seed yield under WW and DS conditions indicated robustness and possible practical utility of the identified markers in finger millet breeding programme. The markers identified in this study are useful genomic resources to initiate marker-assisted selection and trait introgression of finger millet under drought-stressed and well watered conditions, and for fine mapping of QTL. Further studies are required to validate the significant markers identified in the present study.

4.3 Effect of early reproductive growth stage stress on flowering, quality and quantity of pollen grains in contrasting finger millet genotypes

Reproductive stage drought stress causes more severe damage and yield loss than any other stage of development of crop plants (Dorion *et al.*, 1996; Sheoran and Saini, 1996). In order to study the effect of moisture stress during early reproductive stage in finger millet, 12 finger millet genotypes comprising 6 drought tolerant viz. GE-1234, GE-1260, GE-1531, GE-5118, GE-2268, GE-909 and 6 drought susceptible genotypes

viz. GE-1080, GE-1417, GE-1641, GE-2439, GE-4172 and GE-4719 were selected. These genotypes were selected based on their ranking in drought tolerance indices (DTIs) over two years (2019 and 2020) experiments. The selected genotypes were subjected to drought stress under field conditions by growing them in a replicated experiment (Plate 4.3). In order to subject the genotypes to reproductive stage moisture stress, the irrigation was withheld for 20 days from 47 days after sowing. The control block was irrigated regularly without any stress till maturity.

The results from analysis of variance showed significant differences for number of pollen grains per anther, pollen sterility, relative water content and SCMR value excluding membrane leakage among 12 finger millet genotypes evaluated under well watered (WW) and drought stress (DS) condition. The genotype, stress treatment and genotype $\times$ stress treatment effect were significant for number of pollen grains per anther, pollen sterility, relative water content and SCMR value (Table 4.26). The mean performance of 12 genotypes for each of the traits recorded together under WW and DS condition is tabulated in table 4.27. The mean values for number of pollen grains per anther, relative water content and SCMR value showed the significant reduction under DS condition compared to well watered treatment suggest that the drought treatment significantly affected the performance of the genotypes (Table 4.28). The pollen sterility significantly increased under drought stress treatment. The genotype $\times$ stress treatment interaction was also significant the differential effect of treatment on genotypes indicated that the different genotypes responded differentially to stress treatment (Table 4.29).

a. Number of pollen grains per anther

The mean value of all the genotypes for number of pollen grains per anther recorded was 779.49 under WW conditions which was significantly reduced to 605.32 under DS condition (Table 4.28) suggesting that, drought stress at reproductive stage resulted in 22.43 *per cent* reduction in number of pollen grains per anther. The number of pollen grains per anther ranged from 686.20 (GE-4719) to 912.55 (GE-909) under WW condition whereas, under DS it was ranged from 517.7 (GE-2268) to 695.75 (GE-4172) (Table 4.29). The genotype $\times$ stress treatment was also significant. The number of pollen grains per anther was significantly reduced under drought stress in the genotypes viz. GE-

WW

DS



Plate 4.3 Field view of selected 12 finger millet genotypes evaluated under well watered (WW) and drought stress (DS) conditions at reproductive stage

1531, GE-5118, GE-2268, GE-909, GE-1641 and GE-2439. However, the genotypes GE-1234, GE-1260, GE-1080, GE-1417, GE-4172 and GE-4719 did not show significant reduction in number of pollen grains per anther under drought stress suggesting their tolerance. Many of these genotypes were also tolerant under field conditions over two years and hence number of pollen grains under drought stress environment reflects on the

Table 4.26 Analysis of variance of number of pollen grains per anther, pollen sterility (%), relative water content (%), SCMR value, and membrane leakage (%) among the 12 genotypes evaluated under WW and DS conditions

Source of variation	df	PA	PS	RWC	SCMR value	ML
Genotypes (G)	11	178677.83**	629.89**	183.54**	258.09**	309.41
Treatment (T)	1	364007.15**	1671.58**	2757.27**	2509.68**	7.43
G × T	11	40684.42**	301.83**	132.67**	111.17**	186.65
Replication	1	10360.54	1.90	5.29	0.05	2.79
Error	23	15304.24	9.08	68.23	15.56	352.65
Total	47	609034.18	2614.29	3747.02	2894.57	858.95
CD @5%						
Genotypes (G)		123.96	12.69	14.23	8.94	N/A
Treatment (T)		45.72	3.86	6.37	3.83	N/A
G × T		169.89	15.96	21.86	12.95	N/A

* indicates significant at 5%, ** indicates significant at 1%

Where, PA: Number of pollen grains per anther, PS: Pollen sterility (%), RWC: Relative water content (%), SCMR value: SPAD chlorophyll meter reading value, ML: Membrane leakage (%)

Table 4.27 Mean values of 12 finger millet genotypes evaluated for number of pollen grains per anther, pollen sterility (%), relative water content (%), SCMR value, and membrane leakage (%) under well watered and drought stress conditions

Genotypes (GE No.)	PA	PS (%)	RWC (%)	SCMR value	ML (%)
1234	746.20±7.46	12.60±1.67	74.29±2.46	48.81±1.88	11.65±1.52
1260	756.18±6.94	16.52±1.65	72.56±2.44	45.53±2.25	16.69±0.95
1531	576.13±9.00	18.11±1.68	73.96±2.50	44.96±1.94	12.09±1.21
5118	679.98±8.54	15.92±1.72	73.12±2.33	45.82±1.92	16.96±1.56
2268	630.88±8.95	12.89±1.69	68.89±3.05	44.86±2.09	13.15±0.61
909	784.93±9.50	16.14±2.27	71.46±2.81	44.40±2.12	11.55±1.09
1080	699.30±7.43	23.85±2.90	66.39±3.36	40.66±2.32	17.12±0.73
1417	686.00±4.22	22.76±2.36	66.80±3.12	41.53±2.51	22.40±1.71
1641	686.25±8.30	23.16±2.91	63.88±3.22	42.19±2.58	17.93±1.29
2439	648.25±8.94	26.90±3.12	67.19±2.95	42.07±2.46	17.97±0.81
4172	775.08±7.49	22.58±2.81	69.41±3.01	41.79±2.41	17.15±1.53
4719	639.70±5.73	30.12±3.24	67.16±3.35	41.54±2.50	19.79±1.76

Table 4.28 Mean and range performance of the 12 genotypes of finger millet under WW and DS conditions

Traits	WW		DS	
	Mean	Range (min-max)	Mean	Range (min-max)
PA	779.49±2.37	912.55-686.20	605.32±2.47	517.7-695.75
PS (%)	11.89±0.43	8.67-15.24	28.37±0.89	16.53-45.00
RWC (%)	81.53±0.34	78.55-83.03	57.65±0.72	49.22-65.73
SCMR value	50.91±0.33	48.25-53.79	36.45±0.56	32.68-43.82
ML (%)	16.82±0.63	9.87-26.54	15.59±0.57	8.39-24.18

Where, PA: Number of pollen grains per anther, PS: Pollen sterility (%), RWC: Relative water content (%), SCMR value: SPAD chlorophyll meter reading value, ML: Membrane leakage (%),

Table 4.29 Performance of 12 finger millet genotypes of finger millet for number of pollen grains per anther, pollen sterility, relative water content, SCMR value and membrane leakage in well-watered and stress condition and their difference

Genotype (GE No.)	PA			PS (%)			RWC (%)			SCMR value			ML (%)		
	WW	DS	Diff.	WW	DS	Diff.	WW	DS	Diff.	WW	DS	Diff.	WW	DS	Diff.
1234	824.90	667.50	157.40	8.67	16.53	7.87	82.84	65.73	17.11	53.79	43.82	9.97	14.91	8.39	6.52
1260	824.35	688.00	136.35	12.67	20.37	7.70	80.94	64.17	16.77	52.71	38.35	14.36*	17.95	15.42	2.53
1531	690.65	461.60	229.05*	14.09	22.12	8.03	82.83	65.10	17.73	50.26	39.65	10.61	10.02	14.17	4.15
5118	783.20	676.75	106.45	11.72	20.12	8.40	80.78	65.46	15.32	51.04	40.59	10.45	20.39	13.53	6.86
2268	744.05	517.70	226.35*	8.85	16.94	8.09	82.07	55.71	26.37*	51.03	38.69	12.34	12.62	13.69	1.06
909	912.55	657.30	255.25*	8.83	23.45	14.62	82.66	60.25	22.41*	50.75	38.04	12.71	9.87	13.23	3.36
1080	777.45	621.15	156.30	11.95	35.75	23.80*	82.39	50.39	32.00*	48.25	33.07	15.18*	16.36	17.88	1.52
1417	711.15	660.85	50.30	14.89	30.64	15.74	80.55	53.04	27.51*	50.47	32.59	17.88*	26.54	18.26	8.28
1641	783.60	588.90	194.70*	11.22	35.11	23.89*	78.55	49.22	29.33*	51.59	32.79	18.80*	20.27	15.59	4.68
2439	761.35	535.15	226.20*	13.15	40.66	27.51*	79.50	54.88	24.61*	50.65	33.49	17.16*	17.04	18.90	1.86
4172	854.40	620.75	233.65*	11.39	33.76	22.37*	82.24	56.59	25.66*	49.97	33.60	16.37*	20.44	13.85	6.60
4719	686.20	593.20	93.00	15.24	45.00	29.76*	83.03	51.29	31.74*	50.39	32.68	17.71*	15.41	24.18	8.77
CD@5%	169.89			15.96			21.86			12.95			N/A		

Where, * indicates significant at 5%, PA: Number of pollen grains per anther, PS: Pollen sterility (%), RWC: Leaf relative water content (%), SCMR value: SPAD chlorophyll meter reading value, ML: Membrane leakage (%), WW: well watered (No stress), DS: Drought stress

tolerance of the genotypes for seed yield under drought stress. The similar findings reported in maize, where the pollen numbers per anther significantly reduced under stress environment and resulted in reduction in yield and yield components contributing traits (Mohapatra *et al.*, 2020). However, Singh *et al.* (2020a) showed that, number of pollen grain per anther serve as an important trait for development of heat tolerant lines with improved agro-morphological performance for seed yield under stress environment. The results also confirmed that, reduction in pollen number is severe under drought stress conditions irrespective of the drought tolerant and susceptible genotypes. Saragih *et al.* (2013) showed that pollen quality traits like pollen number per anther and pollen viability decreased under moisture stress in weedy and cultivated rice genotypes. They reported that tolerant genotypes were able to maintain pollen quality traits compared to susceptible cultivated rice genotypes. The reduction in number of pollen grains per anther during abiotic stress mainly drought and heat at early reproductive stages significantly reduces grain number in cereal crops was also reported by Dolferus *et al.* (2011), Fischer, (2011) and Liu *et al.* (2015b). Similarly, greater reduction in fertile pollen was observed in drought sensitive genotype compared to drought tolerant genotype in wheat (Dong *et al.*, 2017). The reduction in pollen number per anther under drought also attributed to reduced activity of enzymes involved in carbohydrate metabolism namely vacuolar and cell wall invertases and other enzymes related to starch biosynthesis which plays crucial role in maintaining energy supply to developing anther tissues (Sheoran and Saini 1996; Roitsch and González, 2004; Koonjul *et al.*, 2005; Oliver *et al.*, 2005).

b. Pollen sterility (%)

In each genotype, the fully circular (turgid), non-transparent pollen grains were counted in each sample and were considered as fertile pollen grains while the irregular shaped (flaccid), transparent pollen grains were considered as sterile pollen grains (Plate 4.4). The pollen sterility was found to be high as 28.37 *per cent* under DS conditions as compared to WW condition was 11.89 *per cent* (Table 4.28) suggesting that, drought stress resulted in significant increase in pollen sterility in genotypes. The pollen sterility percentage under WW condition was ranged from 8.67 (GE-1234) to 15.24 (GE-4719), whereas, under DS it was ranged from 16.53 (GE-1234) to 45.00 (GE-4719) (Table 4.29).

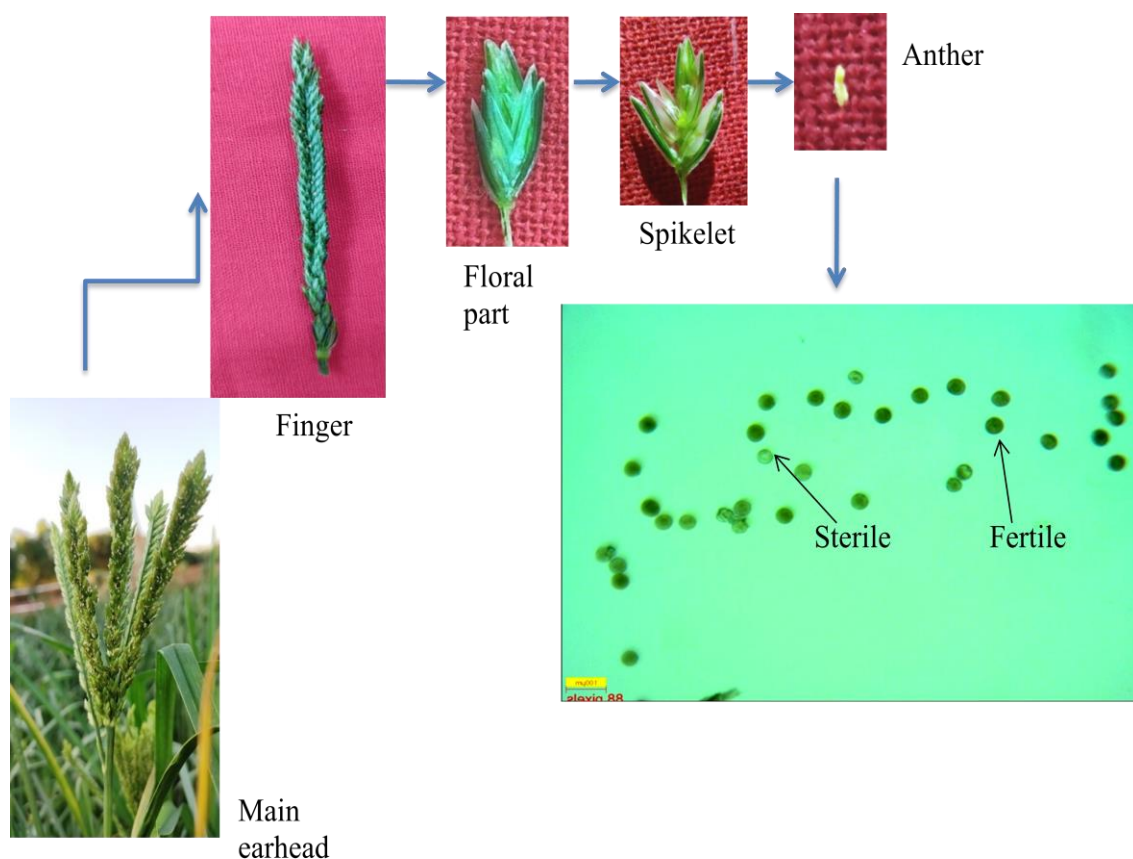


Plate 4.4 Pictorial representation of data recorded for number of pollen grain per anther and pollen sterility. Starting from left, finger collected from immature main earhead of field grown plant and then the spikelets dissected to remove anthers carefully. Each anther were kept in tween-20 solution followed by sonication to release the pollen grains which observed under microscope. Fully circular (turgid), non-transparent pollen grains counted as fertile pollen grains, whereas, irregular shaped (flaccid), transparent pollen grains were considered as sterile pollen grains

The genotype $\times$ stress interaction was also significant for pollen sterility percentage. The overall mean performance of 12 genotypes under well watered and drought stress conditions exhibited that, pollen sterility percentage increased under drought stress conditions. However, the significant pollen sterility percentage was observed in genotypes viz. GE-1080, GE-1641, GE-2439, GE-4172 and GE-4719. The highest percentage of pollen sterility was recorded in GE-4719 (45.00%) followed by GE-2439 (40.66%) under drought stress conditions. The significant differences and high percentage of pollen sterility was recorded under drought stress as compared to well watered condition in the genotype GE-4719 (29.76%) followed by GE-2439 (27.51%). Significant differences for pollen sterility also recorded in GE-1641 (23.89%), GE-1080 (23.80%) and GE-4172 (22.37%). However, in other genotypes, viz. GE-1234, GE-1260, GE-1531, GE-5118, GE-2268, GE909 and GE-1417 significant differences were not recorded for pollen sterility percentage under well watered and drought stress treatment. The results clearly showed that, pollen sterility has been increased under drought stress conditions in all the genotypes. Interestingly, the pollen sterility percentage was found to be high in drought susceptible genotypes as compared to tolerant ones. Dong *et al.* (2017) observed the genotypic differences for higher pollen sterility, where high pollen sterility recorded in drought sensitive genotype than the drought tolerant under early reproductive stage drought stress. Drought stress at early reproductive stage significantly affects the development of floral meristem and causes the reduction in spikelet initiation and abortion of developing microspores. It has been shown that, drought stress at young microspore stage dramatically reduces the yield of the crop (Saini *et al.*, 1984) which has been linked to pollen sterility during the early reproductive stage (Ji *et al.*, 2010b). Drought stress induced abnormal anther development and pollen sterility observed may be due to reduced starch accumulation under drought stress and diminished sugar utilization in anther tissues (Lamin-samu *et al.*, 2021). Studies with cereals have shown that disturbances in carbohydrate metabolism lead to pollen sterility associated mainly with abnormal starch accumulation in the pollen and repression of genes involved in sucrose and starch metabolism in anthers under drought and cold stress (Koonjul *et al.*, 2005; Oliver *et al.*, 2005). Higher pollen abortion and greater reduction in grain associated with higher abscisic acid (ABA) accumulation in anthers of drought-

susceptible than drought-tolerant reported in wheat cultivars (Ji *et al.*, 2011; Dong *et al.*, 2017). Apart from ABA level, the tapetum degeneration could be the primary cause of pollen sterility under drought stress and therefore, grain number loss (Dong *et al.*, 2017; Lamin-Samu *et al.*, 2021).

c. Relative water content (%)

Leaf relative water content (RWC) is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration rate (Rachmilevitch *et al.*, 2006). RWC plays an important role in moisture stress tolerance as it defines the ability of plant to maintain higher leaf water status to maintain turgidity and yield stability under moisture stress conditions (Mullan and Pietragalla, 2012). The mean leaf RWC was recorded as 81.53 *per cent* under WW condition and as that of 57.65 *per cent* under DS conditions (Table 4.28) suggesting that drought stress significantly reduced the leaf RWC. The leaf RWC was ranged from 78.55 (GE-1641) to 83.03 (GE-4719) *per cent* under WW condition and from 49.22 (GE-1641) to 65.73 (GE-1234) *per cent* under DS conditions (Table 4.29). The genotype $\times$ stress interaction was also significant for leaf RWC. The results also indicated that, each genotype has different ability to maintain relative RWC under drought stress conditions. The significant differences and highest reduction under drought stress in leaf RWC was recorded in GE-1080 (32.00%) followed by GE-4719 (31.74%). The significant reduction was also observed in the other genotypes GE-1641 (29.33%), GE-1417 (27.51%), GE-2268 (26.37%), GE-4172 (25.66%), GE-2439 (24.61%) and GE-909 (22.41%) under drought stress condition. However, the genotypes viz. GE-1234, GE-1260, GE-1531, GE-5118 did not show significant reduction under DS environment. It also has been observed that, the leaf RWC was significantly reduced in drought susceptible genotypes as compared to the tolerant genotypes. The results are in agreement with Atteya, (2003) found the moisture stress resulted in decreased relative water content and water potential in leaf. Valentovic *et al.* (2006) affirm that upon induction of moisture stress the leaf RWC decreased in both tolerant and susceptible cultivars. Similarly, Chen *et al.* (2012) observed high leaf relative water content in tolerant inbred lines and low leaf relative water content in sensitive lines. However, drought tolerant genotypes are capable of retaining leaf RWC

as compared to drought sensitive genotypes. It is manifested from the results that, the relative water content was significantly affected in majority of genotypes due to moisture stress. It is possible that the tolerant genotypes may be able to cope up with moisture stress for retention of relative water content through higher root growth and uptake of water. This was in agreement with the results obtained by Mude *et al.* (2020) in finger millet, reported that four-finger millet varieties had the different ability for absorption of water from the soil and its retention capacity in the leaves under drought stress.

d. SPAD chlorophyll meter reading value (SCMR value)

Chlorophyll (Chl) is an important photosynthetic pigment that largely determines photosynthetic capacity and growth of a plant (Barutcular *et al.*, 2015; Croft and Chen 2018). The chlorophyll meter or SPAD meter is a simple portable diagnostic tool that can measure the greenness or relative chlorophyll content of leaves. The mean SPAD chlorophyll meter reading (SCMR) value under DS stress was found to be lower 50.91 as compare to that of WW conditions where it was 36.45 (Table 4.28). The SCMR value under WW ranges from 48.25 (GE-1080) to 53.29 (GE-1234) and from 32.59 (GE-1417) to 43.82 (GE-1234) DS conditions (Table 4.29). This indicated that, chlorophyll content in leaves reduced under DS conditions in all genotypes. The significant difference and highest reduction in SCMR value recorded in genotype GE-1641 (18.80) followed by GE-1714 (17.88), GE-4719 (17.71), GE-4172 (16.37), GE-1080 (15.18) and GE-1260 (14.36). However, significant reduction was not recorded in the genotypes viz. GE-1234, GE-1531, GE-5118, GE-2268 and GE-909 suggesting that each genotype performed differently under stress environment. Drought stress significantly reduced the SCMR value in all the genotypes suggested that chlorophyll degradation was more in drought stress conditions and that was possibly attributable to free radicals generated under drought stress (Sarkar *et al.*, 2013; Mishra and Panda 2017; Mathobo *et al.*, 2017). Several studies in sorghum, maize and wheat have indicated that higher chlorophyll content (*i.e.*, stay green trait) contributes to improved yield under drought conditions (Benbella and Paulsen, 1998; Borrell *et al.*, 2000; Haussmann *et al.*, 2002; Verma *et al.*, 2004). The reduction in total chlorophyll content in drought stress condition was due to a significant decrease in both chlorophyll a (Chl a) and b (Chl b) as reported in pearl millet

(Kholova *et al.* 2011). Under drought stress, SCMR value decreased significantly and the similar results were recorded by Suma (2014), Abad *et al.* (2017), Anitha *et al.* (2019), Mohanabharathi *et al.* (2019) and Krishna *et al.* (2021) in finger millet genotypes. The reduced chlorophyll contents under drought stress may trigger the inactivation of photosynthesis. A decrease in chlorophyll content would result in decreased photosynthetic rate by more than 50% and consequent biomass and yield (Anitha *et al.*, 2019) which further lead to pigment photo-oxidation and chlorophyll degradation, is considered a symptom of oxidative stress (Ma *et al.*, 2020).

e. Membrane leakage (%)

The permeability and leakage of ions out of the cell has been used as a measure of cell membrane stability and as a screen test for stress tolerance. Usually the coexistence of heat and drought stresses affects plant biochemical and physiological processes including cell membrane function (ElBasyoni *et al.*, 2017). The mean of membrane leakage was found to be lower as 15.59 *per cent* DS whereas, 16.82*per cent* under WW condition (Table 4.28). The membrane leakage under WW condition was ranged between 9.87 (GE-909) to 26.54 (GE-1417) *per cent*, whereas under DS it was ranged between 8.39 (GE-1234) to 24.18 (GE-4719) *per cent* (Table 4.28). The overall mean performance in all the genotypes for membrane leakage was lowered under drought stress conditions though the differences were not significant (Table 4.29).

However, it is well documented that, the drought stress has negative effect on the plant cell by damaging the selective permeability of the plasma membrane. Thus the cell cannot maintain its internal composition (Barnabás *et al.*, 2007). This damage affects the plant growth and development (Bajji *et al.*, 2002). Measurement of solute leakage from the plant tissue was used to estimate the damage to the cell membrane caused by drought (Tardieu *et al.*, 2012; Wang *et al.*, 2016) and heat (Reynolds *et al.*, 1994; Suzuki *et al.*, 2014; Jamali *et al.*, 2015) under field conditions. Cell membrane stability was found to be a quantitative trait that was moderately heritable by Hemantaranjan, (2014), with a high genetic correlation with grain yield (Ali *et al.*, 2009; Asif, 2011) and that a small number of genes control a large portion of the variation in the cell membrane permeability (Talukder *et al.*, 2014). Levi *et al.* (2009) demonstrated that the genotypes with higher

chlorophyll content, higher relative water content and less cell membrane damage produced increase in 40 *per cent* of yield as compare to drought susceptible genotypes under drought stress conditions. In another study, cell membrane-thermo stability and relative water content used to select tolerant varieties under drought (Elbasyoni *et al.*, 2017).

In conclusion, the drought tolerant genotypes showed the maintained number of pollen grains per anther and less reduction in pollen sterility under drought stress environment suggesting the usefulness of these two traits for selection and screening of genotypes for drought improvement. Along with these, physiological traits such as leaf relative water content, SCMR value and membrane leakage were also proved the important traits for drought tolerance in finger millet. From this experiment, two contrasting finger millet genotypes viz. GE-1234 (as a drought tolerant) and GE-4719 (as drought susceptible) were identified for drought stress based on their overall performance number of pollen grains per anther, pollen sterility, relative water content, SCMR value and membrane leakage under drought stress condition. The drought tolerant genotype GE-1234 recorded *on par* performance under drought stress compared to well watered conditions for number of pollen grains per anther, pollen sterility, relative water content, SCMR value and membrane leakage. On the other hand, drought susceptible genotype GE-4719 recorded significantly highest percentage of pollen sterility, higher reduction in leaf RWC and SCMR value under drought stress conditions. These two genotypes were further used to study expression of a few stress related genes in the reproductive tissues under moisture stress condition. Conclusively, the drought tolerant genotypes performed better for number of pollen grains per anther, pollen sterility, leaf relative water content, SCMR value and membrane leakage as compared to the susceptible genotypes under drought stress conditions.

4.4 Expression of a few stress related genes in the reproductive tissues under moisture stress

The impact of drought stress on crop productivity depends on the specific developmental stage of the crop. In plants, reproductive phase involves many processes, including floral initiation, anther and pollen development, pistil development, blossom,

fertilization and seed development (Saini and Lalonde 1997). Of all the reproductive processes, the stage of anther and pollen development is the most sensitive to drought (Guo *et al.*, 2016). During male reproductive development under moisture stress, extensive transcriptional rebuilding and gene regulation have been reported in anthers in various crops (De-Storme and Geelen, 2014; Yu *et al.*, 2019). Gene expression analysis is effective in investigating the molecular mechanism underlying anther developmental process. Efforts have been made to characterize the key genes involved in drought tolerance in finger millet at vegetative stages and to utilize them for further applications (Singh *et al.*, 2015; Parvathi and Nataraja, 2017; Ramegowda *et al.*, 2017). With this background, an attempt was made to understand the relative expression of selected few orthologous genes in maize related to pollen development in anthers viz. Sucrose synthase1 (*SUS1*) (Kakumanu *et al.*, 2012), Cell wall invertase (*INCW*), Invertase-1 (*IVR1*) (Ji *et al.*, 2010), GRMZM2G162968 and GRMZM5G844309 (Zhang *et al.*, 2017) under moisture stress conditions. The gene expression analysis was carried out in the developing reproductive tissues *i.e.* immature anthers from developing earhead of two contrasting finger millet genotypes GE-4719 (drought susceptible) and GE-1234 (drought tolerant) (Plate 4.5). The analysis was carried out in the immature anthers collected from well watered and drought stressed plants of both the genotypes.

Semi quantitative polymerase chain reaction was performed to study the expression of the selected genes. It has been observed that, the expression of selected four genes was found to be lower under moisture stress condition as compared to well watered conditions in both the genotypes. However, the reduction in the expression of genes *SUS1*, *INCW*, *IVR1* and GRMZM2G162968 were more in drought susceptible genotype GE-4719 under moisture stress condition as compare to tolerant genotype GE-1234 as well as in well watered conditions. The results indicated that, the drought stress reduce the expression of selected genes in the reproductive tissues. The other gene, GRMZM5G844309 did not show any significant reduction in the expression under moisture stress condition in the drought susceptible genotype GE-4719. Although, expression level of the selected genes viz. *SUS1*, *INCW*, *IVR1*, GRMZM2G162968 and GRMZM5G844309 were lower under moisture stress conditions in both the genotypes, the reduction was not highly significant compared to well watered condition in the

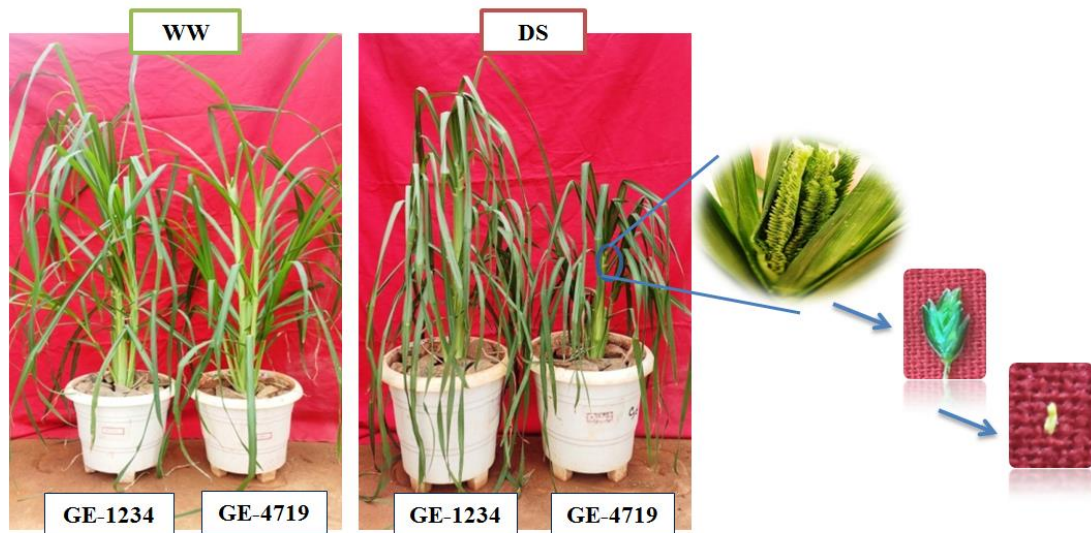


Plate 4.5 Two contrasting genotypes GE-1234 (drought tolerant) and GE-4719 (drought susceptible) showing drought symptoms under well watered (WW) and moisture stress (DS) conditions. The circle depicting the developing immature earheads followed by spikelet and anther (rectangle picture). Anthers were used for RNA extraction

drought tolerant genotype GE-1234. The results indicate that, the drought tolerant genotype GE-1234 maintain the gene expression level for the selected genes under stress compared to drought susceptible genotype GE-4719. *Sucrose synthase 1 (SUS1)* catalyses the reversible reaction in the conversion of sucrose into UDP-glucose and fructose which is the first step in the conversion of sucrose to starch. Thus, it acts as a source of energy and has an impact on reproductive growth in plants (Stein and Granot, 2019). In plants, formation of mature and fertile pollen grains depends on supply of assimilate in the form of sucrose transported from the leaves. Limited studies are available with respect to the understanding of sucrose metabolism in microspores. Oliver *et al.* (2005) reported that deficiency in sugars can lead to pollen abortion. Disruption in the sugar metabolism of the anther could impair the development of pollen and eventually lead to male sterility (Datta *et al.*, 2002). In present study the relative expression of *SUS1* was lowered in stressed condition as compared to control in both genotypes GE-4719 and GE-1234. However, the higher reduction of *SUS1* level was observed in drought susceptible genotype GE-4719 under moisture stress condition (Fig. 4.17; Plate 4.6). Probably, the reduced expression of *SUS1* has resulted the increased pollen sterility in the susceptible genotype GE-4719 (45.00%) and *vice versa* in the tolerant genotype GE-1234 (8.67%). This lowered expression of *SUS1* under stress condition in both the genotypes could be realised in decreased number of pollen grains produced under stress. *SUS1* is an important gene in microsporogenesis in anther and the expression of which influence the quality and quantity of pollen grains. Dorion *et al.*, (1996) and Sheoran and Saini (1996) reported low sucrose synthase activity in moisture stressed anthers of wheat and rice respectively. Moisture stress during reproductive development causes disturbances in carbohydrate metabolism, its distribution within anthers. The inhibition of acid invertases and *sucrose synthase* limits the sucrose supply to developing anthers thereby affecting pollen production and viability (Saini and Lalonde, 1998). Sucrose accumulation, inadequate starch build-up in pollen grains and subsequent abortion of pollen development are attributed to repression of genes involved in sucrose and starch metabolism in drought and cold-stressed anthers (Koonjul *et al.*, 2005; Oliver *et al.*, 2005). The decrease in the *Sucrose synthase 1 (SUS1)* expression has also resulted in the reduction in pollen numbers per anther under stressed condition. However, Zhuang *et al.*

(2007) observed repression in *SUS1* expression under stress and it was compensated by the increase in the expression of *SUS3* during meiosis in the immature reproductive tissues of maize suggesting the compensatory role of *SUS3*.

The synthesis, transport and degradation of sucrose are determining factors for biomass allocation and yield of crop plants. Invertases are a key enzyme of carbon metabolism in both source and sink tissues. Invertase irreversibly catalyses the cleavage of sucrose into glucose and fructose. Two vacuolar invertases viz. *IVR1* and *IVR2* and four cell wall invertases viz. *INCW1*, *INCW2*, *INCW3*, and *INCW4* (Juárez-Colunga *et al.*, 2018) were annotated in maize. Impairment of invertase activity is suspected to be a key causal step in the induction of pollen sterility in wheat under water deficit condition (Dorion *et al.*, 1996). In the present study, the significant reduction in gene expression of *IVR1* under moisture stress condition was observed in drought susceptible genotype GE-4719 as compared to tolerant GE-1234 (Fig. 4.17; Plate 4.6). The relative expression of another invertase gene, cell wall invertase (*INCW*) expression was also studied in both genotypes. The relative expression of *INCW* found to be lower under moisture stress condition in both the genotypes. However, a significant reduction under moisture stress was observed in drought susceptible genotype GE-4719. Reduced invertase activity would minimize the proper intake of sucrose, which harms the metabolic and developmental processes in anthers, resulting in pollen abortion (Dorion *et al.*, 1996). Drought during meiosis selectively represses the expression of vacuolar isoform *IVR5* and cell wall isoform *IVR1*, but not the expression of *IVR3* in wheat (Koonjul *et al.*, 2005). Transcriptional down-regulation of cell wall invertases and active sugar carriers have been claimed to cause the blockage of sugar supply to developing microspores, consequently leading to a failure of starch deposition in unviable pollen (Oliver *et al.*, 2005). The results of the present study are in agreement with the earlier results observed in wheat where in drought-sensitive wheat varieties, the anther invertase gene *IVR1* is significantly reduced under moisture stressed anthers, while the expression remains the same in a drought-tolerant variety (Koonjul *et al.*, 2005; Ji *et al.*, 2010). Male fertility was dramatically affected with differing pollen viability in flowers of the same panicle due to aberrant anther development under water stress in rice (Jin *et al.*, 2013). As mentioned in earlier studies, the higher pollen sterility under moisture stress could be due

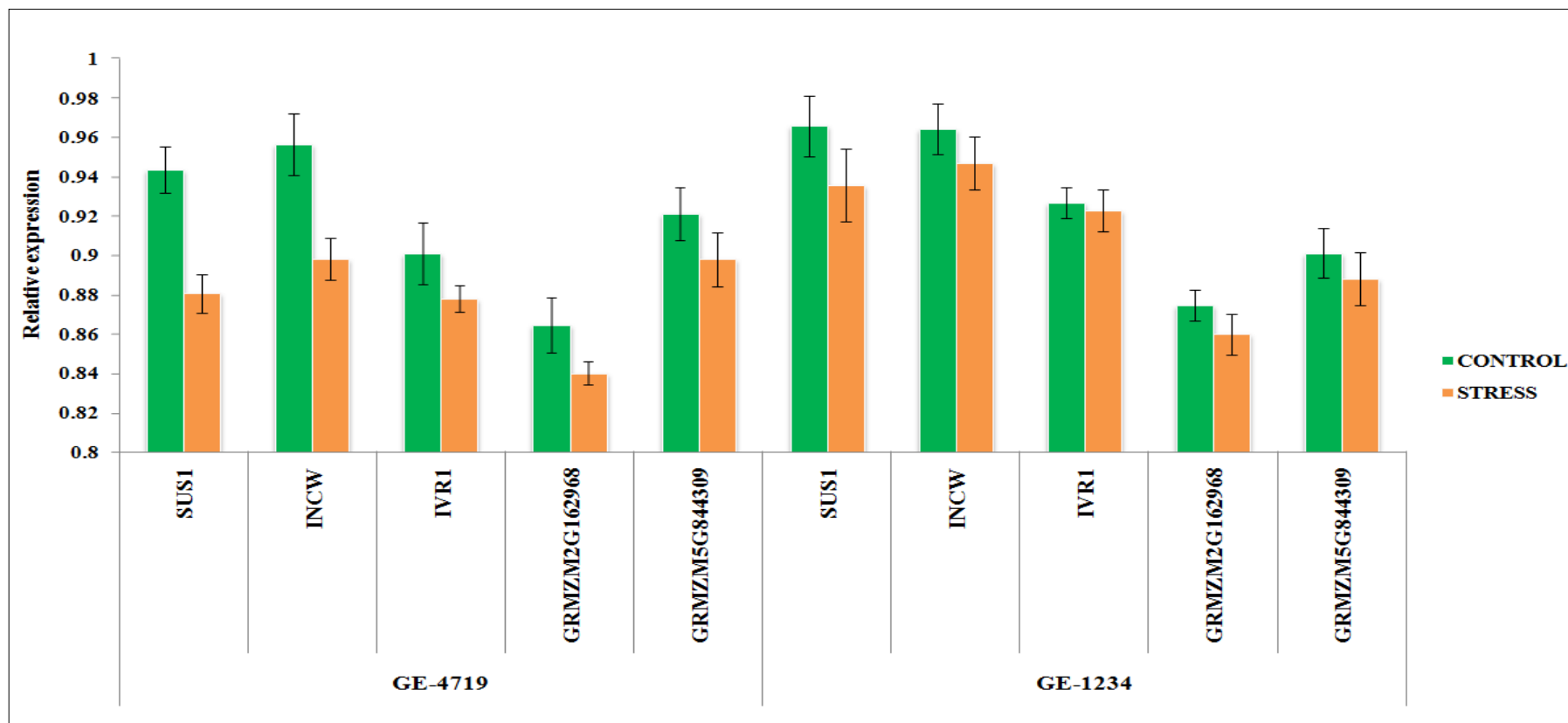


Fig. 4.17 Relative expression of *SUS1*, *INCW*, *IVR1*, *GRMZM2G162968* and *GRMZM5G844309* genes in the anthers of finger millet genotypes GE-4719 and GE-1234 under control and drought stress conditions. *SUS1* (Sucrose synthase1), *INCW* (Cell wall invertase), *IVR1*(Invertase-1), *GRMZM2G162968* and *GRMZM5G844309* (uncharacterised genes)

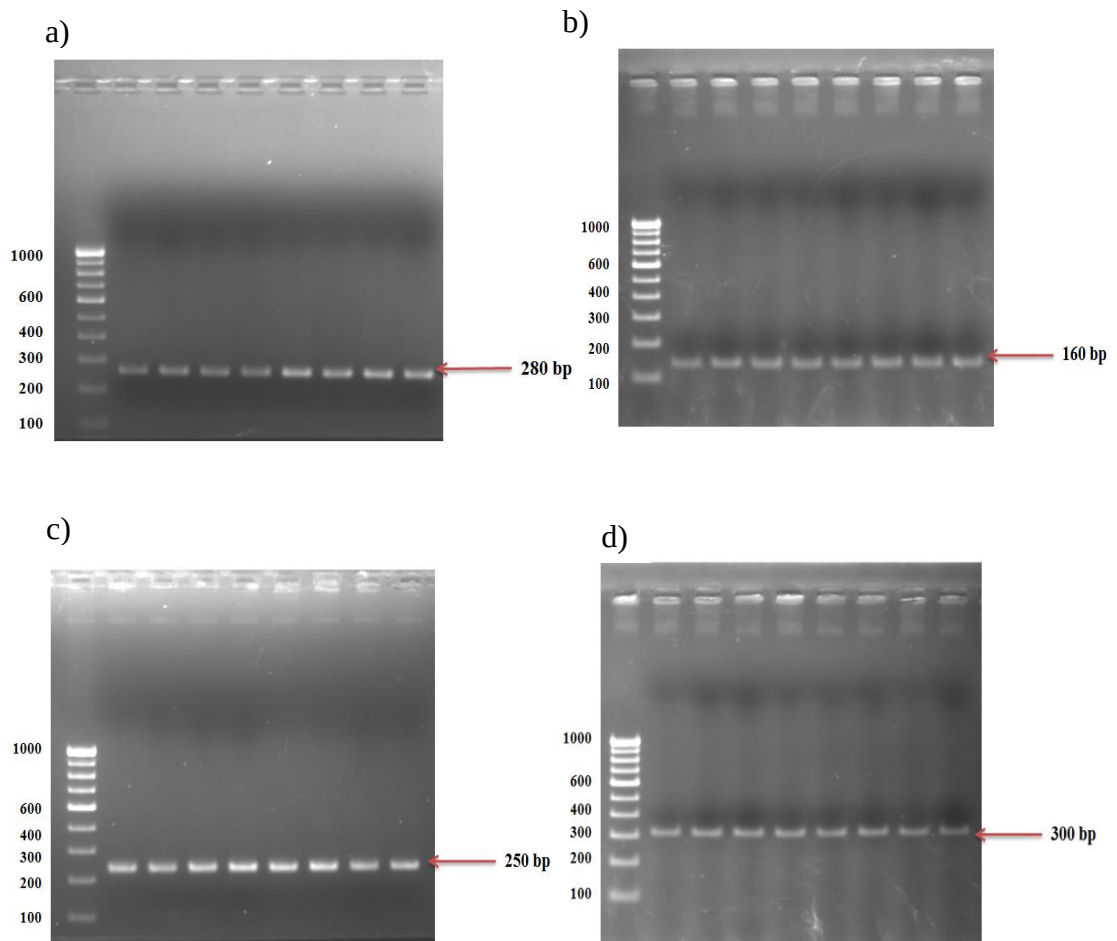


Plate 4.6 Relative gene expression of selected genes in the anthers of genotype GE-4719 and GE1234 under well watered and moisture stress conditions. Where, a) *SUS1* (Sucrose synthase 1), b) *IVR1* (Invertase-1), c) *INCW* (Cell wall invertase), d) *GAPDH* (Glyceraldehyde 3-phosphate dehydrogenase)-Reference gene

L:100bp ladder, well 1-2:GE4719 (moisture stress), 3-4:GE1234 (moisture stress), 5-6:GE4719 (well watered), 7-8:GE1234 (well watered)

to decreased accumulation of starch in pollen grains which is attributed by the decreased activity of vacuolar and cell wall invertases and other enzymes involved in carbohydrate metabolism (Koonjul *et al.*, 2005; Oliver *et al.*, 2005). These results also indicated that invertase activity might result in depleted glucose levels and ATP supplies, which might be lethal to reproductive tissues anthers under drought in crops such as maize (Nan *et al.*, 2018).

The overall relative expression of drought induced uncharacterised genes GRMZM2G162968 and GRMZM5G844309 were slightly lower in stress treatment as compared to control in both the genotypes. The differential expression of these genes was reported by Zhang *et al.* (2017) among contrasting inbred lines of maize under moisture stress. These genes are not characterised until now and hence their role in tolerance mechanism or role in pathways is still unknown. Conclusively, the variation in the expression of *SUS1*, *IVR1* and *INCW* in anther during drought stress treatment depends on the tolerance of the genotypes and the allelic variation can be exploited in crop improvement programs.

V SUMMARY

The present investigation entitled “Genetic association mapping to identify QTLs for drought tolerance and expression analysis of selected genes under drought environment in Finger millet [*Eleusine coracana* (L.) Gaertn]” was carried out at Department of Plant Biotechnology, UAS, GKVK, Bangalore. The primary objective of the present study was to identify stable QTLs associated with agro-morphological traits, seed yield and drought tolerance and the expression analysis of selected genes in contrasting genotypes under drought stress. In the process of attempt, we also made to identify stable drought tolerant genotypes across seasons and important traits associated with seed yield and drought tolerance. In this direction, an association mapping panel of 350 finger millet genotypes representing worldwide diversity of finger millet growing regions were assembled and evaluated for sixteen agro-morphological traits related to seed yield under drought stress (DS) and well watered (WW) conditions over the two years. Further genome wide association studies (GWAS) were carried out using polymorphic SNP data available through resequencing of 350 genotypes (from ISCB project) to identify QTLs associated with agro-morphological traits evaluated under WW and DS conditions in each year separately. The effect of early reproductive stage drought stress on pollen quality and quantity were also assessed in selected contrasting finger millet genotypes. Additionally, the variation in the relative expression of drought stress related genes in immature anthers of drought stress tolerant and susceptible genotypes were also studied. The silent features of the investigation are briefly summarized here:

A significant reduction in the mean values of sixteen quantitative traits were recorded under DS condition compared to WW condition in both the years. The seed yield reduction was 41.45 and 35.62 *per cent* under DS19 and DS20 conditions compared to WW19 and WW20 respectively. Significant variability was recorded among the genotypes for all the 16 quantitative traits in both WW and DS conditions and both the years. It was also observed that, the drought stress at initial reproductive stage affected the performance of the genotypes for yield and related component traits.

The genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance as *per cent* of mean (GAM) for all the sixteen

traits were estimated for WW and DS separately for each year. The GCV and PCV estimates were high for the total number of earheads per plant, main earhead length, total earhead weight per plant, finger length, seed yield per plant and total number of productive tillers per plant under WW and DS conditions in both the years indicating the existence of high variability among the genotypes studied. High heritability estimates were consistently recorded for days to flowering, total number of tillers per plant, main earhead length, finger length, test weight and total biomass under WW and DS conditions in both the years. High heritability of these traits indicating that the variation observed was mainly under genetic control and was less influenced by environment.

High heritability coupled with high GAM was recorded consistently for plant height at reproductive stage, plant height at harvest, total number of tillers per plant, productive tillers per plant, number of earheads per plant, main earhead length, total earhead weight per plant, finger numbers per earhead, finger length, finger width, biomass and seed yield per plant under WW conditions in both the years. Under DS conditions, high heritability with high GAM was observed for total number of tillers per plant, main earhead length, finger length, test weight and total biomass in both the years. High GAM with high heritability under WW and DS conditions was observed for number of tillers per plant, main earhead length, finger length and total biomass. Hence, direct selection can be made through these traits for the improvement of genotypes for drought tolerance and higher seed yield.

The comparison of Pearson's correlation coefficient between seed yield per plant and others traits under WW and DS conditions suggest that, total earhead weight per plant, main earhead weight, total biomass, earhead length and finger width had positive correlation under both the conditions. The results indicated that the improvement in these traits is important for improvement in seed yield under drought stress conditions. In order to reduce the number of variables and to identify important traits associated with seed yield under WW and DS conditions, stepwise multiple linear regression with backward elimination was carried. Results from stepwise multiple linear regression under WW and DS conditions indicated that, total earhead weight per plant and main erahead weight are the major traits under both the environment consistently over the two years. The two

traits are very important for the improvement of seed yield under both WW and DS environment. Apart from these, total biomass and total number of productive tillers per plant also identified as important traits for seed yield under WW conditions. Whereas, under DS conditions, plant height at harvest and finger length are found to be major traits contributing to the seed yield.

Principal component analysis (PCA) is used to identify the traits which contribute maximum to the most observed variation within a group of 350 genotypes. The results of PCA revealed that, out of the 16 PCs, first five PCs with eigenvalue of greater than 1.00 accounted for 76.25 and 68.27 *per cent* of total variability for WW19 and WW20 respectively. On the other hand, under drought stress conditions, first five principle components having eigenvalue greater than 1 accounted for 74.90 and 68.23 *per cent* of the total variability in 350 genotypes during 2019 and 2020 respectively. It has been seen that main earhead weight followed plant height at harvest were the major traits contributed to PC1 and total number of productive tillers per plant and number of earheads per plant were the major traits contributed to PC2 under WW conditions in both the years. Whereas, under DS conditions, main earhead weight followed by main earhead length were the major traits contributed to the PC1 and total number of productive tillers per plant and number of earheads per plant were the major traits contributed to PC2 in both the years. Conclusively, main earhead weight for PC1 and total number of productive tillers per plant and number of earheads per plant for PC2 were identified as important traits under WW and DS conditions.

The PCA-biplot used to assess the genotypes based on multiple traits and to identify the genotypes those are superior in the desired variables. The genotypes G187, G3, G106, G89, G170, G130 and G3 were found to be superior for total earhead weight per plant, main earhead weight, total biomass, finger width, plant height at harvest, plant height at reproductive stage, main earhead length and finger width under WW19 along with genotype G187, G311, G172, G177, G340, G346, G61 and G325 under WW20 conditions. The genotype G187 found to be a stable genotype under WW conditions over the two years for the traits viz. total earhead weight per plant, main earhead weight, total biomass, finger width, plant height at harvest, plant height at reproductive stage, main

earhead length and finger width. On the other hand, genotypes G3, G187, G302, G304, G326, G303 and G311 were found to be superior for total earhead weight per plant, main earhead weight, plant height at harvest, culm length, total biomass and finger width under DS19 along with genotypes G107, G304 and G106 under DS20 conditions. The genotypes G3, G187 and G311 are found to higher performing genotypes for total earhead weight per plant, main earhead weight, plant height at harvest, culm length, total biomass and finger width across the WW and DS conditions.

The choice of genotypes must be based on multiple traits rather than limiting to single trait. A genotype by yield*trait (GYT) biplot approach provides useful information for genotype evaluation on multiple traits rather than seed yield only. The superiority of a genotype is measured not only by its levels in individual traits but more importantly by its levels in combining yield with other target traits. GYT biplot used to identify genotypes for multiple combination of yield*traits to achieve higher yield under WW and DS conditions. The genotypes G31, G55, G136, G239, G223, G58, G135, G158, G166 under WW conditions, on the other hand genotype G239, G81, G237, G290, G228, G86, G141, G89 under DS and the genotype G81, G239, G136, G31, G309, G135 and G158 across both well watered and drought stress (WWDS) exhibited highest yield trait combination value for Y*EHP, Y*TLP and Y*PTLP meaning that, these genotypes are best in combining seed yield per plant with number of earheads per plant, total number of tillers per plant and total number of productive tillers per plant. The genotype G239 and G31 found to be stable genotypes under WW and DS conditions. Thus these genotypes can be used as a donor parents to combine these traits with higher seed yield.

The ideal genotypes in terms of environmental adaptation for the entire yield*trait combinations exhibiting balanced trait profile were also identified. The genotype G307 exhibited highest balanced trait profile for all yield*trait combinations followed by G333, G8, G89, G340, G61, G99, G311, G92, G47 and G22 under WW conditions over the two years. Whereas under DS conditions, the genotype G155 recorded highest balanced trait profile for overall yield*trait combinations followed by G170, G304, G115, G326, G302 and G239. The results indicated that, G170, G89, G155, G33G, G311, G340,

G333, G307 and G92 found to be best balanced trait profile genotypes for overall yield*trait combinations across WWDS conditions.

The genotype main effect plus genotype $\times$ environment interaction (GGE) biplot was used to understand interrelationship between genotypes, environment and the interaction and thus widely used to identify the stable and high yielding genotypes under WW, DS and across WWDS conditions. The high yielding and stable genotypes under these conditions were identified using GGE biplot method based on the common traits viz. total earhead weight per plant, main earhead weight and seed yield per plant which were identified through multiple stepwise regression analysis. The genotype G307 and G170 recorded higher mean performance and with high stability for seed yield per plant under WW, DS and across the WWDS environment over the two years. The higher mean performance for total earhead weight per plant was recorded in genotype G311 under WW and G239 under DS and across WWDS conditions respectively. Similarly, the higher mean performance and stability for main earhead weight were recorded in the genotype G172 and G71 under WW and DS conditions respectively. However, the genotype G340 found to be highly stable with high value of main earhead weight across WWDS environments.

In order to understand the molecular diversity and population structure of 350 genotypes assembled, the SSR and SNP markers were employed. Genetic diversity among the 350 finger millet genotypes was assessed by fifteen genomic SSR markers. The dendrogram constructed using marker data based on UPGMA method, grouped the 350 genotypes into two main clusters. The cluster analysis showed the mixture of genotypes from Asian and African regions indicating that the genotypes grouped independently irrespective of their geographical regions into cluster. The population structure analysis based on SSR markers revealed that 350 genotypes grouped in to three distinct sub-populations. Population structure and clustering patterns of finger millet genotypes used in the study are not largely based on a geographical region. The admixture of African germplasm lines found in the Indian accessions and *vice versa* was probably as a result of intercrossing between Indian and African germplasm. It suggest

that, there was a lot of exchange of finger millet germplasm between two continents from the early stages.

For identification of polymorphic SNPs, the 350 genotypes were resequenced which revealed a total of 80,82,179 SNPs (516Mb) in sub-genome A and 90,71,360 SNPs (650Mb) in sub-genome B. However, after hard filtering and quality check, a total of 32,04,543 SNPs from sub-genome A and 22,71,900 SNP's from sub-genome B were used for the studies. The understanding of linkage disequilibrium (LD) decay of A sub-genome was quite complicated because of admixture happened over domestication and introduction compared to B sub-genome. The B sub-genome was stable and constant population structure was observed. When we compared LD decay of A and B sub-genome, LD decay observed in A sub-genome at 1500 kb at $0.2 r^2$, whereas in B sub-genome at around 1000 kb at $0.2 r^2$. This indicated that the LD decay was faster in B sub-genome than the A sub-genome. This may be due to the genes of the wild species are introgressed or some admixtures in A sub-genome but not in B sub-genome.

The population structure analysis was performed using 54,76,443 SNPs from both A and B sub-genome based on Bayesian model to determine the genetic relatedness among the genotypes. The cross-validation errors depicts that $K=5$ i.e. five sub-populations were inferred according to the admixture analysis. The graphical output of analysis shows that genotypes from Indian source and African source are mixed together in the population.

Marker trait association (MTA) analysis using genome wide association studies (GWAS) with SNP markers resulted in total of 83 SNPs associated through mixed linear model (MLM) at the significant Bonferroni threshold level of $p < 1.826 \times 10^{-7}$ on FDR corrected p-value for sixteen agro morphological traits recorded under WW and DS conditions over the two years. The analysis was carried separately for WW and DS for each year. The 49 SNPs under WW conditions and 34 SNPs under DS conditions were identified for significant MTAs over the two years. The chromosomes from A sub-genome exhibited 46 and from B sub-genome exhibited 37 significant MTAs for different agro morphological traits under WW and DS conditions.

The higher number of MTAs were detected for finger width (12) followed by culm length (10), number of earheads per plant (08), finger length (08) main earhead weight (04), main earhead length (02), days to flowering (01), total number of tillers per plant (01), total number of productive tillers per plant (01), total earhead weight per plant (01) and seed yield per plant (01) under WW conditions.

Twelve MTAs observed for finger width under WW20 by the SNP SSB0010_10948960 with the highest phenotypic variance (18.00%) and the lowest by SSB0007_21895928 (13.00%) under WW20 condition.

Ten significant MTAs were observed for culm length with the highest phenotypic variance explained by SSA0016_6626688 (16.00%) and lowest by SSA0005_10053094 (13.00%) under WW20. Five significant MTAs observed for finger length with the higher phenotypic variance explained by SSA0009_2114820 (10.00%) and lowest by SSA0009_2076536 (9.00%).

Eight significant MTAs were observed for number of earheads per plant by SSA0004_6476006 under WW19 conditions with the highest phenotypic variance 11.00 *per cent* and lowest 10.00 *per cent* by SSA0004_7802930.

A common SNP SSA0001_35610702 on chromosome SSA0001 for number of earheads per plant and total number of productive tillers per plant was observed with phenotypic variance of 11.00 *per cent* and 12.00 *per cent* respectively under WW19 condition. Similarly, another common SNP SSA0012_6750700 was identified for total earhead weight per plant and seed yield per plant with the phenotypic variance of 10.00 *per cent* and 11.00 *per cent* respectively under WW19 condition.

Thirteen MTAs were detected for finger width followed by total number of tillers per plant (07), days to flowering (04 SNPs), number of earheads per plant (03), test weight (02), main earhead length (01), main earhead weight (01) and finger length (01) under DS conditions.

Thirteen significant MTAs were observed for finger width with the highest phenotypic variance explained by SSA0013_6980927 (14.00%) and lowest by SSB0014_9466771 (10.00%) under DS20 conditions.

Four significant MTAs were observed for days to flowering under DS20 with phenotypic variance ranging from 14.00 *per cent* by SSA0016_6626492 to 13.00 *per cent* by SSB0030_4969655.

Two significant MTAs were observed for number of earheads per plant phenotypic variance 13.00 *per cent* (SSA0017_12236508) and 11.00 *per cent* (SSB0017_6712142). Two significant MTAs were also observed for test weight under DS20 condition by SSB0006_8350137 and SSB0023_416374 with the phenotypic variance of 10.00 *per cent* each SNP.

Seven significant MTAs were observed for total number of tillers per plant with the highest phenotypic variance explained by SSB0041_365099 (10.00%) and lowest by SSA0013_19151605 (9.00%) under DS19.

A common SNP SSA0009_2114820 on chromosome SA0009 was found to be significantly associated with main earhead length and finger length with the phenotypic variance range from 11.00 to 10.00 *per cent* under DS20 conditions, which also found to be associated with main earhead length under and with finger length across WW and DS conditions with the phenotypic variance ranging from 8.00 to 11.00 *per cent*.

In order to select drought tolerant and drought susceptible genotypes, six drought tolerance indices (DTI) viz. geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HM), drought resistance index (DRI), yield index (YI), yield stability index (YSI) were used by combining the seed yield performance of genotypes in both the years and across all the DTI. Based on the ranking in each DTI, twelve finger millet genotypes comprising six drought tolerant genotypes viz. GE-1234, GE-1260, GE-1531, GE-5118, GE-2268, GE-909 and six drought susceptible genotypes viz. GE-1080, GE-1417, GE-1641, GE-2439, GE-4172 and GE-4719 were selected.

Six each drought tolerant and drought susceptible genotypes were evaluated under field conditions to study the effect of early reproductive growth stage stress on flowering, quality and quantity of pollen grains. Drought stress showed significant effect on number of pollen grains per anther, relative water content and SCMR value. There was a significant reduction in values under DS condition compared to WW condition. The pollen sterility was increased under DS condition. The effect was not uniform across the genotypes viz. GE-1234, GE-1260, GE-1531, GE-5118, GE-2268, GE-909 and GE-1417.

Two contrasting finger millet genotypes viz. GE-1234 (as a drought tolerant) and GE-4719 (as drought susceptible) were identified from the previous experiment. The drought tolerant genotype GE-1234 did not show significant reduction in the number of pollen grains per anther, relative water content, SCMR value and membrane leakage compared to WW condition. On the other hand, drought susceptible genotype GE-4719 recorded highest percentage of pollen sterility, higher reduction in pollen grains per anther, leaf RWC and SCMR value under DS condition compared to its values under WW condition.

Relative gene expression analysis was performed using semi quantitative polymerase chain reaction to study the gene expression profiles of the selected genes at reproductive stage drought stress. The gene expression analysis was carried in the selected contrasting genotypes GE-1234 (drought tolerant) and GE-4719 (drought susceptible) in the immature anthers collected from developing inflorescences (or earhead). The expression level of the selected genes viz. Sucrose synthase-1 (*SUS1*), Cell wall invertase (*INCW*), Invertase-1 (*IVR1*), GRMZM2G162968 and GRMZM5G844309 were lower under moisture stress conditions in both the genotypes. However, the reduction was not highly significant compared to well watered conditions in the drought tolerant genotype GE-1234. The results indicated that, the drought tolerant genotype GE-1234 maintain the gene expression level for the selected genes in reproductive tissue under moisture stress conditions compared to drought susceptible genotype GE-4719 hence maintain the pollen quality and quantity.

Future line of work:

- A few superior genotypes performing better across drought stress and well watered environments over the two years have been identified. The selected genotypes may be evaluated for the tolerance to drought under large plots and across environments.
- Several SNPs associated with many quantitative traits have been identified using association mapping which needs to be validated using other methods like linkage mapping.
- Mapping the identified SNPs with their flanking sequences on the reference to identify candidate genes and to develop gene based markers.
- The drought tolerant genotypes and trait donors identified from this study may be used in the future breeding programs.

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Appendix I

Genotypes of association mapping panel used in the present study with their place of collection

Genotype code	Genotype name (GE number)	Place of collection	Genotype code	Genotype name (GE number)	Place of collection
G1	12	Tamil Nadu	G40	616	Uttar Pradesh
G2	50	Tamil Nadu	G41	665	Karnataka
G3	69	Uttar Pradesh	G42	669	Africa
G4	70	Tamil Nadu	G43	730	Uttar Pradesh
G5	86	Africa	G44	731	Tamil Nadu
G6	91	Uttar Pradesh	G45	736	Karnataka
G7	104	Tamil Nadu	G46	751	Tamil Nadu
G8	106	Tamil Nadu	G47	763	Uttar Pradesh
G9	113	NEFA	G48	772	Karnataka
G10	116	Madhya Pradesh	G49	774	
G11	125	Sikkim	G50	778	Karnataka
G12	127	Uttar Pradesh	G51	794	Karnataka
G13	128	Bihar	G52	805	
G14	144	Tamil Nadu	G53	808	
G15	149	Bihar	G54	824	Karnataka
G16	156	Jammu & Kashmir	G55	832	Tamil Nadu
G17	199	Andhra Pradesh	G56	834	Karnataka
G18	202	Tamil Nadu	G57	838	Uttar Pradesh
G19	225	Uttar Pradesh	G58	845	Karnataka
G20	255	Peninsular India	G59	877	Tamil Nadu
G21	258	Uttar Pradesh	G60	902	Uttar Pradesh
G22	290	Peninsular India	G61	909	Uttar Pradesh
G23	292	Uttar Pradesh	G62	926	Uttar Pradesh
G24	314	Andhra Pradesh	G63	942	
G25	358	Madhya Pradesh	G64	987	Uttar Pradesh
G26	384	Andhra Pradesh	G65	995	Uttar Pradesh
G27	388	Tamil Nadu	G66	996	Uttar Pradesh
G28	404	Karnataka	G67	997	Uttar Pradesh
G29	436	Tamil Nadu	G68	1004	Uttar Pradesh
G30	468	Kerala (KRL)	G69	1013	Uttar Pradesh

G31	469	Kerala (KRL)		G70	1014	Karnataka
G32	482	Madhya Pradesh		G71	1026	Karnataka
G33	489	Kerala		G72	1033	Karnataka
G34	492	Uttar Pradesh		G73	1034	Karnataka
G35	554	Uttar Pradesh		G74	1050	Karnataka
G36	568			G75	1052	Karnataka
G37	595	Tamil Nadu		G76	1059	Karnataka
G38	597	Andhra Pradesh		G77	1071	Uttar Pradesh
G39	608	Tamil Nadu		G78	1077	Karnataka
G79	1080	Uttar Pradesh		G124	1664	Karnataka
G80	1090	Uttar Pradesh		G125	1673	Karnataka
G81	1093	Uttar Pradesh		G126	1681	Karnataka
G82	1102	Uttar Pradesh		G127	1684	Karnataka
G83	1126	Uttar Pradesh		G128	1779	Uttar Pradesh
G84	1172	Uttar Pradesh		G129	1815	Tamil Nadu
G85	1200	Uttar Pradesh		G130	1853	Tamil Nadu
G86	1234	Karnataka		G131	1855	Tamil Nadu
G87	1235	Uttar Pradesh		G132	1893	Uttar Pradesh
G88	1240	Tamil Nadu		G133	1899	Uttar Pradesh
G89	1260	Tamil Nadu		G134	1914	Uttar Pradesh
G90	1274	Tamil Nadu		G135	1915	Uttar Pradesh
G91	1282	Tamil nadu		G136	1936	Uttar Pradesh
G92	1286	Uttar Pradesh		G137	1944	Tamil Nadu
G93	1298	Uttar Pradesh		G138	1956	Tamil Nadu
G94	1300	Karnataka		G139	2002	Uttar Pradesh
G95	1309	Africa		G140	2037	Uttar Pradesh
G96	1322	Uttar Pradesh		G141	2056	Tamil Nadu
G97	1332	Karnataka		G142	2063	Tamil Nadu
G98	1338	Africa		G143	2069	Tamil Nadu
G99	1354	Uttar Pradesh		G144	2073	Tamil Nadu
G100	1356	Africa		G145	2075	Uttar Pradesh
G101	1360	Andhra Pradesh		G146	2078	Uttar Pradesh
G102	1366	Karnataka		G147	2127	Uttar Pradesh
G103	1376	Uttar Pradesh		G148	2136	Tamil Nadu
G104	1377	Uttar Pradesh		G149	2154	Uttar Pradesh
G105	1380	Uganda		G150	2162	Tamil Nadu
G106	1381	Africa		G151	2215	Uttar Pradesh

G107	1399	Uttar Pradesh		G152	2216	Tamil Nadu
G108	1417	Uttar Pradesh		G153	2238	Tamil Nadu
G109	1423	Karnataka		G154	2262	Tamil Nadu
G110	1424	Karnataka		G155	2268	Tamil Nadu
G111	1437	Uttar Pradesh		G156	2272	Uttar Pradesh
G112	1483	Uttar Pradesh		G157	2275	Uttar Pradesh
G113	1486	Karnataka		G158	2329	Karnataka
G114	1531	Karnataka		G159	2358	Karnataka
G115	1537	Uttar Pradesh		G160	2398	Uttar Pradesh
G116	1557	Karnataka		G161	2439	Uttar Pradesh
G117	1559	Karnataka		G162	2447	Uttar Pradesh
G118	1596	Karnataka		G163	2477	Uttar Pradesh
G119	1606	Karnataka		G164	2486	Uttar Pradesh
G120	1634	Karnataka		G165	2572	Uttar Pradesh
G121	1640	Karnataka		G166	2619	Uttar Pradesh
G122	1641	Uttar Pradesh		G167	2624	Uttar Pradesh
G123	1662	Uttar Pradesh		G168	2628	Uttar Pradesh
G169	2634	Uttar Pradesh		G214	3363	Karnataka
G170	2644	Kenya, Africa		G215	3369	Karnataka
G171	2650	Kenya, Africa		G216	3371	Karnataka
G172	2664	Kenya, Africa		G217	3434	Malawi, Africa
G173	2666	Kenya, Africa		G218	3443	Malawi, Africa
G174	2674	Kenya, Africa		G219	3454	Malawi, Africa
G175	2693	Kenya, Africa		G220	3461	Malawi, Africa
G176	2707	Kenya, Africa		G221	3463	Malawi, Africa
G177	2735	Kenya, Africa		G222	3474	Malawi, Africa
G178	2770	Kenya, Africa		G223	3499	Malawi, Africa
G179	2811	Kenya, Africa		G224	3510	Malawi, Africa
G180	2819	Kenya, Africa		G225	3516	Malawi, Africa
G181	2842	Kenya, Africa		G226	3605	Malawi, Africa
G182	2866	Sri lanka		G227	3628	Malawi, Africa
G183	2882	Maharashtra		G228	3630	Malawi, Africa
G184	2888	Maharashtra		G229	3638	Malawi, Africa
G185	2911	Sikkim		G230	3666	Maharashtra
G186	2931	Malawi, Africa		G231	3674	Maharashtra
G187	2940	Malawi, Africa		G232	3678	Maharashtra
G188	2948	Malawi, Africa		G233	3689	Maharashtra

G189	2963	Malawi, Africa		G234	3701	Maharashtra
G190	2999	Malawi, Africa		G235	3703	Maharashtra
G191	3000	Malawi, Africa		G236	3722	Maharashtra
G192	3003	Malawi, Africa		G237	3741	Maharashtra
G193	3067	Malawi, Africa		G238	3752	Maharashtra
G194	3090	Malawi, Africa		G239	3758	Maharashtra
G195	3091	Malawi, Africa		G240	3764	Maharashtra
G196	3094	Malawi, Africa		G241	3767	Maharashtra
G197	3096	Malawi, Africa		G242	4004	Maharashtra
G198	3099	Malawi, Africa		G243	4011	Maharashtra
G199	3101	Malawi, Africa		G244	4013	Maharashtra
G200	3112	Malawi, Africa		G245	4129	Sikkim
G201	3140	Karnataka		G246	4136	Andhra Pradesh
G202	3147	Karnataka		G247	4140	Andhra Pradesh
G203	3179	Andhra Pradesh		G248	4149	Andhra Pradesh
G204	3197	Andhra Pradesh		G249	4172	Andhra Pradesh
G205	3218	Andhra Pradesh		G250	4201	Madhya Pradesh
G206	3219	Andhra Pradesh		G251	4222	Madhya Pradesh
G207	3235	Andhra Pradesh		G252	4248	Madhya Pradesh
G208	3248	Andhra Pradesh		G253	4398	Bihar
G209	3265	Andhra Pradesh		G254	4404	Bihar
G210	3294	Andhra Pradesh		G255	4415	Bihar
G211	3314	Karnataka		G256	4434	Orissa
G212	3322	Karnataka		G257	4437	Orissa
G213	3353	Karnataka		G258	4440	Orissa
G259	4448	Orissa		G303	5003	Tanzania, Africa
G260	4468	Orissa		G304	5004	Tanzania, Africa
G261	4478	Orissa		G305	5052	Zimbabwe, Africa
G262	4501	Peninsular India		G306	5067	Zimbabwe, Africa
G263	4517	Madhya Pradesh		G307	5078	Zimbabwe, Africa
G264	4547	JAPAN		G308	5095	Zimbabwe, Africa
G265	4568	JAPAN		G309	5116	Kenya, Africa
G266	4596	Karnataka		G310	5117	Kenya, Africa
G267	4597	Karnataka		G311	5118	Kenya, Africa
G268	4600	Karnataka		G312	5134	Kenya, Africa
G269	4601	Karnataka		G313	5140	Uganda, Africa
G270	4678	Uganda, Africa		G314	5146	Zambia, Africa

G271	4683	Uganda, Africa		G315	5148	Zambia, Africa
G272	4685	Uganda, Africa		G316	5166	Uganda, Africa
G273	4690	Uganda, Africa		G317	5175	Uganda, Africa
G274	4693	Uganda, Africa		G318	5209	Uganda, Africa
G275	4703	Ethiopia, Africa		G319	5237	Uganda, Africa
G276	4707	Uganda, Africa		G320	5241	Uganda, Africa
G277	4719	Kenya, Africa		G321	5252	Uganda, Africa
G278	4720	Kenya, Africa		G322	5421	Uganda, Africa
G279	4729	Kenya, Africa		G323	6150	
G280	4731	Kenya, Africa		G324	6336	
G281	4732	Kenya, Africa		G325	6370	
G282	4736	Kenya, Africa		G326	6595	USA
G283	4738	Kenya, Africa		G327	6998	Kenya, Africa
G284	4741	Kenya, Africa		G328	1264-A	
G285	4745	Kenya, Africa		G329	1265-B	
G286	4748	Kenya, Africa		G330	554-B	
G287	4761	Kenya, Africa		G331	GPU-28	
G288	4777	Kenya, Africa		G332	GPU-48	
G289	4793	Kenya, Africa		G333	GPU-66	
G290	4829	Malawi, Africa		G334	GPU-67	
G291	4832	Malawi, Africa		G335	HYBRID-G	
G292	4833	Malawi, Africa		G336	INDAF-15	
G293	4839	Malawi, Africa		G337	KEMPURAGI	
G294	4846	Malawi, Africa		G338	KJNS-52	Chinchawad, Kolhapur
G295	4903	Ethiopia, Africa		G339	KMR-316	
G296	4922	Zambia, Africa		G340	KOPN 330	
G297	4951	Zambia, Africa		G341	L-5	Karnataka
G298	4962	Zambia, Africa		G342	PES 110	Pantnagar (UP)
G299	4963	Zambia, Africa		G343	POORNA	
G300	4976	Zambia, Africa		G344	PR 202	
G301	4983	Zambia, Africa		G345	RAU-8	Bihar
G302	4995	Tanzania, Africa		G346	SHARADHA	
G347	SUNAMANI					
G348	VL-146					
G349						
G350						

PUBLICATIONS

Mysore J. Agric. Sci., 55 (4) : 340-348 (2021)

Selection of Stable Drought Tolerant Genotypes in Finger Millet [*Eleusine coracana* (L.) Gaertn] using Combination of Drought Tolerance Indices

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ABSTRACT

Drought stress may continue to be a major limitation for the crop productivity world wide and severity may increase with the changing climate scenario. Although the finger millet is renowned for its drought tolerance, drought stress at reproductive stage dramatically affects the grain yield. A field experiment was conducted over the two years during summer 2019 and summer 2020 to identify stable drought tolerant finger millet genotypes. Three hundred and fifty genotypes representing wide genetic variability available in finger millet were evaluated for grain yield per plant under drought stress and well watered conditions by following augmented design. Significant yield reduction was observed due to drought stress in both the years. The grain yield per plant under drought stress and well watered conditions were used to determine different drought tolerance indices (DTI) viz. geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HM), drought resistance index (DRI), yield index (YI), yield stability index (YSI) for each genotype and year. The combination of drought tolerance indices over the two years were used to identify the stable drought tolerant genotypes. The genotypes viz. GE-1234, GE-1286 and GE-3003 were found to be the most drought tolerant across DTI and GE-1641, GE-4719 and GE-3722 were found to be highly susceptible. The tolerant genotypes recorded less than 26 per cent yield reduction under drought stress over the two years. On the other hand, the yield reduction was more than 76 per cent over the two years in susceptible genotypes. In the present study using combination of DTI, contrasting genotypes for drought tolerance were identified and were confirmed by yield reduction under stress environment for further use.

Keywords: Finger millet, Drought indices, Grain yield, Tolerant genotypes

FINGER millet [*Eleusine coracana* (L.) Gaertn] is an important food crop in the arid zones of Africa and South Asia. In India, it is cultivated in an area of 1.19 million hectares with a production of 1.98 million tonnes and productivity of 1661 kg per hectare. Karnataka accounts for 56.21 per cent of area and 59.52 per cent of production in India (Sakamma *et al.*, 2018). It is the third most important millet after sorghum and pearl millet in India. Finger millet is mainly grown under rainfed conditions in India and South Asia where drought stress for 15 to 30 days is a common feature and will remain a major abiotic limitation for productivity. Drought stress for 25 to 30 days invariably occurs during either stage of crop growth every year and decreases the grain yield significantly in finger millet (Maqsood & Azam Ali, 2007 and Anonymous, 2011). The simulation models predict that drought stress reduces the grain yield of wheat and maize by 21 to

40 per cent (Daryanto *et al.*, 2016). It is predicted that moisture stress will increase in most arid and semi-arid regions of the world under future climate-change scenarios (Wassmann *et al.*, 2009) significantly affecting the crop productivity in these regions.

The rainfall pattern in the semi-arid conditions of ragi growing areas is unpredictable and intermittent drought stress occurs at all the growth stages of the crop. However, it is reported that the reproductive and grain filling stages are the most sensitive to moisture stress reducing the yield significantly (Talwar *et al.*, 2020). Achieving a yield increase and stability under drought environment has been recognized to be a difficult challenge, while progress in yield has been much higher in favorable environments (Richards *et al.*, 2002). Drought tolerance is a complex trait influenced by

combinations of physiological and productivity related traits (Tuberosa, 2012). The relative yield performance of genotypes in drought and non-stress environments appear to be a common starting point for identifying desirable genotypes for unpredictable conditions (Mohammadi *et al.*, 2012 and Nouri *et al.*, 2011). However, many methods of determination of drought tolerance indices (DTIs) have been proposed to identify drought tolerant genotypes in different crops. The commonly used drought tolerance indices are mean productivity (MP), yield stability index (YSI), geometric mean productivity (GMP), yield index (YI), harmonic mean (HM) and drought resistance index (DRI) to identify drought tolerant genotypes under stress conditions (Mau *et al.*, 2019 and Ferde *et al.*, 2020). In the present study an attempt has been made to identify finger millet genotypes tolerant to reproductive stage moisture stress by evaluating

diverse genotypes over two years under drought stress and well watered conditions.

MATERIAL AND METHODS

Three hundred and fifty diverse genotypes of finger millet representing wide diversity were selected for the present study. The genotypes were grown at the experimental field of Department of Plant Biotechnology, UAS, GKVK, Bengaluru over the two years *viz.* Summer 2019 and Summer 2020. Each year the genotypes were grown in two main blocks separately in augmented design. One block was subjected to drought stress (DS) and another was without any stress or well watered (WW). The seedlings raised in small pots in greenhouse and 21 days old seedlings of 350 genotypes along with checks were transplanted in the field by following augmented design. Each genotype was grown in a

TABLE 1
Different drought tolerance indices used in the present study

Drought indices	Formula	Description	Reference
Geometric Mean Productivity (GMP)	$GMP = \sqrt{Y_p * Y_s}$	The genotypes with high value of this index will be more desirable	Schneider <i>et al.</i> , 1997
Mean Productivity (MP)	$MP = Y_s + Y_p/2$	The genotypes with high value of this index will be more desirable	Rosielle and Hamblin, 1981
Harmonic Mean (HM)	$HM = 2 (Y_p * Y_s) / (Y_p + Y_s)$	The genotypes with high value of this index will be more desirable	Jafari <i>et al.</i> , 2012
Drought Resistance Index (DRI)	$DRI = Y_s (Y_s/Y_p)/Y_p$	The genotypes with high value of this index will be suitable for drought stress condition	Lan, 1998
Yield Index (YI)	$YI = Y_s/Y_p$	The genotypes with high value of this index will be suitable for drought stress condition	Gavuzzi <i>et al.</i> , 1997
Yield Stability Index (YSI)	$YSI = Y_s/Y_p$	The genotypes with high values can be regarded as stable genotypes under stress and non-stress conditions	Bousslama and Schapaugh, 1984

Ys: grain yield under DS for each genotype and Yp: grain yield under WW for each genotype Ys~: average yield of all genotypes under DS and Yp~: average yield of all genotypes under WW

single row of 1.2 m length by maintaining a spacing of 30 cm x 10 cm (row to row and plant to plant within a row, respectively). The drought stress was imposed to one block by withholding water for 20 days from 57th day after sowing. After 20 days of stress, regular irrigation was provided till the maturity for the stress block. The other block was watered regularly once in two days which served as well watered / no stress (WW). The crop was raised by following all the cultural practices including fertilizer and pesticide applications. In each block five plants each per genotype were selected for recording observations on grain yield per plant after harvest in both the years.

Data Analysis

The analysis of variance (ANOVA) by following augmented design using the mean grain yield per plant values of genotypes was carried out for stress and well watered block separately for each year. Further, the mean grain yield per plant of DS and WW block of each genotype was used to determine the different drought tolerance indices (DTI) of genotypes as given in Table 1.

For each DTI the overall mean (μ) and standard deviation (σ) was determined for each year separately and the genotypes were grouped as given below:

DTI value of a genotype	Group
$\mu + 2\sigma$ and above	- Tolerant
$\mu + \sigma$ to $\mu + 2\sigma$	- Moderately tolerant
$\mu + \sigma$ to $\mu - \sigma$	- Moderately susceptible
$\mu - \sigma$ to $\mu - 2\sigma$	- Susceptible
$\mu - 2\sigma$ and less	- Highly susceptible

The tolerant and highly susceptible genotypes for drought stress were identified for each of the DTI for both the years separately. The genotype which falls in tolerant group at least in two different DTI in each year over two years was considered as stable tolerant genotype. Similarly, the genotype which falls in highly susceptible group at least in two different drought tolerant indices over two years was considered as stable drought susceptible genotype.

RESULTS AND DISCUSSION

Drought tolerance is a complex trait and the lack of fast reproducible screening technique based on a reliable inheritable trait linked to drought tolerance remains a challenge to identify tolerant genotype under unpredictable drought stress conditions. Several studies on drought tolerance have frequently used indirect selection indicators such as morphological and physiological responses (Kumar *et al.*, 2015 and Purbajanti *et al.*, 2017). Such indirect selection methods rely on many characters that need to be evaluated and the selected tolerant genotypes may not necessarily be stable for yield *per se* under stress conditions. So there is no reliable trait linked to drought tolerance not only in finger millet but also in other crops. For this reason, selection for drought tolerance based on indices developed from grain yield is considered a more rapid and effective approach for selecting drought tolerant genotypes.

The analysis of variance for grain yield per plant revealed significant mean sum of squares attributable to 'Genotypes' and 'Checks vs. Genotypes' under both WW and DS conditions in both the two years, indicating considerable amount of variability in the experimental material (Table 2). The average grain yield per plant under WW condition was 14.26 ± 0.22 and 16.16 ± 0.21 during 2019 and 2020, respectively. The mean grain yield under DS condition greatly reduced to 8.93 ± 0.17 and 10.31 ± 0.19 gm per plant during 2019 and 2020, respectively (Table 3). The grain yield reduction under DS compared to WW conditions in both the seasons indicated that drought stress at initial reproductive stage affected performance of the genotypes. At reproductive stage, drought stress alters the physiological mechanism which ultimately affects the yield and related traits of finger millet (Krishna *et al.*, 2021). Earlier findings reported that the drought stress reduces the grain yield in finger millet up to 18 to 25 per cent (Anonymous, 2008 and Reddy *et al.*, 2020). The grain yield reduction also depends on the severity, duration and the stage of the crop. In the present study grain yield per plant under WW condition varied from 4.38 to 25.96 and from 5.98 to 30.00 in 2019 and 2020, respectively. Whereas, under DS the

TABLE 2
Mean sum of squares in 350 genotypes of finger millet for grain yield per plant under drought stress and well watered conditions

Source of variation	2019			2020		
	df	WW	DS	df	WW	DS
Blocks	4	2.17	14.5	6	1.36	3.94
Entries (Genotypes + Checks)	355	17.33	10.74	354	18.68 **	14.93 *
Checks	5	12.75	15.48	4	72.26 *	38.24 **
Genotypes	349	17.22 *	10.39 *	349	16.78 **	12.59 *
Checks vs. Genotypes	1	78.10 *	109.08 **	1	465.78 **	738.71 **
Error	20	16.12	5.91	24	6.43	8.06

* P <= 0.05; ** P <= 0.01; WW: well watered; DS: drought stress

grain yield per plant ranged from 1.66 to 17.86 and from 2.64 to 22.26 during 2019 and 2020, respectively (Table 3). Overall, the genotypes recorded significant variability for grain yield under stress environment suggesting genotypic tolerance to drought stress. Some genotypes produced moderate to high grain yield under stress environment. However it is difficult to conclude the tolerance of a genotype by *per se* performance under stress environment (Edmeades, 2013). Plants cope with drought stress by a mixture of strategies that vary with genotypes and growth stage of the crop. In this regard, several criteria have been proposed to select genotypes based on their behavior in an environment under drought stress or without stress conditions (Naghavi *et al.*, 2013).

Stable yield performance of genotypes under both favorable and drought stress conditions is vital for plant

breeders to identify drought tolerant genotypes. The high-yielding genotypes under optimum conditions may not be drought tolerant (Mardeh *et al.*, 2006); therefore, many studies preferred the selection based on drought tolerant indices that provides a measure of drought based loss in yield in comparison to well watered condition for screening the genotypes (Ali and El-Sadek, 2016). Different drought tolerance methods have been developed by scientist as selection criteria. The use of drought tolerance indices such as geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HM), drought resistance index (DRI), yield index (YI), yield stability index (YSI) has been widely studied to identify drought tolerant genotypes in different crops (Naghavi *et al.*, 2013; Jalilvandy & Rozrokh, 2013; Menezes *et al.*, 2014 and Darkwa *et al.*, 2016). In the present study, grain yield under stress and well watered conditions were primarily used to determine the drought tolerance indices of the genotypes screened. The drought tolerance indices viz. GMP, MP, HM, DRI, YI and YSI were calculated for each genotype for both the years separately. High variability was observed among genotypes for all the drought tolerance indices studied (Table 4). The 350 genotypes were classified into five groups viz. tolerant, moderately tolerant, moderately susceptible, susceptible and highly susceptible based on their DTI values (Table 5). In both the years, majority of the genotypes were grouped under moderately susceptible followed by susceptible group

TABLE 3
Mean grain yield per plant under drought stress and well watered conditions during 2019 and 2020

Year	Treatment	Mean $\pm$ SE	Range (min-max)
2019	WW	14.26 $\pm$ 0.22	4.38 - 25.96
	DS	8.93 $\pm$ 0.17	1.66 - 17.86
2020	WW	16.16 $\pm$ 0.21	5.98 - 30.00
	DS	10.31 $\pm$ 0.19	2.64 - 22.26

WW: well watered; DS: drought stress

TABLE 4
Mean and range of different drought tolerance indices over the two years

DTI	Year	Mean $\pm$ SE	Range (min-max)
GMP	2019	10.79 $\pm$ 0.18	3.47 - 19.65
	2020	11.49 $\pm$ 0.19	3.16 - 22.54
MP	2019	11.32 $\pm$ 0.17	4.11 - 20.09
	2020	11.99 $\pm$ 0.18	4.01 - 22.84
HM	2019	10.31 $\pm$ 0.18	2.80 - 19.56
	2020	11.05 $\pm$ 0.19	2.27 - 22.48
DRI	2019	0.37 $\pm$ 0.01	0.01 - 1.03
	2020	0.38 $\pm$ 0.01	0.01 - 1.24
YI	2019	1.00 $\pm$ 0.02	0.19 - 2.12
	2020	0.99 $\pm$ 0.02	0.14 - 0.32
YSI	2019	0.60 $\pm$ 0.01	0.11 - 0.98
	2020	0.59 $\pm$ 0.01	0.14 - 0.98

and a very few genotypes recorded high susceptibility (Table 5). During 2019, in GMP 12 genotypes, MP 11 genotypes, HM 13 genotypes, DRI 15 genotypes, YI 13 genotypes and YSI only one genotype and in 2020, 13 genotypes in GMP, 11 genotypes in MP, 12 genotypes in HM, 17 genotypes in DRI, 15 genotypes in YI and 05 genotypes in YSI were found in drought tolerant group. In both the years, the number of genotypes in tolerant group was least for YSI compared to other DTI (Table 6). However some studies pointed out that the genotypes showing low

fluctuation of yield under various levels of drought stress can be considered as drought tolerant along with drought tolerance indices for the stability of tolerance in the genotype (Ali and El-Sadek, 2016). Thus, by combining the performance of genotypes in both the years and across all the DTI, three genotypes *viz.* GE-1234, GE-1286 and GE-3003 were considered as drought tolerant genotypes. Among the three tolerant genotypes, the genotype GE-3003 found consistently in tolerant group for all the DTI and in both the years. Similarly, GE-1234 was also found in tolerant group for all the DTI in both the year except for YSI in 2019. Whereas, across all the DTI in both the years GE-1641, GE-3722 and GE-4719 genotypes were considered as highly drought susceptible genotypes. Out of the three susceptible genotypes, the genotype GE-1641 found consistently in susceptible group for all the DTI except for MP in 2020. Similarly, GE-4719 was also found in susceptible group for all the DTI except MP in 2019 (Table 7). The highest grain yield reduction under stress environment among tolerant genotype was observed in GE-1286 (26.42%) during 2020 followed by GE-1234 (25.25%) in 2019. The least reduction in grain yield among tolerant genotypes under stress was observed in GE-1286 (17.46%) during 2019 followed by GE-3003 (3.86%) in 2020 (Table 7). These genotypes recorded high values for drought tolerance indices *viz.* GMP, MP, HM, DRI, YI and YSI in both the years. The lowest grain yield per plant under

TABLE 5
Number of genotypes into different drought tolerance categories based on drought tolerance indices over the two years

Category	Year	GMP	MP	HM	DRI	YI	YSI
Tolerant	2019	12	11	13	15	13	01
	2020	13	11	12	17	15	05
Moderately tolerant	2019	35	45	42	38	36	58
	2020	41	43	40	33	38	48
Moderately susceptible	2019	241	236	230	231	235	233
	2020	239	242	241	252	238	239
Susceptible	2019	57	54	60	54	60	50
	2020	53	48	52	38	57	46
Highly susceptible	2019	05	04	05	12	04	08
	2020	04	06	05	10	02	12

TABLE 6
Drought tolerant and highly susceptible genotypes based on drought tolerance indices over the two years

Year	DTI	Tolerant genotypes	Highly susceptible genotypes
2019	GMP	GE-1286, GE-1260, GE-3758, GE-5118, GE-156, GE-2644, GE-1077, GE-1026, GE-290, GE-3003, GE-1234, GE-1013	GE-2272, VL-146, GE-4719, GE-2215, GE-1641
	MP	GE-1260, GE-1286, GE-3758, GE-5118, GE-290, GE-156, GE-2644, GE-1026, GE-1077, GE-1234, GE-3003	GE-1080, GE-1641, GE-2215, GE-2272
	HM	GE-1286, GE-1260, GE-3758, GE-5118, GE-260, GE-156, GE-2644, GE-1077, GE-3003, GE-1026, GE-1234, GE-5004, GE-290, GE-1013	VL-146, GE-4839, GE-1743, GE-1641, KJNS-52
	DRI	GE-1286, GE-5004, GE-3090, GE-1381, GE-4995, GE-4013, GE-3003, GE-3758, GE-2664, GE-6595, GE-1077, GE-1531, GE-1234, GE-3638, GE-5118	GE-1417, GE-4601, GE-6336, GE-3101, GE-4951, GE-3722, VL-146, GE-1641, GE-4719, GE-1684, GE-4839, KJNS-52
	YI	GE-1286, GE-3758, GE-5004, GE-69, GE-3003, GE-1077, GE-5118, GE-1381, GE-1260, GE-2644, GE-1026, GE-2664, GE-1234	GE-4839, GE-4719, GE-1641, KJNS-52
	YSI	GE-3764	GE-4719, GE-4601, GE-4839, GE-4690, GE-1684, GE-4951, GE-3722, KJNS-52
2020	GMP	GE-489, GE-106, GE-1424, GE-832, GE-127, GE-3003, GPU-28, GE-3454, GE-909, GE-2056, KOPN-330, GE-1286, GE-1234	GE-1641, VL-146, GE-997, GE-4719
	MP	GE-106, GE-489, GE-1424, GE-832, GE-127, GPU-28, GE-909, GE-3003, KOPN-330, GE-3454, GE-2056	GE-4172, GE-1080, GE-70, GE-2215, GE-4719, GE-997
	HM	GE-489, GE-106, GE-1424, GE-832, GE-127, GE-3003, GE-3454, GE-2056, GPU-28, GE-909, GE-1234, GE-1286	GE-6336, GE-4601, GE-1641, GE-4719, VL-146
	DRI	GE-3003, GE-489, GE-469, GE-832, GE-1102, GE-3454, GE-127, GE-1234, GE-1260, GE-1298, GE-2056, GE-4149, GE-1309, GE-1424, GE-1640, GE-2275, GE-1381	GE-597, GE-1933, GE-6336, GE-2398, GE-4951, GE-3722, GE-1641, GE-4601, GE-4719, VL-146
	YI	GE-489, GE-3003, GE-832, GE-127, GE-1424, GE-3454, GE-469, GE-2056, GE-1234, GE-1260, GE-106, GE-4149, GE-1298, GE-1309, GE-1286	VL-146, GE-4719
	YSI	GE-469, GE-1102, GE-3003, GE-4995, GE-1234	GE-1026, GE-6336, GE-2770, GE-1641, GE-1399, GE-4951, GE-597, GE-2398, GE-4719, GE-3722, VL-146, GE-4601

drought stress was recorded in genotype GE-1641 (1.76g) and in GE-4719 (1.34g) during summer 2019 and 2020, respectively. The highest reduction in grain yield per plant over the two years was recorded in GE-3722 (83.68% and 83.50%) followed by GE-4719 (77.11% and 82.08%). The results indicate that all the

three tolerant genotypes consistently showed less than 26 per cent yield reduction under stress environment while the three susceptible genotypes recorded more than 74 per cent yield reduction in both the years. Mousavi *et al.*, (2008) reported the yield stability is more important than high yield for these indices.

TABLE 7
Stable drought tolerant and highly susceptible genotypes selected based on the drought tolerance indices over the two years

Genotypes	DTIs (summer 2019)						DTIs (summer 2020)						GYP (summer 2019)		GYP (summer 2020)	
	GMP	MP	HM	DRI	YI	YSI	GMP	MP	HM	DRI	YI	YSI	Yp	Ys	Yp	Ys
DT GE-1234	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	19.96 (25.25)	14.92 (14.54)	20.22 (14.54)	17.28 (14.54)
GE-1286	✓	✓	✓	✓	✓		✓		✓				21.64 (17.46)	17.86 (17.46)	21.80 (26.42)	16.04 (26.42)
GE-3003	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	19.96 (19.43)	16.08 (19.43)	20.18 (3.86)	19.40 (3.86)
DS GE-1641	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	6.86 (74.34)	1.76 (74.34)	8.30 (76.14)	1.98 (76.14)
GE-3722				✓		✓				✓		✓	18.76 (83.68)	3.06 (83.68)	17.46 (83.50)	2.88 (83.50)
GE-4719	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	8.04 (77.11)	1.84 (77.11)	7.48 (82.08)	1.34 (82.08)
Mean gain yield per plant													14.26	8.39	14.99	8.99

DT: Drought tolerant, DS: Drought susceptible, ✓: indicates the genotype selected across the DTI, GYP: Grain yield per plant (g), Values in parenthesis indicates the *per cent* reduction in grain yield per plant under the drought stress compared to the well watered condition

Ghasemi and Farshandfar (2015) in wheat, Yousefi (2015) in barely, and Mohammadi (2016) in durum wheat confirmed that high values of STI, MP, GMP, YSI, and YI are the best indices for identification of superior genotypes under drought and non-stress conditions. Naghavi *et al.*, (2013) in maize reported that GMP, MP, YI, YSI, and DRI were positively correlated with seed yield under drought stress conditions. Similarly the mean grain yield under drought stress was significantly correlated with GMP, MP, YI, YSI, DRI, and HM in tef (Ferede *et al.*, 2020). In view of consistent performance of the genotypes for different DTI over the two years, the genotype GE-1234, GE-3003 and GE-1286 were considered as stable drought tolerant genotypes and the genotypes GE-1641, GE-4719 and GE-3722 were considered as drought susceptible genotypes.

The present study indicated significant variability among the genotypes for seed yield under stress and well watered conditions. The yield reduction under

stress was also not uniform across genotypes over the two years suggesting genotypes respond differentially for reproductive stage drought stress in finger millet. Similarly, there was a variation in the response of genotypes for drought stress across two years. However, by using a combination of drought tolerance indices which mainly depended on the effect of drought stress on grain yield three stable drought tolerant genotypes *viz.* GE-1234, GE-1286 and GE-3003 were identified. The highly susceptible genotypes *viz.* GE-1641, GE-3722 and GE-4719 were found. The selected drought tolerant genotypes with least reduction in grain yield under stress a vice versa in case of susceptible genotypes with highest reduction in grain yield confirming the usefulness of drought tolerance indices in the identification of stable contrasting genotypes for drought tolerance.

Acknowledgement : Authors are thankful to CAAST-NGT (Activity 1b), ICAR and Indo-Swiss collaboration in Biotechnology (ISCB)-Ragi network, DBT, India

for financial support. We also acknowledge the University Grant Commission (UGC) of India for providing fellowship during the study.

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(Received : August 2021 Accepted : October 2021)