Pre-breeding of Okra (*Abelmoschus esculentus* [L.] Moench) for Drought Tolerance

By

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Thesis Abstract

Okra (*Abelmoschus esculentus* [L.] Moench; 2n = 2x = 130) is an important vegetable and oil crop. It is extensively grown in tropical and subtropical regions with limited and erratic rainfall conditions. A lack of improved cultivars with drought tolerance hinders the production of okra in sub-Saharan Africa (SSA). Considerable phenotypic and genotypic variation present in okra genetic resources from SSA useful for cultivar design with enhanced fresh pod and oil yields and drought tolerance. However, the genetic diversity in SSA's okra germplasm collection is yet to be explored for breeding targeting economic and horticultural traits. There has been limited progress in the breeding of okra for drought tolerance. Therefore, the specific objectives of this study were i) to determine the response of selected okra genotypes to drought stress using fresh fruit yield and yield-related traits to identify and select candidate genotypes for drought tolerance breeding, ii) to determine genetic diversity present among okra accessions using simple sequence repeats (SSR) and complementary phenotypic markers and to select genetically divergent and superior parental accessions for pre-breeding, iii) to assess the levels of drought tolerance in preliminarily selected okra accessions based on leaf gas exchange and chlorophyll fluorescence to determine best-performing genotypes for drought-tolerance breeding and iv) to determine the combining ability and heterosis of selected okra accessions for yield and yield-related traits to identify superior parents and progenies for breeding.

The first part of the study involved 26 okra genotypes that were evaluated in glasshouse and field environments under drought-stressed (DS) and non-stressed (NS) conditions using a 13 × 2 alpha lattice design with two replications. The findings revealed significant ($P < 0.05$) genotype x testing environment x water condition interaction effects for most traits, allowing for the selection of okra genotypes suited for drier conditions. Yield per plant (YPP) positively and significantly correlated with fresh pod length (FPL) ($r = 0.66$; $P \leq 0.001$), dry pod weight (DPW) ($r = 0.80$; $P \leq 0.001$) and number of pods per plant (NPP) ($r = 0.58$; $P \leq 0.001$) under DS condition in the field environment. The study identified genotypes with high yield and other desirable phenotypic attributes, which are useful genetic resources for future crosses and the selection of promising progenies based on combining abilities analyses and heritability under water-limited environments.

In the second study, 26 preliminarily selected okra accessions were assessed using nine highly polymorphic SSR markers and phenotyped under DS and NS environmental conditions using a 13 × 2 alpha lattice design with two replications. The SSR markers revealed a mean heterozygosity value of 0.54, indicating moderate genetic diversity among the tested okra accessions. Cluster analysis based on phenotypic and SSR markers differentiated the accessions into three distinct genetic groups. Pod yield per plant (PYPP) was positively and significantly correlated with fresh pod length (FPL) (r = 0.81), above-ground biomass (ABG) (r = 0.69), and harvest index (HI) (r = 0.67) under DS conditions, and FPL (r = 0.83) and AGB (r = 0.60) under NS conditions. Genetically complementary accessions such as LS04, LS05, LS06, LS07, LS08, LS10, LS11, LS15, LS18, LS23, LS24, and LS26 were identified for their high yield potential and related yield-improving traits under DS conditions. The identified accessions were recommended as parents for hybridization and selection programs to improve the yield potential of okra under drought-stressed environments.

In the third part of the study, 26 genetically diverse okra accessions were screened for physiological traits response under NS and DS conditions in a controlled glasshouse environment using a 13 × 2 alpha lattice design and three replications in two growing seasons. Statistical analyses revealed a significant genotype × water condition interaction effect for transpiration rate (T), net CO_2 assimilation (A), intrinsic water use efficiency (WUE*i*), instantaneous water use efficiency (WUE*ins*), minimum fluorescence (*Fo′*), maximum fluorescence (*Fm′*), maximum quantum efficiency of photosystem II photochemistry (*Fv′/Fm′*), the effective quantum efficiency of *PSII* photochemistry (*ϕPSII)*, photochemical quenching (*qP*), nonphotochemical quenching (*qN*) and relative measure of electron transport to oxygen molecules (ETR/A). The results suggested variable drought tolerance of the studied okra accessions for selection. Seven principal components (PCs) contributing to 82% of the total variation for assessed physiological traits were identified under DS conditions. Leaf gas exchange parameters, T, A and WUE*i*, and chlorophyll fluorescence parameters such as the *ϕPSII*, *Fv′/Fm′*, *qP*, *qN*, ETR and ETR/A had high loading scores and correlated with WUE*i*, the *ϕPSII*, *qP* and ETR under DS conditions. The study identified drought-tolerant accessions, namely LS05, LS06, LS07 and LS08 based on high A, T, *Fm′*, *Fv′/Fm′* and ETR, and LS10, LS11, LS18 and LS23 based on high *AES*, *Ci*, *Ci/Ca*, *WUEi*, *WUEins*, *ϕPSII* and AES. The selected genotypes are high yielding (≥5 g pods/plant) under drought stress conditions. The data presented will complement phenotypic data and guide breeding for water-limited agroecologies.

Eight selected okra genotypes were crossed in the fourth part of the study to generate new genetic combinations and breeding populations. The parents were selected based on their high yield potential and tolerance to drought stress. The genotypes were sourced from the Agricultural Research Council-Vegetable, Industrial and Medicinal Plants (ARC-VIMP), South Africa, assembled from diverse regions of origin. The selected eight parents were crossed using an 8 × 8 half diallel mating design during the 2021 cropping season. The parents were planted under field conditions at the ARC-VIMP research station during the 2021/2022 growing season. Subsequently, 28 new generations were developed. The crosses and eight parents were field evaluated using a 12 × 3 lattice design with three replications. The genotypes were evaluated under NS and DS conditions at two locations, namely the ARC – Loskop and ARC – Brits sites. Significant ($P<0.01$) effects of genotype, environment, and genotype × environment interaction was recorded for fresh pod yield and component traits. General combining ability (GCA) and specific combining ability (SCA) effects were significant ($P<0.05$) for most traits, indicating the role of additive and non-additive gene action underlying the inheritance of the assessed traits. The GCA × environment and SCA × environment interaction effects were significant for days to flowering (DTF), number of leaves per plant (NOL), fresh pod length (FPL), number of fresh pods per plant (NFPP) and pod yield per plant (PYP). Parental genotypes LS09, LS10 and L24 showed positive GCA effects for PYP under DS conditions and were selected to be valuable germplasm for variety design to widen genetic variability for drought tolerance and yield-related traits. Crosses LS01 × LS17, LS01 × LS18, LS09 × LS10, LS09 × LS18, LS09 × LS24, LS15 × LS18, LS15 × LS21, LS15 × LS24 and LS17 × LS21 expressed positive SCA effects for PYP under DS condition and are recommended for genetic advancement, production, and commercialization in water-scarce environments of South Africa.

Overall, the study discerned considerable genetic diversity among the evaluated okra genotypes. Further, the study selected parental lines and new families with good product profiles, drought tolerance and combining ability for genetic advancement and variety design for water-limited environments in South Africa and similar agroecologies.

Declaration

I, Sonto Silindile Mkhabela, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other University.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a. Their words have been re-written, but the general information attributed to them has been referenced.

b. Where their exact words have been used, then their writing has been placed in italics and inside quotation marks and referenced.

5. This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the reference's sections.

Signed:

Sonto Silindile Mkhabela

As the candidate's supervisor, I agree to the submission of this dissertation:

Prof. Hussein Shimelis (Supervisor)

Dr. Abe Shegro Gerrano (Co-Supervisor)

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Dedication

This dissertation is dedicated to my late brother, Eric Mkhabela, and my son, Lwazoluhle.

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Publications pertaining to this Thesis

Chapter one

Mkhabela, S.S., Shimelis, H., Gerrano, A.S. and Mashilo, J. 2020. Phenotypic and genotypic divergence in okra [*Abelmoschus esculentus* (L.) Moench] and implications for drought: A Review. *South African Journal of Botany* 145: 56-64. DOI: https://doi.org/10.1016/j.sajb.2020.12.029

Chapter two

Mkhabela, S.S., Shimelis, H., Gerrano, A.S. and Mashilo, J., 2021. Phenotypic response of okra (*Abelmoschus esculentus* [L.] Moench) under drought-stressed and non-stressed conditions. *South African Journal of Botany* 145:293-302. DOI: https://doi.org/10.1016/j.sajb.2021.11.008

Chapter three

Mkhabela, S.S., Shimelis, H., Gerrano, A.S. and Mashilo, J., 2022. Characterization of okra (*Abelmoschus esculentus* [L.] Moench) accessions with variable drought tolerance through simple sequence repeat markrs and phenotypic traits. *Diversity* 14 (9): 747. DOI: **https://doi.org/10.3390/d14090747**

Chapter four

Mkhabela, S.S., Shimelis, H., Gerrano, A.S. and Mashilo, J., 2023. Drought tolerance assessment of okra (*Abelmoschus esculentus* (L) Moench) accessions based on leaf gas exchange and chlorophyll fluorescence. *Life* 13,682. DOI: https://doi.org/103390/life13030682.

Chapter five

Combining ability and heterosis analyses of selected okra (*Abelmoschus esculentus*) accessions for agronomic traits under non-stressed and drought-stressed conditions. Under review in the *Journal of Crop Improvement*.

Thesis introduction

Background

Okra (*Abelmoschus esculentus* [L.] Moench), belonging to the Malvaceae family, is a highly nutritious and underutilized crop widely grown in Asia, South America, and Africa (Gemede et al., 2015). The crop is an allotetraploid derived from the natural hybridization of a wild progenitor A. *tuberculatus* ($2n$ = 58), with another unidentified species with $2n$ = 72 chromosomes. It is widely cultivated for its fresh and succulent pods consumed as a vegetable, whereas matured and dry seeds are a rich source of edible oils (Jarret et al., 2011; Reddy et al., 2013). The tender green pod is the most economical and vital source of vitamins A, B_1, B_3, B_6, folic acid, C, and K, essential for the human diet (Komolafe et al., 2021). Potassium, magnesium, phosphorus, and calcium are the principal and essential mineral elements in the green and immature okra pods (Abed et al., 2020). In addition, the fresh pod contains 9.7% carbohydrate, 2.2 % protein, and 1% fibre (Saifullah and Rabbani, 2009). Okra grains contain 22.14% protein, rare amino acids (such as lysine and tryptophan), fat, and fibre. The seed oil content of okra varies from 20-40%, and the primary fatty acids of seed oils are linoleic (49.54%), palmitic (28.60%), oleic (16.81%), stearic (3.57%) and linolenic (1.48%) acids (Jarret et al., 2011) . The seeds are rich sources of protein (22.14%), amino acids (i.e., lysine and tryptophan), fibre, vitamins (i.e., A, C and K), and mineral elements (i.e., calcium, potassium, sodium, and magnesium) (Sanjeet et al., 2010; Petropoulos et al., 2018).

Okra fresh pod production accounts for an estimated area of 2 million hectares globally, with a total annual production of 9 million t/ha (FAO, 2020). The African continent accounts for 32.8% of the world's okra production. West and Central African countries contribute over 75% of the total okra production in sub-Saharan Africa (SSA) (Kumar and Reddy, 2016). Despite the significant contribution by SSA towards global okra production, average yields are low in the region. For example, 2.5, 6.2 and 8.8 tons/ha pod yields were reported in West, East, and North Africa, respectively (FAO, 2020). Low and variable yields in SSA are attributed to the cultivation of genetically inferior and unimproved cultivars (Alake, 2020) associated with poor management practices. Further, biotic (i.e., cucumber mosaic virus) and abiotic stress (i.e., drought and heat) are significant constraints to okra production and declining yields (Mkhabela et al., 2021).

Okra is a relatively drought-tolerant crop that can grow successfully under water-limited conditions with minimal supplemental irrigation. Despite being relatively drought-tolerant, the crop fails to reach its maximum yield potential, resulting in low marketable pod yields. Notably, drought stress alone accounts for yield losses ranging between 30% to 100% for okra, primarily when the stress occurs during the flowering and pod-filling stages (Mkhabela et al., 2021). Previously, very few okra genotypes that are tolerant to drought stress were identified (Munir et al., 2016, Adejumo et al., 2018; Abd El-Fattah et al., 2020; Shi et al., 2020). However, there are still limited drought-adapted varieties in SSA, which necessitates developing high-yielding and drought-tolerant okra genotypes adapted to the region. Population development for improved okra genotypes requires adequate genetic background information and product profiling for planning appropriate okra breeding and selection strategies. Further, there is scant information on associations of phenotypic, physiological traits and molecular markers in okra for drought tolerance conditions, limiting the development of drought tolerant varieties and efficient selection criteria for drought tolerance breeding of the crop.

Rationale of the research

There are fewer breeding efforts to develop drought-tolerant okra genotypes for cultivation in arid and semi-arid regions of SSA. This is hampering efforts to improve crop yield and quality for food and nutrition security in the region. Identifying new sources of drought tolerance in okra genotypes is critical for developing drought-tolerant varieties with desirable product profiles. Therefore, exploring the okra genetic resources available in SSA is essential to identify potential sources of valuable phenotypic and market-preferred traits associated with drought tolerance. Assessing genotypic variation for phenotypic traits in okra genetic resources under dry environments may aid in selecting potential genotypes for improvement programmes for drought tolerance breeding. Further, there is a paucity of information on associations of phenotypic traits and SSR markers to guide variety development and efficient selection criteria for okra breeding under drought stress conditions.

Overall research goal

The overall research goal of this research was to identify and select genetically divergent and superior parental accessions for pre-breeding and develop high-yielding okra genotypes under drought-stressed conditions.

Specific objectives

The specific objectives of this study were:

1. To determine the response of selected okra (*Abelmoschus esculentus*) genotypes to drought stress using fresh pod yield and yield-related traits to identify and select candidate genotypes for drought tolerance breeding.

2. To determine genetic diversity present among okra (*Abelmoschus esculentus*) accessions using simple sequence repeats (SSR) and complementary phenotypic markers and to select genetically divergent and superior parental accessions to guide the selection of parental lines for pre-breeding.

3. To assess the levels of drought tolerance in preliminarily selected okra (*Abelmoschus esculentus*) accessions based on leaf gas exchange and chlorophyll fluorescence to identify best-performing genotypes for drought-tolerance breeding.

4. To determine the combining ability and heterosis of selected okra (*Abelmoschus esculentus*) accessions for yield and yield-related traits to identify superior parents and progenies for use in future genetic improvement of crop. .

Hypotheses

The hypotheses of this study were:

i. Considerable genetic variation exists for phenotypic traits under drought and non-stressed conditions among the assessed okra accessions.

ii. Physiological and phenotypic traits and SSR markers are complementary in selecting drought-tolerant okra genotypes.

iii. Okra accessions show good combining ability and heterosis to effectively select superior parents and progenies for future breeding.

Outline of thesis

This thesis consists of five different chapters in accordance with the number of objectives (see Table 0.1). Chapter 1 is written as a separate review paper, while chapters 2 to 5 are written as discrete research papers, each following the format of a stand-alone research paper, followed by a general overview and implications of findings from the study. The literature review and four experimental chapters of the study make the thesis chapters that were condensed into discrete but inter-dependant papers according to the University of KwaZulu-Natal's dominant thesis format. There are some overlaps and unavoidable repetitions of references and some introductory information between chapters. Chapter 1 was published in South African Journal of Botany (2021, 145: 56-64. https://doi.org/10.1016/j.sajb.2020.12.029), chapter 2 in South African Journal of Botany (2022, 145: 293-302. https://doi.org/10.1016/j.sajb.2021.11.008), chapter 3 in Diversity (2022, 14, 747. https://doi.org/10.3390/d14090747), chapter 4 in Life (2023, 13, 682. **https://doi.org/ 10.3390/life13030682**) and chapter 5 is under review in Journal of Crop Improvement.

Table 0.1: Thesis structure

Chapter	Title
-	Introduction to thesis
1	Phenotypic and genotypic divergence in okra [*Abelmoschus esculentus* (L.) Moench] and implications for drought tolerance breeding: A review.
2	Phenotypic response of okra (*Abelmoschus esculentus* [L.] Moench) genotypes under drought-stressed and non-stressed conditions
3	Characterization of okra (*Abelmoschus esculentus* L.) accessions with variable drought tolerance through simple sequence repeat markers (SSR) and phenotypic traits
4	Drought tolerance assessment of okra (*Abelmoschus esculentus* [L.] Moench) genotypes based on leaf gas exchange and chlorophyll fluorescence.
5	Combining ability and heterosis of selected okra (*Abelmoschus esculentus* L. Moench) accessions for yield and yield-related traits under drought-stressed and non-stressed conditions.
-	An overview of research findings and implications from the study

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Chapter 1. Phenotypic and genotypic divergence in Okra [*Abelmoschus esculentus* (L.) Moench] and implications for drought tolerance breeding: A review

Abstract

Okra (*Abelmoschus esculentus* L. Moench) is high-valued vegetable and oil crop serving the food, pharmaceutical, paper and oil industry. The production of okra in sub-Saharan Africa (SSA) and globally are hindered by a lack of improved cultivars with drought tolerance. Considerable phenotypic and genotypic variation present in okra genetic resources from SSA is useful for cultivar design with enhanced pod and oil yields, and drought tolerance. However, the genetic diversity present in SSA's okra germplasm collection is yet to be explored for breeding targeting economic and horticultural traits. The objective of this review was to document the research progresses on phenotypic and genotypic divergence analysis and drought tolerance breeding of okra to facilitate breeding and conservation. The review presented the diversity of okra for key agronomic, horticultural, and physiological traits for ideotype breeding, gene introgression and drought tolerance improvement. This is followed by key summaries on genetic diversity using conventional and genomic tools, and use of genetic variation in selection programs involving drought-adaptive and economic traits. The review serves as a baseline information to guide future okra breeding and cultivar design under water limited environments in SSA or related agro-ecologies.

Keywords: Drought tolerance, Genetic diversity, Genomic assisted selection, Okra germplasm

1.1 Introduction

Okra (*Abelmoschus esculentus* [L.] Moench; 2n = 2x = 130) which belongs to the Malvaceae family is an important vegetable and oil crop (Gemede et al. 2014). Several Abelmoschus species are globally recognized including A. *callei*, A. *moschatus*, A. *crinitis*, A. *ficulneus*, A. *tuberculatus*, A. *enbeepeegearense*, A. *angulosus*, A. *palianus*, A. *rogosus*, A. *rhodopetalus* and A. *manihot* (Jarret et al. 2011; Sutar et al. 2013; Bairwa et al. 2016). A. esculentus is the most common and widely cultivated species for diverse purposes including for food, medicine, and in the manufacturing of paper and oil products (Naveed et al. 2009). Tender and immature okra pods are used to prepare various dishes and consumed as a pod vegetable, whereas mature and dry seeds are processed as a source of edible oils. The seed oil content of okra varies from 20_40%. The major fatty acids of seed oil are linoleic, palmitic, oleic, diacylglycerols and triacylglycerols acids (Jarret et al., 2011). Okra oil is valued for its unique quality for human health and nutrition. The seed is rich in the contents of protein and amino acids notably lysine and tryptophan (Sanjeet et al. 2010), fat, fibre, vitamins (i.e., A, C and K), mineral elements such as calcium, potassium, sodium, and magnesium (Petropoulos et al. 2018), and sugars (Benchasri, 2012). These attributes render okra a nutritious fruit vegetable for reducing malnutrition among low-income populations in Asia and SSA . Despite the various economic values of okra, the crop is underutilized in SSA. The mean pod yield of okra in SSA is 2.5 t/ha compared with the potential yield of the crop reaching up to 8.8 t/ha (FAO, 2018). The factors responsible for the low production and productivity of okra in SSA include abiotic stress factors notably drought and heat stress, pests and diseases and the use of genetically inferior and unimproved varieties which have poor adaptation under the arid and semi-arid environments (Alake, 2020). Therefore, breeding programmes need to develop high yielding and drought adapted cultivars to boost okra productivity.

Okra is believed to be originated in Ethiopia; however West Africa, India and South-East Asia are thought to be centres of genetic richness of the crop (Gemede et al. 2014). The crop is referred using local names such as “lady’s finger” in England, “gumbo” in the United States of America, “bhindiin” in India (Benchasri, 2012) and “bamia” in Ethiopia (Gemede et al. 2015). There is limited research and development emphasis towards okra’s genetic improvement under water limited conditions in the region. This resulted in limited development, release, and commercialisation of improved varieties in SSA. In the region, okra is grown from genetically inferior local genotypes indicating the limited breeding efforts and research support in its improvement and product development. The wide phenotypic and genetic diversity present in

the cultivated okra species in SSA will aid in accelerated breeding of the crop with biotic and abiotic stress tolerance and enhanced yields. Therefore, the objectives of this review were to document the research progresses on the genetic variation of okra for key agronomic, horticultural and physiological traits aiming gene introgression for yield and drought tolerance improvement. The review provided key summaries on genetic diversity analysis using the conventional and genomic tools, and the use of genetic variation in selection programs involving drought-adaptive and economic traits. The review serves as a baseline information to guide future okra breeding and cultivar design under water limited environments.

1.2 Status of okra production in sub-Saharan Africa

Okra production accounts to an estimated area of 2 million ha globally with a total annual output of 9 million tons (Davis, 2019). Asia accounts for 66.3% of the world's total okra production with India being the leading producer of okra, with 6 million tons of total production, produced from 514 000 ha of cultivated lands (Davis, 2019). Africa accounts for 32.8% of the world okra production with West and Central African countries contributing over 75% of total production in SSA. However, the average yields of okra are relatively low in West Africa (2.5 t/ha) compared with East Africa (6.2 t/ ha) and North Africa (8.8 t/ha) (Davis, 2019). In SSA, Nigeria is the largest okra producer (with 2 million tons), followed by Sudan (305 000 tons) and Mali (227 000) per annum (Davis, 2019). The overall yields of okra are low in SSA compared to global averages despite its socio-economic importance in the region (Asare et al. 2016). Factors that attributed to low yield levels in SSA include erratic and poorly distributed rainfall, heat and drought stress, insect pests and diseases, and the use of genetically inferior and unimproved varieties with poor adaptation. The predominant biotic and abiotic stress factors that affect the sustainable and profitable okra production and productivity in SSA are discussed below.

1.3 Biotic constraints to okra production

Biotic stress (e.g., diseases and insect pests) are the major constraints to sustainable okra production. Major viral diseases of okra include yellow vein mosaic virus (YVMV), leaf curl disease (LCD) and root-knot nematodes (RKN). YVMV is the most economically important disease of okra and infection levels can reach up to 100% in susceptible varieties resulting in yield losses ranging from 50-94% (Das et al. 2013). YVMV is transmitted by white-fly (Bemisia tabaci Gen.), and two recessive alleles are reported to confer resistance to YVMV (Ali et al. 2000; Seth et al., 2017). There is marked genotypic variation for resistance to YVMV

useful for host-plant resistance breeding. For example, okra genotypes such as VNR green, Shagun, Barsha Laxmi, Parbhani Kranti and IPSA Okra 1 with high yield potential and relatively low infection rate for YVMV disease were identified for breeding (Ali et al. 2000; Das et al. 2013). Also, okra genotypes such as Adom, Togo, Asutem, Labadi dwarf, Kirikou-F1 and Kwabenya were reportedly tolerant to YVMV (Appiah et al. 2020). Some genotypes of wild Abelmoschus species including A. *caillei* (e.g., EC306731- P and EC305725), A. *manihot* (e.g., IC117175, IC344598, IC433667 and IC331214) and A. *moschatus* (e.g., IC140986, IC141067 and IC470454) with high to moderate resistance to YVMV have been reported (Gangopadhyay et al. 2017). Resistance to YVMV is reportedly associated with high concentrations of phenolic compounds (Prabu and Warade, 2009), suggesting phenolic compounds may serve as in indirect selection criteria for YVMV resistance in okra. Leaf curl disease (LCD) is the second most important viral disease limiting okra production after YVMV and can cause yield losses ranging from 30-100% (Singh et al. 2014). LCD is caused by begomovirus and transmitted by white flies (Singh et al. 2014). Cultivated okra genotypes such as 10/OKYVRES-11 and IC112449 with moderate resistance and IC105675, IC411692, IC417885, IC433445, IC433533, IC433532 and BCO-1 with moderate susceptibility to LCD were reported as useful source of genetic variation for breeding (Ayam et al. 2018). There is no stable sources of resistance to LCD in the cultivated okra species, however some wild species including A. *crinitus* and A. *manihot* have been reported to be resistant to LCD (Yadav et al. 2018). The transfer of resistance from wild relatives to the cultivated okra species has been partially successful due to sterile progenies. This limited development of breeding populations and successful backcrosses. Thus far, there is no stable source of resistance tolerance to LCD (Kumar et al. 2010, Das et al. 2013, Yadav et al. 2018) and okra genotypes possessing high levels of resistance to LCD are yet to be developed. Root-knot nematodes (RKN) caused by *Meloidogyne incognita* is one of the most widespread and damaging pests on okra. RKN causes yield loss of up to 27% (Sikora and Fernandez, 2005). Several okra cultivars resistant to RKN have been identified including Sanam, Dikshah, Arka Anamika, Ikra-1 and Ikra-2, Bamya Akkoy, Bamya Sultani, Perbhani Kranti and Clemson Spineless (Manzoor et al. 2016; Mukhtar et al. 2017). The identified okra genotypes may be sources of resistant genes to breed "new" RKN-resistant genotypes.

1.4 Abiotic constraints to okra production

Drought is the primary cause of yield losses and poor-quality produce in okra (Altaf Romaisa et al. 2015; Zandalinas et al. 2018). In okra, drought stress during flowering and pod-filling stages resulted in pod yield losses of over 70% (Mbagwu and Adesipe, 1987). Some drought tolerant varieties of okra were identified serving as useful germplasm for enhancing drought tolerance (Table 1.1). There is limited improved cultivars of okra with abiotic stress tolerance for cultivation in SSA and globally. Therefore, it is essential to explore the genetic variation present in okra germplasm for traits associated with abiotic stress tolerance for use in improvement programmes. The cultivated and wild okra genetic resources are phenotypically diverse, and they can be useful for developing okra hybrids with desired agronomic, horticultural, physiological, and nutritional traits for drought tolerance breeding (Aladele et al. 200 Muleken et al. 2016, Munir et al. 2016; Gerrano, 2018). Okra is relatively drought tolerant crop. However, the level of drought tolerance could be further enhanced through pre-breeding and pipeline programs targeting drought-adaptive traits.

Table 1.1: Drought tolerant okra genotypes reported globally

Genotype name/designation	Origin	References
Sabz Pari	Pakistan	Ashraf et al. (2002), Munir et al. (2016)
Kano	Iran	Barzegar et al. (2016)
NHAe 47-4	Nigeria	Adejumo et al. (2019)
Chinese-red	China	Ashraf et al. (2002)
Sanam	-	Naveed et al. (2009)
P-1999-31	-	Naveed et al. (2009)
Ikrza 1	-	Naveed et al. (2009)
Pusa sawani	Egypt	Abd El-Fattah et al. (2020)
Iraqi	Egypt	Abd El- Fattah et al. (2020)
Hala	Egypt	Abd El-Fattah et al. (2020)
Xianzhi	China	Shi et al. (2020)
Okr-6	-	Kusvuran, (2012)
Okr-67	-	Kusvuran, (2012)
Okr-105	-	Kusvuran, (2012)

1.5 Okra genetic resources

The world's largest genetic resources of vegetables, including okra is maintained by the World Vegetable Centre (also referred to as WorldVeg) in Taiwan (Ebert and Wu, 2019). WorldVeg and its partners have been responsible for introducing and promoting new cultivars of okra (Kumar et al. 2010). The National Gene Bank at ICAR-National Bureau of Plant Genetic Resources, New Delhi, India is the second largest okra gene bank in the world, maintaining over 3000 accessions comprising of cultivated and wild species (Bairwa et al. 2016). The National Bureau of Plant Genetic Resources maintains 2256 accessions of A. *esculentus*, 121 accessions of A. *ficulneus*, 126 accessions of A. *manihot* spp. *manihot*, 168 accessions of A. *manihot var. tetraphyllus*, 15 accessions of A. *pungens*, 32 accessions of A. *tetraphyllus*, 3 accessions of A. *tetraphyllus var. pungens* and 133 accessions of A. *tuberculatus* (http://genebank.nbpgr.ernet.in/CropSpecieswithICEC Wise.aspx?CropCode=2011). The United States Department of Agriculture- Agricultural Research Services (USDA/ARS) Plant Germplasm Collection in Griffin also maintains the largest okra germplasm comprising of approximately 1100 A. *esculentus* accessions, 310 A. *manihot* accessions, 156 A. *caillei* and 25 A. *moschatus* accessions (Jarret et al. 2011).

Several okra genetic resources are held by various national institutions in several countries including Ethiopia, Ghana, Pakistan and Nigeria (Table 1.2). Various species of *Abelmoschus* are well recognized including the common/cultivated okra (A. esculentus), West African okra (A. *caillei)*, A. *ficulneus*, A. *manihot*, A. *manihot variety tetraphyllus*, A. *moschatus* and A. *tuberculatus* (Kumar et al. 2010). Recently, a new wild okra species named *Abelmoschus angulosus var. mahendragiriensis* has been identified in India (Misra et al. 2018). These genetic resources provide opportunities for okra breeding targeting various economic traits. Among some genotypes of the common okra species, narrow genetic base were reported requiring further efforts to explore the full genetic pool of the okra germplasm resources preserved in gene banks (Reddy et al. 2012).

Table 1.2: Institutions maintaining okra germplasm collections

Institution	Country	References
Institute of Horticultural Sciences	Pakistan	Munir et al. (2016)
Bangladedsh Agricultural Research Institute (BARI)	Bangladesh	Synfullarh et al. (2018)
Indian Institute of Vegetable Research (IIVR)	India	Kumari et al. (2017)
Indian Institute of Horticulture Research (IIHR)	India	Kumari et al. (2017)
Plant Genetic Resource Research Institute (PGRRI)	Ghana	Oppong-sekyere et al. (2011)
Ethiopian Biodiversity Institute (EBI)	Ethiopia	Muleken et al. (2016)
Melkassa Agricultural Research Center	Ethiopia	Binalfewand Alemu (2016)
Agricultural Research Institute	Pakistan	Ashraf et al. (2002)
National Institute of Horticultural Research (NIHORT)	Nigeria	Eshiet and Brisibe (2015)
National Centre for Genetic Resources and Biotechnology	Nigeria	Eshiet amd Brisibe (2015)
World Vegetable Center	Taiwan	Aladele et al. (2008)
National Center for Agricultural Research and Extension (NCARE)	Jordan	Akash et al. (2013) Salameh (2014)
National Bureau of Plant Genetics Resources (NBPGR)	India	Bairwa et al. (2015)

1.5.1 Genetic diversity for drought tolerance breeding

Wide phenotypic diversity exists for agronomic and horticultural traits including days to flowering, plant height, number of branches per plant, number of fresh pods per plant, weight of fresh pods per plant, stem diameter, total fresh pod yield and seed yield (Gerrano, 2018; Petropoulos et al. 2018; Adejumo et al. 2019; Abd El-fattah et al. 2020; Alake, 2020). Phenotypic trait analysis revealed marked genetic variation for traits such as plant height, leaf area, number of leaves per plant, number of branches per plant, number of pods per plant and weight of fresh pods under drought stress conditions (Ariyo 1993; Aladele et al. 2008; Osawaru and Ogwu, 2013; Abd El-fattah et al. 2020). A study by Gerrano (2018) reported the presence

of high phenotypic diversity among South African okra genotypes for phenotypic traits including leaf width, number of branches, number of pods per plant, plant height and leaf length. This allowed for the identification and selection of two okra genotypes, namely VI055421 and VI056450 as useful parental lines for drought tolerance breeding. Silver et al. (2020) reported wide genetic variation in Brazilian okra genotypes based on leaf traits (e.g., leaf length, leaf width and petiole length), stem diameter and flowering rate. Agronomic traits such as number of locules, mature pod length, internode length, pod yield per plant, number of pods per plant, pedicel length and number of branches were identified as the most discriminating traits among Turkish okra landrace accessions (Duzyaman, 2005).

Collection, conservation, and characterization of crop genetic resources is a major component in genetic improvement programs, especially for previously neglected and under-utilized vegetable crops including okra. Wide morphological variation exists among okra genotypes for qualitative traits such as epicalyx segment, nature of pod tip, petiole colour and flowering patterns and quantitative traits including pod size, pod length, pod width, and pod size (Osawaru and Ogwu, 2013; Asare et al. 2016; Rubiang- Yalambing et al. 2016; Gangopadhyay et al. 2017). Qualitative and quantitative traits are useful for germplasm classification, phenotyping, and selection, and for breeding of new okra cultivars.

Okra exhibits a diverse range of stem and leaf pubescence (Binalfew and Alemu, 2016). Stem colour is an important distinguishing phenotypic trait amongst okra species. Binalfew and Alemu (2016), reported that 50% of their okra germplasms had green stem with red patches, whereas 31.5% had green stem and, 17.8% possessed red stem colour. Further, some okra genotypes possess flowers that vary in sizes and shapes (da Silva Costa et al. 2018) which is useful parameter for classification of okra genotypes. Depending on the species, pod size can vary from medium to short with expanded angles or with almost circular sections (da Silva Costa et al. 2018).

In South Africa, A. *esculentus* is successfully cultivated as an exotic species. The most commonly grown cultivar is Clemson spineless, a commercial cultivar. This cultivar is spineless, with medium dark green stem and angular pods that are 12-15 cm long. The plant grows from 1.2 to 1.5 m in height and takes about 55-58 days to pod maturity. Therefore, there is a need for a dedicated okra improvement in the country to develop and release improved and market preferred varieties.

1.6 Genetic diversity analysis

Genetic variation of crop germplasm is useful for the identification of genetically distinctive genotypes possessing desirable agronomic and drought-adaptive traits for drought tolerance breeding. Genetic differentiation in okra genetic resources has been widely studied using various molecular markers such as the random amplified polymorphic DNA (RAPD) (Aladele et al. 2008), inter-simple sequence repeats (ISSR) (Yuan et al. 2014), amplified fragment length polymorphism (AFLP) (Akash et al. 2013; Kyriakopoulou et al. 2014; Massucato et al. 2020), sequence-related amplified polymorphism (SRAP) (Gulsen et al. 2007), and simple sequence repeat (SSR) markers. SSR markers are highly polymorphic and co-dominant (Sawadogo et al. 2009; Shi et al. 2020). The co-dominant nature of SSR primers allows the detection of heterozygosity at specific loci (Prasad et al. 2000). For example, genetic diversity analysis in cultivated okra using SSR markers revealed high (>75%) polymorphism (Ramneek et al. 2012; Kumar et al. 2016) suggesting the efficiency of this marker system for germplasm differentiation and breeding. Okra germplasm from India was reported to be highly diverse compared to germplasms from other countries such as the United States of America and Africa when profiled with SSR markers (Kumar et al. 2017).

Different marker systems used for genetic diversity analysis in okra genetic resources are presented in Table 1.3. Furthermore, a list of highly polymorphic SSR markers useful for future genetic diversity studies to aid cultivar identification and selection in okra breeding programs is shown in Table 1.4. The identified markers will aid efficient germplasm characterization and selection of genetically unique genotypes for improving desirable traits such as fruit and oil yield. The development of drought tolerant cultivars with marker-assisted breeding or through genomic-assisted breeding require an understanding of the genetic constitution and gene functions associated with drought tolerance (Shi et al. 2020). The omics technologies defined as a suite of approaches like genomics, transcriptomics, proteomics, metabolimics and ionomics are potentially important tools for abiotic stress tolerance breeding (Zhan et al. 2019). There is limited information available describing the use of omics technologies in orphan and under-utilized crops including okra.

Table 1.3: Reported marker systems and genetic diversity analysis in okra

Marker type	No of markers	Species	No of genotypes evaluated	References
SSR	18	*A. esculentus,* *A. turberculantus,* *A. manihot,* *A. moschatus,*	24	Fougat et al. (2015)
SSR	19	*A. esculentus,*	69	Kumar et al. (2017)
RAPD	14	*A. esculentus,*	44	Prakash et al. (2010)
ISSR	7	*A. esculentus,*	10	El-Sherbeny et al. (2018)
SRAP	19	*A. esculentus,*	60	Yildiz et al. (2016)
AFLP	-	*A. esculentus,*	50	Kyriakopoulou et al. (2014)
RAPD	13	*A. callei,* *A. esculentus,*	93	Aladele et al. (2008)
RAPD	22	*A. esculentus,* *A. ficulneus,* *A. manihot,* *A. moschatus,* *A. tuberculatus,*	260	Bisht et al. (1995)
SRAP	39	*A. esculentus,*	23	Gulsen et al. (2007)
ISSR	22	*A. esculetus,*	24	Yuan et al. (2014)
Isozyme	34	*A. esculentus,*	22	Torkpo et al. (2006)
SSR	9	*A. esculentus,*	66	Yildiz et al. (2015)
Ipbs retrotransposon	13	*A. esculentus,*	66	Yildiz et al. (2015)
SSR	16	*A. esculentus,*	20	Sawadogo et al. (2009)
RAPD	40	*A. esculentus,*	70	Sawadago et al. (2009)
RAPD	40	*A. esculentus,*	70	Kaur et al. (2013)
RAPD	20	*A. esculentus,*	39	Haq et al. (2013)
AFLP	8	*A. esculetus,*	21	Akash et al. (2013)
RAPD	31	*A. esculentus,*	39	Martinello et al. (2001)

SSR: simple sequence repeats, RAPD: random amplified polymorphic DNA, ISSR: inter-simple sequence repeats, AFLP: amplified fragment length polymorphism, SRAP: sequence-related amplified polymorphism

Table 1.4: List of highly polymorphic SSR markers reported for genetic analysis in okra

Marker	Forward (F) and reverse (R) primers	PIC	References
Okra 111	F: GATGGAATTGAGAAACCAGA R: TGTGTTCTTCACTCTCGTCA	0.89	Fougat et al. (2015)
Okra 152	F: GCTCTATTGATGGCGAGTAA R: AAAGTCATCCAAGGTGACAA	0.81	Fougat et al. (2015)
Okra 166	F: TTCCAGTTGGAGAGGTAAGA R: CTTCCATTTCATCGACTTTC	0.82	Fougat et al. (2015)
Okra 167	F: CGGCACTCACATTTTACATA R: GCTGTGAAGCTCTCAAAAGT	0.76	Fougat et al. (2015)
Okra 165	F: GAGCTAAACCTTGCTTTTGA R: CTCTTATGACTTCGGTCCAG	0.70	Fougat et al. (2015)
SSR 64	F: RAAGGAGGAGAAAGAGAAGGA R: ATTTACTTGAGCAGCAGCAG	0.81	Yildiz et al. (2015)
SSR9	F: ACCTTGAACACCAGGTACAG R: TTGCTCTTATGAAGCAGTGA	0.70	Yildiz et al. (2015)
AVRDC-OKRA70	F: GTAGCTGAACCCTTTGCTTA R: CTATCATGGCGGATTCTTTA	0.98	Kumar et al. (2017)
AVRDC OKRA39	F: TGAGGTGATGATGTGAGAGA R: TTGTAGATGAGGTTTGAACG	0.99	Kumar et al. (2017)
AVRDC-OKRA64	F: AAGGAGGAGAAAGAGAAGGA R: ATTTACTTGAGCAGCAGCAG	0.87	Kumar et al. (2017)
AVRDC-Okra9	F: ACCTTGAACACCAGGTACAG R: TTGCTCTTATGAAGCAGTGA	0.85	Ouedraogo et al. (2018)
AVRDC-Okra56	F: GGCAACTTCGTAATTTCCTA R: TGAGTAAAAGTGGGGTCTGT	0.70	Ouedraogo et al. (2018)
AVRDC-Okra66	F: CACCAGAATTTCCCTTTTG R: ACTGTTGTTTGGCTTATGCT	0.78	Ouedraogo et al. (2018)
Okra137	F: GAGAGAGATTGCTTCGACTG R:TAAACTTTAAACTCAGCGGC	0.80	Kumar et al. (2016)
Okra119	F: GCAGCGGTAGAAATAAATGT R: GGAGGGTTTAGGTATGGTTT	0.74	Kumar et al. (2016)
Okra148	F: TGCTTATTCATGCTGACCTA R: AGCACTTGATATCCAAGGAA	0.75	Kumar et al. (2016)
Okra12	F: AATGAAGTTGGAGTCGACAG R: CAATACTCGTTGTTGTGGTG	0.72	Kumar et al. (2016)
Okra125	F: RCCCCTTCCTCTAGATCTCAT R: GACGGTGGAGATTGAACTT	0.72	Kumar et al. (2016)

PIC: polymorphism information content

1.7 Drought tolerance breeding in okra

Okra is a relatively drought tolerant crop. However severe drought stress can reduce pod yields by 45 to 70%, especially if it occurs during flowering or pod formation (Naveed et al. 2010, Naveed et al. 2012). Progress in the development of drought tolerant okra genotypes has been slow due to a lack of adequate source of genetic variation for drought tolerance breeding (Abd El- Fattah et al. 2020). A combination of different drought adaptive and economic traits should be recombined in a desirable genetic background rather than a single trait as selection criteria for drought stress tolerance breeding (Kumar et al. 2012). Various morphological traits such as plant height, number of pods per plant, average pod size and seed yield have been proven useful to evaluate the effect of drought stress (Asare et al. 2016, Munir et al. 2016; Alake, 2020). Positive correlation was reported between plant height and leaf area, and number of pods per plant and average pod size (Munir et al. 2016). Similarly, pod number per plant was also reported to be strongly and positively correlated with pod yield per plant (Duzyaman, 2005; Gerrano, 2018). Hence, these traits could be exploited for breeding drought tolerant okra genotypes. Drought tolerance can be assessed using traits such as pod length, fresh pod weight, pod dry weight, number of pods per plant and number of seeds per pod (Pravisya and Jayaram, 2015).

Some physiological traits that are reportedly used to assess drought tolerance includes photosynthesis rate, stomatal conductance, chlorophyll content, transpiration rate and chlorophyll fluorescence parameters (Duzyaman, 2005; Premadasa et al. 2019). Drought stress inflicted a significant reduction in net photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency (Ashraf et al. 2002) and chlorophyll content in okra (Premadasa et al. 2019). Photosynthetic rate, internal CO2 concentration, instantaneous water use efficiency, intrinsic water use efficiency, carboxylation efficiency and maximum quantum efficiency of photosystem II were reduced by drought stress in okra (Chaturvedi et al. 2019; dos Santos Farias et al. 2019). Reduction in photosynthetic capacity has been linked to stomatal closure (Adejumo et al. 2019) which is a basic mechanism for reducing the impact of drought as early stomata closure reduces water loss (Bahadur et al. 2011). However, stomatal closure also reduces carbon dioxide intake which results in the reduction of photosynthesis. According to Blum (2011), plant physiological processes are diverted towards production of osmoprotectants and other anti-oxidant proteins for survival under water stress. This leads to reduced level of photosynthesis and production of metabolites (Shu et al. 2013). Physiological processes are affected by water stress, therefore the evaluation and monitoring of these

processes can be used as a tool to quantify and qualify the level of stress and damages associated with it.

1.8 Quantitative trait loci for phenotypic and physiological traits

Breeding drought tolerant okra cultivars will enhance yields under rainfed condition. In the recent past, many plant breeders have explored conventional breeding approaches, which to some extent have provided limited genetic gains for key agronomic traits including pod and oil yield (Magwanga et al. 2020). However, adoption of molecular and genetic engineering techniques will accelerate the ultimate goal of cultivar development that are more adaptable and tolerant to abiotic stress (Ashraf, 2010). Molecular markers techniques such as genomic selection and marker-assisted selection methods facilite mapping of quantitative traits loci (QTL) affecting yield and yield components (Fan et al. 2015). Quantitative trait loci are specific chromosomal loci containing multiple functional genes affecting yield and yield components. A large number of QTL related to drought-adaptive traits have been identified. Also, QTL linked with root traits such as root penetration ability have been identified (Uga et al. 2011).

QTLs linked to drought-adaptive agronomic and physiological traits is useful for marker-assisted breeding in okra (Zheng et al. 2016). QTL assisted breeding can increase the speed and efficiency of developing highly-adapted and best performing okra genotypes for drought-stressed environments. Mapping QTL for both morphological and physiological traits related to drought tolerance help to elucidate the molecular mechanisms controlling drought tolerance and further facilitate the development of new varieties with improved drought tolerance (Zheng et al. 2016). However, research on QTL analysis and gene mapping for drought-adaptive traits in okra has been lacking.

1.9 Application of genetic tools to improve drought tolerance in okra

Breeding for drought tolerance could be enhanced by integrating omics approaches with conventional breeding methods to identify drought responsive genes and signalling pathways. Omics methodologies include genomics, transcriptomics, proteomics, and metabolomics which play a vital role in crop improvement by facilitating the identification of genes, proteins and metabolites associated with drought tolerance (Singh et al. 2014). However, there is limited genome sequence information available for okra. This may be attributable to the complex and allopolyploid genome of the crop (Zhan et al. 2019).

Drought tolerance is associated with transcriptional factors which are targeted in crop improvement programmes for various applications including drought tolerance breeding. Transcriptome analysis using the next generation sequencing (NGS)-based RNA sequencing (RNA-seq) technology identified multiple genes influenced by genotype (G) × environment (E) interaction (Bohra et al. 2015). NGS has been applied for examining gene expression patterns in rice (Minh-Thu et al. 2013), maize (Kakumanu et al. 2012) and sorghum (Dugas et al. 2011). NGS has also been used to identify novel genes associated with drought adaptation. In common bean, a total of 9298 drought responsive genes were identified under drought stress condition by employing RNA-seq and real time qPCR (Wu et al. 2014). Protein expression analysis enables the examination of changes in key proteins that regulate physiological processes under drought stress (Singh et al. 2014). However, the full potential of proteomics with respect to drought tolerance remains to be explored for okra. In a study involving wheat cultivars under drought tolerance, differentially expressed proteins were identified in two drought tolerant cultivars (KDML 105 and NSG19) which were related to phytochrome, a signalling DNA repair and superoxide dismutase in the cultivar KDML105. Further, proteomic analysis of drought responsive proteins in wheat aided identification of 122 proteins associated with several pathways involved in drought tolerance and adaptation (Jiang et al. 2012). Lack of genome sequence and limited genomic resources in okra limit their application for drought tolerance breeding.

1.10 Discussion

Okra is an important vegetable and oil crop with valuable nutritive and pharmacological values. Abiotic stress mainly drought and heat stress are major yield limiting factors in okra production in arid and semi-arid environments (Abd El- Fattah et al. 2020). Further, the use of okra genotypes with poor adaptation and low yield potential contribute to extensive yield losses. Pre-breeding and breeding of okra are important to identify ideal genotypes for future genetic improvement and can result in the development and release of improved okra genotypes with high yield potential and abiotic stress tolerance. The extensive genetic variation that is present in the cultivated and wild okra species offers opportunities for improvement of drought adaptive traits. However, problems due to hybridization barriers such as hybrid sterility and genotype incompatibility between cultivated and wild okra species is the major problem limiting utilization of wild okra species for breeding and cultivar development. For example, very low outcrossing rates have been reported between A. *esculentus* and A. *caillei* (Hamon and Hamon, 1991). Further, F1 hybrids developed by crossing A. *manihot* (L.) Medik and A.

Manihot (L.) Medik ssp. *Manihot* with A. *esculentus* were reported to be sterile (Jambhale and Nerka, 1981). Interspecific crosses between A. esculentus and A. *moschatus* resulted in successful development of F1 hybrids but with low pollen viability and seed set attributed to pre- and postzygotic hybridization barriers and genotypic differences (Abdullah Yousuf Akhond et al. 2000). The sterility of the F1 hybrids developed between the cultivated and wild okra species limited the introgression of useful genes from wild okra species for drought tolerance breeding in target production areas. To overcome hybridization barriers there is need to carry out a relatively large number of crosses between cultivated and wild okra. The use of wild okra species as female parents has been recommended (Abdullah Yousuf Akhond et al. 2000). In tepary bean (*Phaseolus acutifolius* A. Gray) recurrent back-crossing referred to as congruity backcrossing which involves multiple backcrossing between tepary bean and common bean parental genotypes have been used to reduce hybridization barriers and transfer useful genes from tepary bean (Munoz et al. 2004). In addition, interspecific lines developed between tepary bean and common bean using congruity backcrossing improved hybrid fertility and development of lines with better performance compared to parental lines under drought stress condition (Souter et al. 2017). This procedure can be employed in okra breeding programmes for recombination of desirable genotypes using different okra species targeting desired agronomic, biochemical, and physiological traits associated with drought adaptation. Also, intraspecific crosses between elite cultivated okra genotypes may result in desirable combinations of drought adaptive traits in the progeny. For example, okra F1 hybrids tolerant to YVMV were derived by crossing the cultivated okra genotype IPSA Okra 1 which is YVMV resistant against susceptible genotypes (e.g., Parbhani Kranti, SL-44 and SL-46) (Ali et al. 2000). This suggests the possibility to develop cultivated okra genotypes with increased levels of abiotic stress tolerance.

The extensive genetic diversity of okra requires further investigation to fully utilize wild okra genetic resources for breeding. Genetic differentiation and gene flow are useful parameters for understanding the genetic structure of closely related species. The low outcrossing rates and infertile F1 hybrids derived between the cultivated and wild okra suggests low gene flow between these species. However, limited information is available that unravelled the level of gene flow between the different okra species. Also, it is important to examine genetic parameters in the cultivated and wild okra genetic resources using molecular markers to develop efficient breeding strategies including for drought tolerance breeding.

To develop and release improved okra genotypes with desirable traits, speed breeding could be employed to facilitate rapid generation advancement and cultivar release with desired agronomic, physiological, biochemical, and quality traits (Dhankhar et al. 2020). Speed breeding involves the use of multiple techniques to manipulate environmental conditions (e.g., water, artificial lighting, and nutrition) to induce early flowering and seed set in order to advance the next breeding generation rapidly. Speed breeding has been applied successfully in crops such as pea (*Pisum sativum* L.), pigeonpea (*Cajanus cajanifolia* L.), soybean (*Glycine max* L.), rice (*Oryza sativa*) and amaranth (*Amaranthus* spp.) (Saxena et al. 2019; Cazzola et al. 2020; Jahne et al. 2020). In barley (*Hordeum vulgare* L.), introgression lines with multiple resistance to leaf rust, net and spot forms of net blotch and spot blotch were developed using backcrossing and speed breeding (Hickey et al. 2017). In okra, the application of both backcrossing and rapid generation advancement could be employed in breeding programmes to develop drought tolerant cultivars with high and oil yield potential.

Cultivated okra genetic resources exhibit a wide range of variation for phenotypic quantitative traits (Ariyo, 1993; Koutsos et al. 2000; Kyriakopoulou et al. 2014; Abd El-Fattah et al. 2020; Alake, 2020). Also, wild okra species including A. *caillei*, A. *manihot*, A. *moschatus* and A. *tuberculatus* show genetic variation for quantitative phenotypic traits (Gangopadhyay et al. 2017). Genotypic variation for morphological traits provides opportunities for cultivar selection, population development and genetic advancement. Agronomic traits such as number of days to flowering, number of branches per plant, pod weight and number of pods per plant are highly heritable ($H2 \geq 60\%$), whereas pod yield and plant height exhibit low heritability ($H2 < 30\%$) (Ariyo, 1990). Highly heritable traits are fixable through simple phenotypic selection. To improve genetic gains for desired traits, it is important to identify and select okra genotypes with highly heritable phenotypic traits to improve yield responses under limited water condition. In wheat (*Triticum aestivum* L.), an integrated selection criterion of heritable agronomic traits which are highly correlated with grain yield under drought stress condition has been proposed for drought tolerance breeding (Abdolshahi et al. 2015). In okra, number of pods per plant and plant height were reported as the main contributors to seed yield (Akinyele and Osekita, 2006). Ariyo et al. (1987) also reported that agronomic traits including pod weight, pod length and width, number of seeds per plant, hundred seed weight and number of branches per plant are positively correlated with pod yield per plant. Additionally, Abd El-Fattah et al. (2020) reported positive associations between number of pods per plant and pod weight per plant with total yield indicating integrated selection for multiple traits may be possible for

improving okra yield response under drought stress condition. Early flowering and maturity are negatively correlated with yield in okra. Akinyele and Osekita (2006) and Abd El-Fattah et al. (2020) indicated the possibility for developing high-yielding and early maturing okra genotypes for dry and drought-prone environments characterized by occurrences of mid- and late-season drought stress. Trait association under drought stress condition are required to develop an efficient selection criterion for drought tolerance breeding in okra. pod yield response in okra depends on cultivar differences and the phenological stage at which drought stress occurs. Severe pod yield losses due to drought stress occur during the flowering and pod-filling stages (Mbagwu and Adesipe, 1987). Limited studies are available that determined genotypic variation using a relatively large okra germplasm to drought stress during key phenological stages to identify drought-tolerant genotypes based on yield and yield-related traits. Also, physiological, and biochemical markers were not widely used to characterise genotypic responses of a large set of okra genotypes to drought stress tolerance. Furthermore, the relationship between pod yield with physiological and biochemical traits under drought stress condition requires further investigation for effective drought tolerance breeding in this crop.

1.11 Conclusion and future prospects

Okra is a multipurpose crop for food and various industrial products. However, the crop is under-researched despite being an important food and oil crop. Only limited research is conducted on cultivar development and deployment with enhanced abiotic stress tolerance. The present study highlighted the key challenges affecting okra production. It presented key abiotic and biotic stress factors, okra genetic diversity and genetic analysis using phenotypic traits and genomic tools to guide future studies on okra breeding. For drought tolerance breeding, agronomic, physiological, and biochemical traits that could enhance yield potential under water-restricted environments are proposed. Exploitation of okra genetic resources held in various gene banks worldwide which are phenotypically and genetically distinct for traits associated with pod and oil yields under abiotic stress conditions are proposed to improve drought tolerance breeding in this crop. Also, there is a need for hybridization program between the cultivated and wild okra species to incorporate desirable traits using a wide range of genotypes to enhance drought adaptation in okra. Multiple backcrossing and the use of genetically differentiated genotypes of the cultivated and wild okra species are needed to overcome hybridization barriers (i.e., low pod t set and hybrid sterility). This will allow for development of fertile F1 hybrids for further selection and genetic advancement. Speed

breeding techniques to accelerate rapid generation advancement are proposed to improve timeous release of improved okra genotypes with desired traits such as high pod and oil yield for food and industrial uses. Conventional breeding methods, QTL mapping of important agronomic, physiological, and biochemical traits and omics technologies should be integrated and used for drought tolerance improvement in okra. Finally, there is a need on international research collaboration and strategic partnerships among research institutes and breeders to develop market preferred and high performing okra cultivars with drought tolerance and high yields.

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Chapter 2. Phenotypic response of okra (*Abelmoschus esculentus* [L.] Moench) genotypes under drought-stressed and non-stressed conditions

Abstract

Genetic variation is fundamental for breeding drought-adapted cultivars with economic traits. The objective of this study was to determine the response of selected okra genotypes to drought stress using yield and yield-related traits to identify and select candidate genotypes for drought tolerance breeding. Twenty-six okra genotypes were evaluated in glasshouse (GH) and field (FLD) environments under drought-stressed (DS) and non-stressed (NS) conditions using a 13 × 2 α lattice design with two replications. Data were collected on the following phenotypic traits: number of branches per plant (NB), plant height (PH), days to maturity (DTM), stem diameter (SD), fresh pod length (FPL), dry pod weight per plant (DPW), dry pod length per plant (DPL), number of seeds per plant (NSP), number of pods per plant (NPP), pod yield per plant (YPP) and anthocyanin (ATY) pigmentation of the stem. Significant ($P < 0.05$) genotype x testing environment x water condition interaction effects were recorded for most traits allowing for selection of okra genotypes suited for drier conditions. YPP positively and significantly correlated with FPL ($r = 0.66$; $P \leq 0.001$), DPW ($r = 0.80$; $P \leq 0.001$) and NPP ($r = 0.58$; $P \leq 0.001$) under DS condition in the FLD environment. The study identified genotypes with high yield and other desirable phenotypic attributes which are useful genetic resources for future crosses and selection of promising progenies based on combining ability analysis and heritability under water-limited environments.

Keywords: abiotic stress, okra, phenotypic traits, water-deficit

2.1 Introduction

Okra (*Abelmoschus esculentus* [L.] Moench), belonging to the *Malvaceae* family, is a highly nutritious and underutilized crop widely grown in Asia, South America and Africa (Gemede et al., 2015). Tender and immature pods are consumed as a vegetable, whereas matured and dry seeds are a rich source of edible oils and fatty acids (Jarret et al., 2011; Reddy et al. 2013). The seed oil content of okra varies from 20-40%, and the primary fatty acids of seed oils are linoleic (49.54%), palmitic (28.60%), oleic (16.81%), stearic (3.57%) and linolenic (1.48%) acids (Jarret et al., 2011). The seeds are a rich source of protein (22.14%), amino acids (i.e., lysine and tryptophan), fibre, vitamins (i.e., A, C and K), and mineral elements (i.e., calcium, potassium, sodium, and magnesium) (Sanjeet et al. 2010; Petropoulos et al. 2018; Gerrano, 2018). Okra consumption has several human health benefits, including lowering blood sugar levels and reducing serum cholesterol (Gemede et al., 2015) and relieving constipation (Dubey and Mishra, 2017, Liu et al., 2017). The nutritional composition of okra makes it a vital source of nutrition to reduce malnutrition in Asian and sub-Saharan African countries. Furthermore, the crop is cultivated under various agricultural cropping systems due to its ease of cultivation and wide adaptation to diverse environmental conditions (Kumar and Reddy, 2016). The wide adaptation of the crop is attributed to its phenotypic plasticity that favor yield development under harsh growing conditions. Therefore, unique genotypes of the crop can be selected based on phenotypic traits for breeding and large-scale production.

Okra fresh pods production accounts for an estimated area of 2 million ha globally, with a total annual production of 9 million tons (FAO, 2018). The African continent accounts for 32.8% of the world okra production. West and Central African countries contribute to over 75% of total okra production in sub-Saharan Africa (SSA) (Kumar and Reddy, 2016). Despite the significant contribution by SSA towards global okra production, average yields are low in the region. For example, pod yields of 2.5, 6.2 and 8.8 tons/ha were reported in West, East, and North Africa, respectively (FAO, 2018). Low and variable yields in SSA are attributed to the cultivation of genetically inferior and unimproved cultivars (Alake, 2020) associated with poor management practices. Further, biotic (i.e., cucumber mosaic virus) and abiotic stress (i.e., drought and heat) are significant constraints to okra production and declining yields (Mkhabela et al. 2021). In SSA, the crop is often grown under marginal conditions characterized by low and erratic rainfall, lack of agricultural inputs and modern production technologies.

Abiotic stress factors, notably drought stress, account for okra yield losses ranging between 30-100% (Mbagwu and Adesipe, 1987). Drought stress occurring during the flowering and pod-filling stages cause severe yield losses (Mbagwu and Adesipe, 1987). As a result, yield losses in okra are dependent on cultivar differences and the phenological stage at which drought stress occurs. Previously, very few okra genotypes that are tolerant to drought stress were identified (Munir et al. 2016, Adejumo et al., 2018, Abd El-Fattah et al. 2020; Shi et al. 2020).

Moreover, there are fewer breeding efforts to develop drought-tolerant okra genotypes for cultivation in arid and semi-arid regions of SSA. This is hampering efforts to improve crop yield and quality for food and nutrition security in the region. Identifying new sources of drought tolerance in okra genotypes is critical to understand the adaptation of this crop to drought stress and broaden the genetic base of drought tolerance in okra through breeding. Therefore, it is essential to explore the okra genetic resources available in SSA to identify potential sources of valuable phenotypic traits associated with drought tolerance for use in improvement programmes.

Extensive phenotypic plasticity is present in okra germplasm, including plant height, a higher number of branches per plant, hundred seed weight, increased fresh pod length and pod yield (Asare et al. 2016; Gerrano, 2018; Alake et al. 2020; Ali et al. 2020). Number of pods per plant, total pod production, pod length and width, number of seeds per pod, hundred seed weight, number of branches per plant and plant height are critical yield contributing traits that can be selected for okra improvement in water-restricted environments (Ariyo et al. 1987; Akinyele and Osekita, 2006; Abd El- Fattah et al. 2020). Population development for improved okra genotypes requires adequate genetic background information for planning appropriate okra breeding and selection strategies. Therefore, assessing genotypic variation for phenotypic traits in okra genetic resources under dry environments may aid in selecting potential genotypes for use in improvement programmes for drought tolerance breeding. Further, there is scant information on associations of phenotypic traits in okra under drought stress conditions, limiting development and efficient selection criteria for drought tolerance breeding. Considering the above background, the objective of this study was to determine the response of selected okra genotypes to drought stress using yield and yield-related traits to identify and select candidate genotypes for drought tolerance breeding.

2.2 Materials and methods

2.2.1 Plant materials and study site

Twenty-six okra genotypes obtained from the Agricultural Research Council-Vegetable, Industrial and Medicinal Plants (ARCVIMP) gene bank and one widely grown local variety in South Africa were used for the study. Names and pedigree information of the evaluated okra genotypes are presented in Table 2.1. The experiment was conducted under field environment (FLD) at Ukulinga research farm of the University of KwaZulu-Natal (UKZN) (290 40′ S, 300 24′ E, 806 above sea level) and under glasshouse (GH) environment at the controlled environment facility (CEF) at UKZN during the 2018/19 and 2019/20 growing seasons. The genotypes were grown under non-stressed (NS) and drought-stressed (DS) conditions under both GH and FLD environments.

Table 2.1: Names and pedigree information of okra genotypes used in the study

Genotype code	Pedigree information	Source
LS01	VI033775	ARC-VIMP
LS02	VI033797	ARC-VIMP
LS03	VI056457	ARC-VIMP
LS04	VI039651	ARC-VIMP
LS05	VI046561	ARC-VIMP
LS06	VI047672	ARC-VIMP
LS07	VI050150	ARC-VIMP
LS08	VI050957	ARC-VIMP
LS09	VI050960	ARC-VIMP
LS10	VI055110	ARC-VIMP
LS11	VI055119	ARC-VIMP
LS12	VI055219	ARC-VIMP
LS13	VI055220	ARC-VIMP
LS14	VI055421	ARC-VIMP
LS15	VI056069	ARC-VIMP
LS16	VI056079	ARC-VIMP
LS17	VI056081	ARC-VIMP
LS18	VI056449	ARC-VIMP
LS19	VI060131	ARC-VIMP
LS20	VI060313	ARC-VIMP
LS21	VI060679	ARC-VIMP
LS22	VI060803	ARC-VIMP
LS23	VI060817	ARC-VIMP
LS24	VI060822	ARC-VIMP
LS25	VI060823	ARC-VIMP
LS26	Clemson Spineless	Local variety

ARC-VIMP: Agricultural Research Council - Vegetable, Industrial and Medicinal Plants, South Africa

2.2.2 Experimental design and crop establishment

For glasshouse (GH) trial two seeds of each genotype were grown in 5L capacity plastic pots filled with composted pine bark growing media. Two plants were established per pot for each genotype and thinned to one plant per pot after emergence. Day/night temperatures in the GH environment were 30°C/20°C and relative humidity ranged between 45 and 55% during the

study. The field experiment was conducted on plots covered with a custom-made plastic mulch to eliminate rainfall and soil water evaporation. The soil type is made of Westleigh form which is from orthic A and soft plinthic B with a bulk density of 1.23 $g.cm^{-3}$, and clay, sand, and silt contents of 52.1, 24.3 and 23.6%, respectively. The soil had the following chemical properties: organic carbon (1.50%), nitrogen (0.13%), phosphorus (30 mg/L), potassium (108 mg/L), calcium (844 mg/L), magnesium (314 mg/L), exchangeable acidity (1.14 cmol/L) and pH (KCl) of 3.94. Field plots were 1.5 m long with inter-row and intra-row spacing of 60 and 10cm, respectively. Two seeds were planted at a depth of 3cm per hole and thinned to one plant after emergence. Inorganic fertilizers consisting of Nitrogen (N), phosphorus (P) and potassium (K) were respectively applied at a rate of 120, 30 and 30 kg ha^{-1} based on soil fertility recommendations under both environments using urea (46-0-0), phosphorus pentoxide (P_2O_5) and potassium oxide (P_2O). The 26 okra genotypes were evaluated using a 13×2 alpha lattice design under both DS and NS conditions. DS was imposed at 50% flowering until physiological maturity to mimic terminal drought stress and ensure that the pod formation stage occurs while plants are exposed to sufficient water stress The NS condition involved maintaining soil moisture content at field capacity by supplying water through the irrigation system until physiological maturity under both GH and FLD environments. Tensiometers (Spectrum Technologies, Inc, USA, Illinois) were used to monitor soil moisture status during the experiment.

2.2.3 Data collection

Data was collected from three randomly selected and tagged plants for each genotype under FLD and GH environments. Data was collected on the following phenotypic traits: the number of branches (NB) per plant, stem diameter (SD) was measured at 50% flowering stage using a calliper aiming 30 cm above the soil surface and values were expressed in mm, plant height (PH) in cm measured from the ground level to the apex of the plant on the main stem during physiological maturity of the crop. The number of days to maturity (DTM) was calculated as the number of days from sowing to the day when the pods became fleshy, bright green and non-fibrous and approximately 1.5 cm long. Pods were harvested when 50% of the pods were 3-5 cm long, regarded as marketable size (Petropoulos et al., 2018). Harvests were done every third day by hand. The number of pods, pod length in cm, and pod weight per plant in grams were recorded at each harvest. At the end of the experiment, data were computed on the number of pods per plant (NPP), fresh pod length (FPL), and yield per plant (YPP). Under field conditions, three plants from the second raw of each plot were sampled, tagged, and left until maturity to

collect data on mature pod length (DPL), mature pod weight (DPW) and number of seeds per plant (NSP). The number of pods per plant were counted. Number of seeds per plant were counted after pod shelling. Yield per plant was determined by weighing fresh pods harvested per plant and expressed in grams. At pod formation stage, anthocyanin (ATY) was determined visually using a scale of 0 to 1, where 0 = no anthocyanin and 1 = anthocyanin present. ATY was recorded as a selectable marker for future breeding.

2.2.4 Data analysis

Data were subjected to analysis of variance (ANOVA) using a lattice procedure with GenStat 18th Edition (VSN International, Hempstead, UK). Treatment means were separated using the least significant difference (LSD) test at 5% level of significance. Pearson's correlation coefficients (r) were calculated using IBM SPSS Statistics 25.0 (SPSS, 2017) to determine the magnitude of the relationship among phenotypic traits. Principal component analysis (PCA) was computed using average data across test environments. The PCA was used to identify influential traits under NS and DS conditions. The correlations were calculated separately for NS and DS conditions across test environments. Biplots were constructed using R version 4.0 (R Development Core Team, 2008) to determine relationships between the genotypes and the assessed phenotypic traits.

2.3 Results

2.3.1 Genotype, water condition, testing environment and their interaction effects on phenotypic traits

Analysis of variance revealed significant (P<0.05) differences among genotypes, water conditions, environments, and their interactions for assessed phenotypic traits (Table 2.2). Genotypic differences were significant for most traits except for SD. Significant (P<0.05) effect of water condition was recorded on most assessed traits except for DTM. Non-significant interaction of genotype ×water condition was observed for NB, SD, DTM, FPL, DPL, NSP and NPP. Genotype × environment interaction effect was significant (P<0.05) for PH, DTM, DPW and YPP. In addition, a significant (P<0.05) genotype × water condition × environment interaction effect was observed for DPW and YPP.

Table 2.2: Combined analysis of variance showing mean squares and significant tests of 26 okra genotypes assessed for phenotypic responses in glasshouse and field environments under drought-stressed and non-stressed conditions

Source of variation	df	NB	SD	PH	DTM	FPL	DPW	DPL	NSP	NPP	YPP
Rep	1	23.31 ^{ns}	11.33 ^{ns}	89.60 ^{ns}	101.78^{ns}	7.69 ^{ns}	0.05 ^{ns}	0.00 ^{ns}	392.60 ^{ns}	44.03 ^{ns}	541.00 ^{ns}
Block	1	4.36 ^{ns}	7.32 ^{ns}	8146.70 **	677.89*	39.97 ^{ns}	35.01 ^{ns}	132.33 *	1324 **	240.59 ^{ns}	900.80 *
Env	1	750.60 **	1362.45 **	504.70 ^{ns}	130.10^{ns}	833.01 **	352.22 **	3248.63 **	30428.4 **	13856.66 **	9142.40 **
WC	1	685.58 **	1242.69 **	107214.2 **	0.15^{ns}	1338.93 **	872.51 **	1361.37 **	6737.10 **	1918.49 **	16953.7 **
Gen	25	41.41 *	18.08 ^{ns}	3194.80**	292.00**	34.04 *	39.35 **	65.00 *	361.60 *	156.73 *	584.30 **
Gen.WC	1	34.02 ^{ns}	2.17 ^{ns}	105.30 ^{ns}	5.28^{ns}	56.84 ^{ns}	104.27 *	177.03 *	906.50 *	23.76 ^{ns}	746.20 *
Gen.Env	25	23.14 ^{ns}	22.33 ^{ns}	1487.50 *	215.72**	17.09 ^{ns}	34.28 **	37.32 ^{ns}	232.90 ^{ns}	78.93 ^{ns}	397.60 **
Env.WC	25	28.43 ^{ns}	19.91 ^{ns}	2187.80 **	65.96^{ns}	22.16 ^{ns}	28.06 **	28.11 ^{ns}	292.50 ^{ns}	83.45 ^{ns}	308.10 *
Gen.WC.Env	25	33.85 ^{ns}	6.62 ^{ns}	1342.90 ^{ns}	104.48^{ns}	25.05 ^{ns}	27.11 *	23.70 ^{ns}	221.40 ^{ns}	79.91 ^{ns}	465.90 **
Residual	102	22.18	14.69	910.50	75.97	19.53	14.48	28.07	207.70	80.44	167.90

df: degrees of freedom; NB: number of branches, SD: stem diameter, PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPW: dry pod weight, DPL: dry pod length, NSP: number of seeds per plant, NPP: number of pods per plant, YPP: yield per plant, Env: environment, Gen: genotype, WC: water condition, * significant at 5% level of significance, ** significant at 1% level of significance, ^{ns} non-significant.

2.3.2 Performance of okra genotypes for phenotypic traits under drought-stressed and non-stressed conditions

Mean values of phenotypic traits among 26 okra genotypes evaluated under DS and NS conditions evaluated in GH and FLD environments are presented in Tables 2.3 and 2.4, respectively. Significant genotypic differences (P<0.05) were observed for NB under DS condition in the GH environment. Genotypes LS14, LS15, LS17, LS18 and LS21 recorded the highest NB (>10), compared to genotypes LS07, LS12, LS13 and LS23, which recorded the lowest NB (≤ 3) under DS condition in GH environment. For SD, genotypes LS16, LS20 and LS24 recorded significantly higher values (>15 mm) than LS06 and LS26, which produced the lowest SD (≤5 mm) under NS conditions in GH environment.

Significant (P<0.05) genotypic differences were recorded for PH under both DS and NS conditions in GH environment. Genotypes LS01, LS15, LS17 and LS19 were taller (>110 cm), whereas genotypes LS07, LS12, LS13, LS23 and LS24 were shorter (< 40 cm) under DS conditions in the GH environment. Under NS conditions in GH environment, genotypes LS11, LS14, LS15, LS16, LS18, LS20 and LS23 were taller (>150 cm). The longest DTM (>70 days) was recorded for genotypes LS09, LS12, LS17, LS22, LS23, LS24 and LS26 under DS condition, whereas under NS condition, genotypes LS11 and LS24 were the earliest to mature (<50 days) in the GH environment. Okra genotypes LS01, LS09, LS14, LS15, LS16, LS20 and LS23 recorded significantly higher FPL (>15 cm) under NS conditions in GH environment. Genotypes LS01, LS14 and LS19 recorded the highest DPW (>10 g) than other test genotypes under DS conditions in GH environment. Genotypes LS11, LS14, LS16, LS18, LS20, LS21 and LS23 recorded the highest DPW (>15 g) compared to other test genotypes under NS condition in GH environment. Non-significant differences were observed for DPL, NSP and NPP under both DS and NS conditions in GH environment.

Significant (P<0.05) differences were found for YPP among the studied okra genotypes under DS and NS conditions in GH environment. Genotypes LS01, LS06, LS15, LS17, LS19, LS20 and LS22 had the highest YPP (>20 g), whereas genotypes LS10, LS12, LS13 and LS24 had the lowest YPP (< 5 g) under DS condition in the GH environment. Genotypes LS01, LS11, LS14, LS16, LS20 and LS21 recorded the highest YPP (>50 g), whereas genotypes LS02 and LS03 recorded the lowest YPP (<20 g) under NS condition in GH environment. ATY score of 1 was observed for most studied genotypes under DS condition. Under NS condition, genotypes

LS02, LS03, LS05, LS10, LS12, LS15, LS18 and LS26 recorded ATY scores of 1, whereas the other genotypes recorded ATY score of 0 without ATY colouration of the stem.

Under FLD environment, significant (P<0.05) differences were found among the test genotypes for NB only under NS conditions where genotypes LS01, LS09, LS17 and LS18 recorded the highest NB (≥ 20). For SD, genotypes LS01, LS05, LS09, LS12, and LS18 recorded the highest SD (≥18 mm) under NS conditions in the FLD environment.

Significant (P<0.05) differences were observed among studied okra genotypes for PH in the FLD environment. Genotypes LS01, LS09, LS12, LS17 and LS20 had the tallest plants (≥150 cm) under NS conditions in the FLD environment. The longest DTM (>70 days) under DS condition was found for genotypes LS04, LS17, LS23 and LS26, whereas genotypes LS05, LS08 and LS10 had longer days to maturity (>70 days) under NS condition in the FLD environment. LS01, LS05, LS08, LS09, LS11, LS15 and LS18 recorded the highest FPL (≥20 cm) under NS conditions in the FLD environment. Regarding DPW, genotypes LS01, LS08 and LS09 recorded the highest DPW (≥15 g) under NS conditions in the FLD environment.

Highly significant (P<0.001) differences were observed for DPL among okra genotypes under NS condition in the FLD environment where the highest DPL (>25 cm) was recorded for genotypes LS01, LS05, LS08, LS09, LS12, LS16, LS17 and LS26. Significant (P<0.05) differences were observed for NSP under DS and NS conditions in the FLD environment. Under DS condition the highest NSP (>40) was observed for genotypes LS14, LS17, LS21, LS24 and LS26. Genotypes LS01, LS05, LS08, LS11, LS12, LS17 and LS18 recorded the highest NSP (> 60) under NS condition in the FLD environment. For NPP, okra genotypes differed significantly (P<0.05) under DS and NS conditions in the FLD environment. Higher NPP was found for genotypes LS06, LS11, LS17, LS18, LS22 and LS24 (≥25) under DS condition in the FLD environment. Genotypes LS01, LS05, LS09, LS17, LS18 and LS20 found significantly higher NPP (≥40), under NS condition in the FLD environment.

Under NS condition in the FLD environment, significant (P<0.05) differences were observed among studied okra genotypes for YPP. Genotypes LS09, LS12, LS18 and LS20 recorded the highest YPP (≥70 g), whereas genotypes LS04, LS06, LS22 and LS23 recorded the lowest YPP (<25 g) under NS condition in the FLD environment. Genotypes without ATY pigmentation included LS07, LS09, LS13, LS16, LS22 and LS25 under DS conditions in the FLD environment. Genotypes LS02, LS03, LS05, LS10, LS12, LS15, LS18 and LS25 had ATY pigmentation under DS in the FLD environment. Genotypes LS01, LS02, LS03, LS05, LS07,

LS10, LS12, LS13, LS15. LS18, LS20, LS23 and LS26 had ATY pigmentation under NS condition in the FLD environment, whereas genotypes LS04, LS06, LS08, LS09, LS11, LS14, LS16, LS17, LS19, LS21, LS22, LS24 and LS25 did not exhibit ATY, as no ATY colouration was observed under DS condition in the FLD environment.

Table 2.3: Mean values of phenotypic traits among 26 okra genotypes evaluated under drought-stressed (DS) and non-stressed (NS) conditions under glasshouse environment

Genotype code	NB		SD(mm)		PH(cm)		DTM (days)		FPL(cm)		DPW(g)		DPL(cm)		NSP		NPP		YPP(g)		ATY	
	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS
LS01	5.9	10.6	3.7	7.8	132.1	149.3	67.0	71.3	12.4	15.5	14.2	11.0	13.6	14.5	13.3	15.5	6.0	14.5	43.4	62.5	1.0	0.0
LS02	4.4	6.1	6.3	7.8	69.3	91.4	67.0	66.5	9.3	7.9	1.1	4.8	9.8	12.5	7.5	14.8	3.5	11.0	6.4	18.0	1.0	1.0
LS03	9.4	7.5	5.1	7.4	84.8	87.9	69.0	77.5	7.8	8.5	3.5	3.8	6.3	6.8	10.1	12.8	2.5	4.5	8.0	17.1	1.0	1.0
LS04	4.5	11.3	4.6	6.8	65.9	77.0	64.8	67.3	6.2	13.7	3.3	13.2	1.2	9.7	13.5	9.0	3.5	8.0	8.4	37.6	1.0	0.0
LS05	9.8	6.0	6.4	7.1	90.5	85.1	64.8	66.5	9.0	10.0	4.1	5.6	11.0	9.6	11.5	9.8	5.5	7.0	13.3	22.5	1.0	1.0
LS06	9.4	6.6	7.2	5.9	107.5	81.4	63.0	67.0	9.9	10.7	6.4	4.7	12.4	7.9	7.7	10.5	3.5	8.0	26.0	16.0	1.0	0.0
LS07	1.6	5.9	2.5	6.3	33.5	65.0	64.8	71.5	2.7	12.8	0.9	4.2	6.4	7.4	7.1	4.3	2.0	7.5	11.9	18.3	0.0	0.0
LS08	4.1	6.1	2.4	7.9	46.5	90.2	75.0	73.3	6.8	10.1	2.5	4.3	9.0	13.6	7.2	11.5	3.5	8.0	7.4	14.9	1.0	0.0
LS09	4.0	5.5	4.2	10.9	61.1	115.7	70.5	70.0	5.4	16.8	0.9	1.9	6.6	9.1	3.0	10.5	1.5	9.0	18.9	23.4	0.0	0.0
LS10	5.0	6.1	5.9	9.1	92.8	83.0	67.0	80.0	4.7	6.4	1.6	4.7	5.9	8.4	2.5	18.0	4.0	6.0	3.6	32.4	1.0	1.0
LS11	3.1	14.6	4.4	12.2	67.6	155.4	0.0	33.3	6.6	12.5	2.6	15.3	11.6	7.4	8.8	12.0	2.0	11.5	9.5	53.8	1.0	0.0
LS12	2.5	8.6	0.9	9.2	32.9	114.0	73.0	63.0	4.5	9.0	1.1	9.0	1.4	11.0	7.8	7.0	2.0	9.0	4.5	24.6	1.0	1.0
LS13	2.4	5.3	2.5	7.4	39.4	74.4	66.5	63.0	5.8	12.8	0.5	6.6	3.4	12.9	3.3	15.3	1.5	3.0	3.9	46.0	0.0	0.0
LS14	12.8	15.0	8.3	16.8	78.9	197.2	80.0	67.0	9.2	18.8	10.1	18.0	10.3	17.3	7.0	19.8	3.0	15.0	16.0	74.9	1.0	0.0
LS15	11.3	11.1	8.3	8.4	113.6	166.6	69.0	71.5	14.0	16.3	7.1	8.6	6.7	7.6	6.8	18.5	11.5	7.5	21.9	37.2	1.0	1.0
LS16	3.6	16.4	3.5	16.9	85.1	190.2	63.0	66.5	11.1	17.5	2.6	20.0	11.4	19.0	4.9	25.3	3.5	15.5	19.4	70.7	0.0	0.0
LS17	14.3	10.3	7.7	12.9	127.1	106.5	77.5	69.0	11.1	14.5	6.4	12.6	10.6	15.0	9.3	32.5	8.5	14.5	22.5	45.6	1.0	0.0
LS18	10.1	9.9	7.1	8.8	70.4	166.5	73.0	67.3	11.1	11.0	2.7	16.0	6.2	14.8	2.5	20.5	6.5	7.5	17.9	39.8	1.0	1.0
LS19	6.4	6.1	4.9	7.6	128.4	83.7	66.5	70.8	11.9	6.9	10.4	5.6	8.9	9.2	12.5	5.5	9.5	4.0	38.2	17.2	1.0	0.0
LS20	6.1	10.4	6.8	17.4	57.6	185.4	67.0	75.0	7.6	19.7	1.8	18.3	8.1	16.4	3.8	28.8	0.7	17.5	22.8	57.8	1.0	0.0
LS21	12.9	16.3	8.1	11.1	77.4	140.9	67.0	66.5	13.0	10.4	6.3	17.4	10.4	13.4	4.5	13.3	4.0	11.0	18.2	47.4	1.0	0.0
LS22	7.3	7.8	6.0	9.2	56.5	128.5	70.5	64.8	7.1	6.6	8.5	8.6	6.7	5.0	4.0	3.8	4.5	8.5	21.1	34.4	0.0	0.0
LS23	1.8	10.9	4.0	10.8	35.6	153.1	71.0	69.0	6.4	17.4	1.4	16.9	5.4	14.5	5.0	22.5	2.5	9.0	14.6	53.0	1.0	0.0
LS24	5.4	8.0	3.1	10.6	33.3	125.8	75.0	40.0	5.6	14.2	1.1	6.6	7.5	15.6	6.0	24.3	0.5	10.0	2.8	29.3	1.0	0.0
LS25	4.5	10.3	5.9	13.1	94.5	156.4	63.0	63.0	7.0	8.9	0.8	3.2	4.2	2.8	8.3	9.3	1.0	5.5	5.9	43.0	0.0	0.0

LS26	3.1	6.3	5.5	5.5	43.4	72.4	73.3	63.0	7.7	9.3	0.9	5.0	4.1	3.1	15.8	6.0	2.0	6.5	5.7	22.6	1.0	1.0
Mean	6.4	9.2	5.3	9.8	74.1	120.9	66.5	66.4	8.2	12.2	3.9	9.5	7.7	10.9	7.4	14.6	3.8	9.2	15.1	36.9	0.8	0.4
P-value	<.05	0.2	0.9	<.001	<.05	<.05	<.001	0.7	0.8	<.05	<.05	<.05	0.7	0.6	0.7	0.5	0.3	0.2	<.05	<.05		
SED	3.2	3.8	4.0	2.4	27.4	33.8	3.6	15.2	4.8	4.0	3.3	4.7	5.3	6.4	5.8	10.4	3.5	4.1	11.7	15.3		
LSD (5%)	6.7	10.0	8.0	5.0	56.6	69.7	7.3	32.4	12.0	8.2	6.7	9.7	12.4	17.2	11.8	29.0	7.2	14.0	24.1	31.6		
CV (%)	50.6	41.4	57.0	24.9	37.0	28.0	5.4	23.0	58.7	32.4	47.7	49.0	69.5	58.3	76.2	70.9	59.1	44.6	77.3	41.5		

NB: number of branches, SD: stem diameter, PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPW: dry pod weight, DPL: dry pod length, NSP: number of seeds per plant, NPP: number of pods per plant, YPP: yield per plant, ATY: anthocyanin, LSD: least significant difference; SED: Standard error of difference; CV: coefficient of variation.

Table 2.4: Mean values of phenotypic traits among 26 okra genotypes evaluated under drought-stressed (DS) and non-stressed (NS) conditions under field environment

Genotype	NB		SD(mm)		PH(cm)		DTM (days)		FPL(cm)		DPW(g)		DPL(cm)		NSP		NPP		YPP(g)		ATY	
	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS
LS01	10.8	22.9	12.8	19.2	88.3	166.9	63.5	66.3	11.0	24.8	8.9	15.9	12.7	27.1	30.0	71.5	22.5	40.0	38.0	66.4	1.0	1.0
LS02	10.6	8.4	6.7	11.9	86.2	71.2	57.8	59.5	7.9	13.7	7.1	6.8	11.3	14.0	27.5	28.5	19.5	15.5	36.6	30.9	1.0	0.0
LS03	8.6	5.6	7.2	9.1	56.3	42.4	68.5	63.5	8.5	16.0	6.0	7.3	8.1	15.3	30.0	47.5	15.5	17.5	19.2	38.3	1.0	0.0
LS04	11.5	7.9	13.1	12.4	56.8	79.8	76.5	68.5	12.5	9.7	9.9	7.9	13.3	14.9	31.0	29.5	15.0	18.0	30.2	24.4	0.0	0.0
LS05	5.4	19.6	7.5	20.6	66.9	129.9	60.3	73.0	9.1	21.3	5.3	14.7	8.8	26.7	28.0	64.5	12.0	41.0	19.2	65.2	1.0	0.0
LS06	12.7	9.7	13.1	14.1	87.4	100.4	57.8	66.0	10.9	14.5	10.3	6.0	20.9	15.5	15.0	14.0	30.5	16.5	31.9	21.4	0.0	0.0
LS07	7.4	9.6	8.9	14.9	48.2	90.3	59.5	65.5	8.5	14.6	6.7	5.3	9.1	14.8	8.0	28.5	8.0	7.0	13.4	29.3	1.0	0.0
LS08	7.8	19.8	10.1	17.2	47.2	145.3	63.8	70.5	12.0	21.7	6.5	15.3	13.0	25.6	10.0	62.0	19.5	34.5	26.1	68.1	0.0	0.0
LS09	5.4	20.7	14.0	18.0	91.0	150.1	64.5	63.8	14.6	21.5	11.5	15.7	18.8	28.2	37.5	52.0	23.5	40.5	45.0	70.4	0.0	0.0
LS10	12.1	8.2	9.5	11.7	77.4	68.4	80.0	72.0	11.3	13.6	10.8	6.7	15.8	14.5	34.0	27.0	13.0	17.0	38.9	30.1	1.0	1.0
LS11	11.6	14.9	8.8	16.2	73.9	136.0	56.0	63.8	13.7	21.4	6.4	12.7	14.3	22.8	31.0	61.0	29.5	32.0	28.3	34.6	0.0	0.0
LS12	10.3	18.6	9.8	21.4	86.9	182.9	68.5	64.3	10.0	21.5	7.0	13.9	15.0	26.6	26.5	64.0	23.5	38.5	29.3	70.4	1.0	1.0
LS13	12.3	7.8	9.6	14.3	73.1	96.9	63.0	64.3	8.9	14.6	9.5	9.9	16.6	16.0	21.5	27.0	18.0	11.0	26.9	30.8	1.0	0.0
LS14	11.5	16.7	12.2	13.9	69.4	139.4	62.0	61.8	15.3	14.8	7.4	9.7	12.4	20.0	47.5	41.0	22.0	26.0	37.1	39.6	0.0	0.0
LS15	9.3	16.9	12.4	16.0	77.4	112.8	65.5	60.0	6.5	22.8	7.3	13.8	8.6	23.7	27.5	55.0	18.0	36.5	34.0	66.9	1.0	0.0
LS16	6.3	16.3	4.4	17.5	56.7	140.7	59.5	63.5	12.3	19.8	4.3	14.2	13.9	26.2	14.5	58.0	19.0	34.5	17.9	54.0	0.0	0.0
LS17	9.2	20.2	9.5	16.4	84.4	166.4	73.0	66.3	15.8	19.6	9.2	15.4	15.0	27.3	47.0	65.5	25.5	43.5	40.0	68.1	0.0	0.0
LS18	11.3	20.3	17.3	20.0	105.8	138.9	66.3	59.5	12.6	20.3	12.6	12.0	22.0	27.5	31.0	63.5	26.5	40.0	45.3	72.5	1.0	1.0
LS19	4.9	19.8	9.0	13.8	61.9	135.2	64.3	64.5	9.4	18.4	5.3	13.0	13.4	24.9	7.5	36.5	5.0	28.0	21.3	68.3	0.0	0.0
LS20	9.8	18.9	7.8	17.1	72.4	168.1	60.3	60.3	5.8	18.4	9.0	11.7	13.9	24.3	6.0	56.0	14.0	41.0	28.6	70.8	1.0	0.0
LS21	12.0	8.6	11.0	11.0	85.8	96.3	56.0	57.8	15.2	14.7	8.8	7.4	14.9	15.1	45.5	25.5	21.5	18.5	46.2	29.5	0.0	1.0
LS22	10.4	7.9	11.1	12.0	71.9	100.6	59.5	64.5	11.8	15.4	7.8	7.6	11.8	14.0	31.0	24.0	25.0	12.5	44.1	24.4	0.0	0.0
LS23	6.3	7.9	6.5	13.5	77.4	77.3	71.5	64.0	7.9	13.6	2.9	5.3	8.0	9.8	6.5	11.0	13.0	9.5	21.6	20.6	1.0	0.0

LS24	10.0	13.3	10.3	14.9	65.8	132.8	63.8	66.3	16.5	16.1	7.4	10.8	17.1	20.9	42.0	40.0	26.5	28.0	50.0	44.8	0.0	0.0
LS25	4.4	7.4	6.6	11.1	50.8	59.8	73.0	60.0	5.7	13.2	5.4	6.8	9.2	14.7	28.0	27.5	15.5	12.0	23.0	27.8	0.0	0.0
LS26	11.2	10.9	11.5	14.8	62.6	96.1	71.5	66.0	17.1	13.8	14.1	11.0	18.7	27.4	49.5	37.5	23.5	22.0	43.3	38.5	1.0	0.0
Mean	9.4	13.8	10.0	15.1	72.4	116.3	64.8	64.4	11.2	17.3	8.0	10.7	13.7	20.7	27.4	43.0	19.4	26.2	32.1	46.4	0.5	0.2
P-value	1.0	<.05	0.9	<.05	0.8	<.05	<.05	0.7	0.8	<.05	0.6	<.05	0.6	<.001	<.05	<.05	<.05	<.05	0.2	<.001		
SED	6.3	5.1	5.4	2.6	25.0	31.1	11.6	5.8	5.5	3.4	3.9	3.2	5.5	4.0	16.5	18.8	12.4	11.1	11.9	12.2		
LSD (5%)	13.0	10.5	13.0	5.4	59.8	64.2	5.6	12.0	11.8	7.1	10.5	6.7	13.0	8.3	33.3	43.6	25.5	22.9	38.0	25.2		
CV (%)	67.1	36.9	54.2	17.3	34.6	26.7	8.7	9.0	49.5	19.9	49.5	30.5	39.8	19.6	60.0	43.7	63.9	42.4	37.0	26.3		

NB: number of branches, SD: stem diameter, PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPW: dry pod weight, DPL: dry pod length, NSP: number of seeds per plant, NPP: number of pods per plant, YPP: yield per plant, ATY: anthocyanin, LSD: least significant difference; SED: Standard error of difference; CV: coefficient of variation.

2.3.3 Associations among phenotypic traits under drought-stressed and non-stressed and conditions

The levels of associations of the assessed phenotypic traits among okra genotypes under DS and NS conditions in GH and FLD environments are presented in Tables 2.5 and 2.6, respectively. SD positively and significantly correlated with NB (r = 0.81; $P \leq 0.001$) under DS condition in GH environment. Positive and significant correlations were found between FPL and NB (r = 0.68, $P \leq 0.001$), SD (r = 0.61, $P \leq 0.001$) and PH (r = 0.73, $P \leq 0.001$) under DS condition in GH environment. Under DS condition in GH environment, there were positive and significant associations between YPP with PH (r = 0.67, $P \leq 0.001$), FLP (r = 0.66, $P \leq 0.001$), DPW (r = 0.80, $P \leq 0.001$), DPL (r = 0.57, $P \leq 0.001$) and NPP (r = 0.58, $P \leq 0.001$). Positive and highly significant difference were observed between YPP with NB (r = 0.80, $P \leq 0.001$), SD (r = 0.75, $P \leq 0.001$), PH (r = 0.79, P ≤ 0.001), FPL (r = 0.66, P ≤ 0.001), DPW (r = 0.82, $P \leq 0.001$) and NPP (r = 0.66, $P \leq 0.001$) under NS condition in the GH environment.

Positive and significant correlations were observed between SD and NB under DS (r = 0.46, $P \leq 0.05$) and NS (r = 0.83, $P \leq 0.001$) conditions in the FLD environment. Significant correlations were observed between NPP and FPL (r = 0.59, $P \leq 0.001$), and NPP and DPL (r = 0.60, $P \leq 0.001$) under DS condition in the FLD environment. Also, YPP significantly and positively correlated with FPL (r = 0.83, $P \leq 0.001$) under NS in the FLD environment. Significant correlations were recorded between YPP with PH (r = 0.60, $P \leq 0.001$), FPL (r = 0.64 $P \leq 0.001$) and NPP (r = 0.65, $P \leq 0.001$) under DS condition in the FLD environment.

Table 2.5: Pearson correlation coefficients showing the magnitude of associations of agronomic traits among okra genotypes under drought-stressed (upper diagonal) and non-stressed (lower diagonal) conditions under the glasshouse environment

Traits	NB	SD (mm)	PH (cm)	DTM	FPL(cm)	DPW(g)	DPL(cm)	NSP	NPP	YPP(g)
NB		0.81^{**}	0.584^{**}	0.26^{ns}	0.68^{**}	0.56^{**}	0.42^{*}	-0.01^{ns}	0.56^{**}	0.36^{ns}
SD	0.67^{**}		0.53^{**}	0.10^{ns}	0.61^{**}	0.43^{*}	0.34^{ns}	-0.07^{ns}	0.44^{*}	0.31^{ns}
PH	0.77^{**}	0.80^{**}		-0.03^{ns}	0.73^{**}	0.69^{**}	0.54^{**}	0.31^{ns}	0.71^{**}	0.67^{**}
DTM	-0.25^{ns}	-0.12^{ns}	-0.16^{ns}		0.11^{ns}	0.11^{ns}	-0.24^{ns}	-0.88^{**}	0.14^{ns}	0.06^{ns}
FPL	0.48^{*}	0.59^{**}	0.60^{**}	-0.03^{ns}		0.66^{**}	0.60^{**}	0.20^{ns}	0.73^{**}	0.66^{**}
DPW	0.85^{**}	0.66^{**}	0.75^{**}	-0.13^{ns}	0.58^{**}		0.54^{**}	0.31^{ns}	0.62^{**}	0.80^{**}
DPL	0.42^{*}	0.53^{**}	0.47^{*}	0.03^{ns}	0.60^{**}	0.65^{**}		0.06^{ns}	0.28^{ns}	0.57^{**}
NSP	0.41^{*}	0.63^{**}	0.54^{**}	0.02^{ns}	0.62^{**}	0.57^{**}	0.78^{**}		0.20^{ns}	0.16^{ns}
NPP	0.62^{**}	0.72^{**}	0.64^{**}	-0.09^{ns}	0.65^{**}	0.67^{**}	0.66^{**}	0.61^{**}		0.58^{**}
YPP	0.80^{**}	0.75^{**}	0.79^{**}	-0.14^{ns}	0.66^{**}	0.82^{**}	0.55^{**}	0.57^{**}	0.66^{**}	

NB: number of branches, SD: stem diameter, PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPW: dry pod weight, DPL: dry pod length, NSP: number of seeds per plant, NPP: number of pods per plant, YPP: yield per plant, * significant at 5% level of significance, ** significant at 1% level of significance, ^{ns} non-significant difference.

Table 2.6: Pearson correlation coefficients showing associations of agronomic traits among okra genotypes under drought-stressed (upper diagonal) and non-stressed (lower diagonal) conditions under the field environments

Traits	NB	SD(mm)	PH (cm)	DTM	FPL(cm)	DPW(g)	DPL(cm)	NSP	NPP	YPP(g)
NB		0.46*	0.41*	-0.66**	0.34ns	0.57**	0.47*	0.39*	0.54**	0.52**
SD	0.83**		0.52**	0.06ns	0.42*	0.73**	0.60**	0.38ns	0.47*	0.64**
PH	0.90**	0.83**		-0.11ns	0.17ns	0.44*	0.51**	0.29ns	0.52**	0.60**
DTM	0.09ns	0.20ns	0.05ns		0.01ns	0.21ns	-0.02ns	0.21ns	-0.24ns	0.05ns
FPL	0.85**	0.78**	0.75**	0.044ns		0.46*	0.58**	0.70**	0.59**	0.64**
DPW	0.90**	0.78**	0.82**	0.17ns	0.86**		0.76**	0.49*	0.42*	0.67 **
DPL	0.90**	0.81**	0.81**	0.12ns	0.79**	0.93**		0.28ns	0.60**	0.59**
NSP	0.83**	0.76**	0.74**	0.10ns	0.86**	0.86**	0.86**		0.52**	0.72**
NPP	0.92**	0.80**	0.85**	0.07ns	0.90**	0.92**	0.91**	0.91**		0.65**
YPP	0.92**	0.77**	0.80**	0.03ns	0.83**	0.90**	0.90**	0.90**	0.93**	

NB: number of branches, SD: stem diameter, PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPW: dry pod weight, DPL: dry pod length, NSP: number of seeds per plant, NPP: number of pods per plant, YPP: yield per plant, * significant at 5% level of significance, ** significant at 1% level of significance, ns non-significant difference.

2.3.4 Principal component analysis (PCA) of studied phenotypic traits

PCA showing loading scores, explained and cumulative variation for phenotypic traits assessed among the studied okra genotypes under DS and NS conditions across GH and FLD environments are presented in Table 2.7. Three and two principal components (PCs) were identified for assessed traits for DS and NS conditions, respectively in the GH and FLD environments. The PCs accounted for a cumulative variance of 76.04% and 73.81%, under DS and NS conditions, respectively. Under DS condition, PC1 positively correlated with NB, SD, DTM, FPL, DPW, DPL, NPP and YPP, which accounted for 50.74% total variation. PH was positively correlated with PC2 and NSP was negatively correlated with PC2, which accounted for 14.21% of total variation under DS condition in the GH environment. Under NS condition, PC1 positively correlated with NB, SD, DTM, FPL, DPW, DPL, NSP, NPP, and YPP, accounting for 61.64% of total variation. PC2 positively correlated with PH accounting for 12.16% of total variation. The PCs accounted for a cumulative variance of 75.03% and 87.99% in the FLD environment under DS and NS conditions, respectively. Under DS condition, PC1 positively correlated with NB, SD, DTM, FPL, DPW, DPL, NPP and YPP, which accounted for 51.82% total variation. PC2 positively correlated with PH accounting for 12.89% of total

variation. Under NS condition, PC1 positively correlated with NB, SD, DTM, FPL, DPW, DPL, NSP, NPP and YPP which accounted for 77.80% of total variation. PC2 positively correlated with PH accounting for 10.19% of the total variation.

The relationship between okra genotypes and the studied phenotypic traits is illustrated using principal component biplots under DS and NS conditions in the GH and FLD environments are depicted in Figures 2.1 and 2.2, respectively. Angles lesser than 450 between the dimensions of two variables indicate high traits associations, whereas longer vectors show the discriminating ability of a particular trait. As a result, genotypes excelling in a particular trait were plotted closer and furthest to the vector line. Genotypes LS17 and LS15 were grouped together based on the high values for NB and SD under DS in the FLD environment. Under NS condition, genotypes LS 14, LS16, LS18, LS23 and LS01 were clustered together based on high values of NB, SD, PH, FPL, DPW, DPL, NSP, NPP and YPP. Under FLD environment, genotypes LS21, LS01, LS12, LS14 and LS09 were found in the same cluster based on high values for NB, SD, PH, FPL, DPW, DPL, NSP, NPP and YPP under DS condition. Genotypes LS17, LS12, LS09, LS19 and LS09 were allocated together based on the high values of NB, SD, PH, FPL, DPW, DPL, NSP, NPP and YPP under NS condition in the FLD environment.

Table 2.7: Rotated principal component loading scores, explained and cumulative variances of phenotypic traits among 26 okra genotypes under drought-stressed and non-stressed conditions across glasshouse and field environments

Glasshouse environment	Drought-stressed			Non-stressed	
Traits	PC1	PC2	PC3	PC1	PC2
NB	**0.78**	0.47	-0.12	**0.82**	-0.38
SD (mm)	**0.67**	0.48	-0.30	**0.86**	-0.05
DTM (days)	**0.87**	-0.15	0.02	**0.86**	-0.20
PH (cm)	0.10	**0.65**	**0.62**	-0.15	**0.78**
FPL (cm)	**0.90**	0.06	-0.02	**0.77**	0.22
DPW(g)	**0.85**	-0.20	0.18	**0.88**	-0.11
DPL (cm)	**0.65**	-0.31	-0.48	**0.74**	0.42
NSP	0.24	**-0.56**	0.54	**0.75**	0.40
NPP	**0.79**	0.05	0.25	**0.84**	0.10
YPP(g)	**0.79**	-0.27	0.09	**0.90**	-0.13
Explained variance (eigenvalue)	5.07	1.42	1.11	6.16	1.22
Proportion of total variance (%)	50.74	14.21	11.09	61.64	12.16
Cumulative variance (%)	50.74	64.95	76.04	61.64	73.81
Field environment	Drought-stressed			Non-stressed	
Traits	PC1	PC2	PC3	PC1	PC2
NB	**0.68**	-0.17	0.10	**0.96**	-0.03
SD (mm)	**0.77**	0.01	0.34	**0.88**	0.11
DTM (days)	**0.64**	-0.35	0.33	**0.89**	-0.06
PH (cm)	0.03	**0.88**	0.31	0.12	**0.99**
FPL (cm)	**0.72**	0.20	-0.52	**0.90**	-0.07
DPW(g)	**0.82**	0.20	0.35	**0.95**	0.07
DPL (cm)	**0.80**	-0.12	0.21	**0.95**	0.02
NSP	**0.70**	0.39	-0.45	**0.92**	-0.01
NPP	**0.77**	-0.32	-0.29	**0.96**	-0.05
YPP(g)	**0.89**	0.08	-0.10	**0.94**	-0.10
Explained variance (eigenvalue)	5.18	1.29	1.06	7.78	1.02
Proportion of total variance (%)	51.82	12.89	10.60	77.80	10.19
Cumulative variance (%)	51.82	64.70	75.30	77.80	87.99

SD: stem diameter, PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPW: dry pod weight, DPL: dry pod length, NSP: number of seeds per plant, NPP: number of pods per plant, YPP: yield per plant, PC's with ≥ 0.5 loading scores are boldfaced.

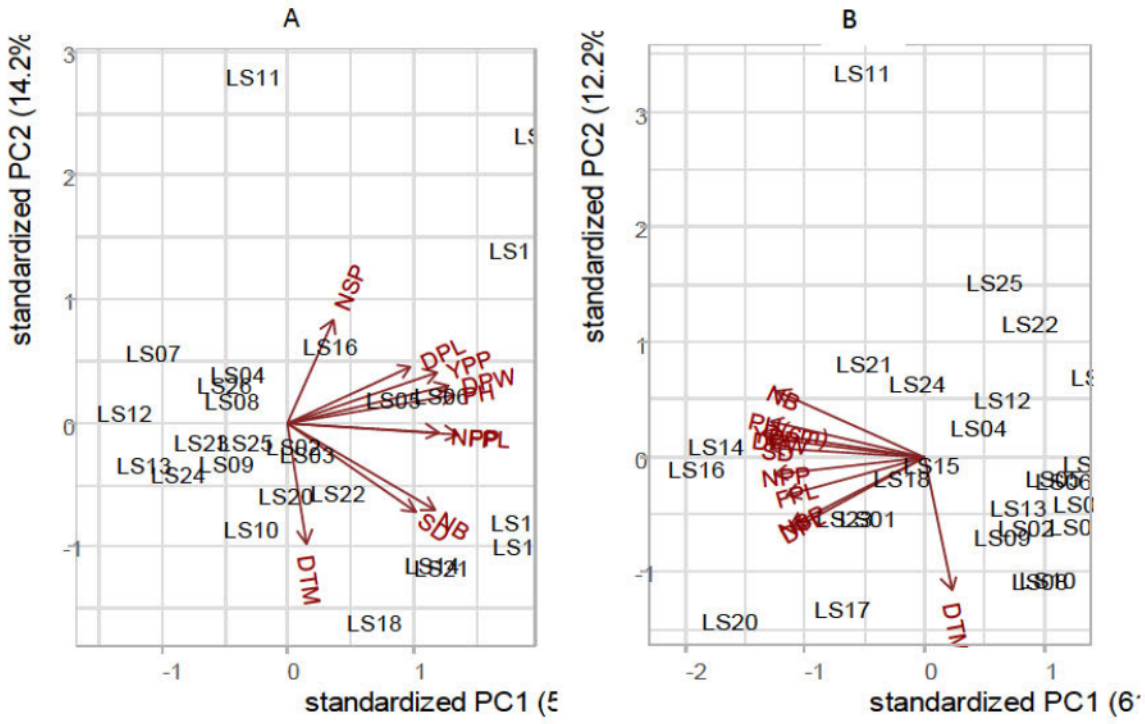

Figure 2.1: Principal component bi-plot of PC1 vs PC2 showing groupings of okra genotypes based on phenotypic traits under drought-stressed (A) and non-stressed (B) conditions in the FLD environment. NB: number of branches, SD: stem diameter, PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPW: dry pod weight, DPL: dry pod length, NSP: number of seeds per plant, NPP: number of pods per plant, YPP: yield per plant.

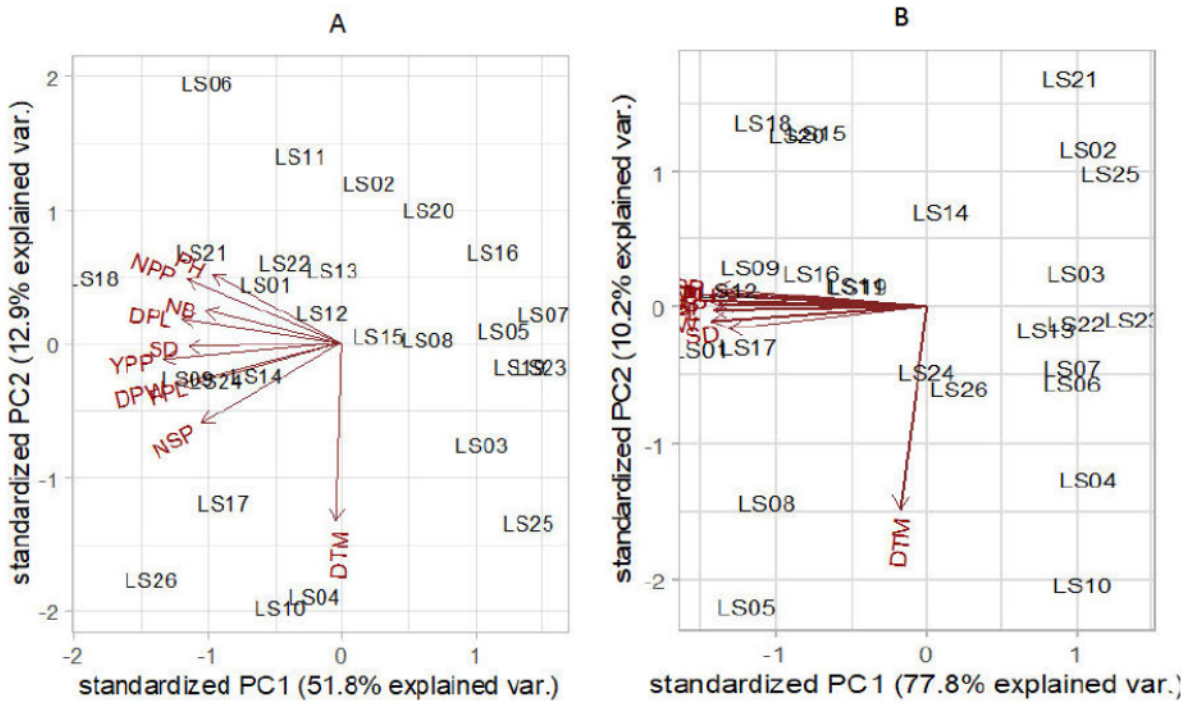

Figure 2.2: Principal component bi-plot of PC1 vs PC2 showing groupings of okra genotypes based on phenotypic traits under drought-stressed (A) and non-stressed (B) conditions in the FLD environment. NB: number of branches, SD: stem diameter, PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPW: dry pod weight, DPL: dry pod length, NSP: number of seeds per plant, NPP: number of pods per plant, YPP: yield per plant.

2.4 Discussion

Drought is the major yield limiting constraint of okra in water restricted environments. Breeding for drought tolerant okra genotypes has been relatively slow due to a lack of genetic variation for drought adaptive traits and evaluations of a limited number of genotypes. Drought tolerance assessment involving a relatively representative set of genetically diverse okra genotypes is useful for selecting potential candidates for cultivation and for use in breeding programs to develop progenies to improve crop yield potential in drought-stressed environments. Plants undergo various morphological changes in response to abiotic stress, which may serve as indicators of drought tolerance. In the present study, the response of okra genotypes was assessed using phenotypic traits to select promising genotypes with desirable levels of drought tolerance to design appropriate breeding strategies in okra for improving yield potential under water-limited conditions.

In the present study, significant genotype × water condition effect and genotype × water condition × environment interaction effect was observed for fresh pod yield that will allow for the selection of okra genotypes for moisture stress tolerance . For example, okra genotypes such as LS01, LS06 and LS19 were high yielding (≥ 25 g per plant) under drought stress conditions in a glasshouse environment, whereas genotypes such as LS17, LS18, LS21, LS22, LS24 and LS26 were best performers with yield levels of ≥ 40g per plant under drought stress condition in field environment. Therefore, these genotypes are useful genetic resources and recommended for cultivar development adapted to water-stressed environment.

Phenotypic traits associated with yield and yield-components traits are useful to improve yield potential for water-constrained environments (Slafer et al. 2014). However, these inter-relationships are poorly understood in okra, limiting genetic improvement for these traits. In okra, various yield components, including plant height, number of branches per plant, fresh pod length and width, number of pods per plant and pod weight influence pod productivity (Akinyele and Osekita, 2006; Abd El-Fattah et al. 2020). Branching capacity in okra has a direct effect on pod yield (Kumar and Reddy, 2016). The positive correlation observed between the number of branches and pod yield under drought stress conditions suggested that profuse branching ability is a valuable trait that could be selected to improve pod yield. Although okra is a tall statured plant, varieties that have been developed are short in stature. However, plant height between 150-170 cm is crucial to accommodate more significant number of pods on the main stem, and this has a direct effect on pod yield in okra (Reddy et al. 2012). Drought stress causes wilting, and stomatal closure leading to reduced transpiration, cell growth, turgor pressure, and lower water potential and hence reduced plant height. Furthermore, drought reduces cell division and cell differentiation in okra resulting in reduced pod weight and plant height. Plant height and above-ground biomass production influence photosynthetic response, which affects yield expression. Drought causes leaf senescence, reduced photosynthesis, and low biomass production (Ayub et al., 2020). Okra genotypes such as LS09, LS18 and LS21 with tall plants (> 90 cm) recorded higher fresh pod yield (> 40 g per plant). Further, the positive associations between plant height and fresh pod yield in the present study under drought-stress conditions indicated direct selection for plant height improves yield response making this trait useful for cultivar development geared towards drier environments. Some tall okra genotypes including LS06, LS09, LS15, LS18 and LS19 could serve as useful genetic resources targeting the development of taller okra genotypes for drought-stressed environments.

Days to maturity is an essential trait in the selection of drought-tolerant okra genotypes. During drought stress, plants synthesize phytohormones which regulate the transition from vegetative to reproductive stages. The synthesis allows plants to regulate flowering and maturity periods and yield productivity (Campos-Rivero et al., 2017). Under water-limiting conditions, okra can accumulate sufficient photo-assimilates resulting in higher pod yields (Chaturvedi et al. 2019). Drought stress reduced the number of days to maturity for most genotypes, including LS01, LS02, LS06, LS09, LS14, LS18, LS19 and LS21 in the glasshouse and field environments. However, such genotypes were high yielders (Tables 2.3 and 2.4), suggesting that okra genotypes with shorter days to maturity have yield advantage in water-limited conditions and can be valuable genetic stocks in breeding short maturation genotypes. The relatively low correlations recorded between days to maturity with pod yield under drought stress condition in the contrasting environments suggests limited trade-offs on yield responses (Slafer et al. 2014). Also, poor associations were recorded between days to maturity with dry pod length and number of pods per plant, suggesting small trade-offs on pod yield. This implies that that okra breeding can be undertaken for drier environments targeting phenological responses (i.e., targeted selection for early or late maturing genotypes), without significant yield penalties.

Stem diameter is an important agronomic trait for improving yield potential in okra (Yadav et al. 2010, Kumar et al. 2011, Asare et al. 2016). According to Eshiet and Brisibe, (2015) thin stems are not desirable because they are prone to lodging, leading to a decrease in fresh pod yield. Reduced stem diameter under drought stress is attributed to changes in turgidity of cells through osmoregulation which could be a mechanism to stress tolerance. However, reduced stem diameter restricts the distribution of water and nutrients to the pods, partly explaining the slightly low correlations between this trait and other yield components under drought stress compared to non-stressed conditions (Tables 2.5 and 2.6). Alam et al. (2020) reported a reduction in stem diameter for okra plants exposed to drought stress. Nevertheless, the positive association observed between stem diameter with pod yield per plant and other pod yield component traits such as plant height and the number of pods per plant under drought stress conditions suggested that okra genotypes with thick stems can be selected to improve yield potential under drought stress conditions. The high variation in stem diameter among the tested okra genotypes is probably related to varied concentrations of photosynthates that accumulate in the stem (Barzegar et al. 2016). The present study identified okra genotypes such as LS04, LS06, LS09, LS14, LS15, LS18 and LS21 with thicker stems for breeding.

The green tender okra pod is the most important and economical product useful as a green vegetable (Reddy et al. 2013; Eshiet and Brisibe, 2015). Cultivar selection and breeding of okra is mainly focussed on developing genotypes with desirable pod characteristics including length, width, and size. The reduction of the number of pods per plant under drought stress conditions is attributed to the physiological altercation that disturbs plant growth processes and reduces carbon assimilation (Chaturvedi et al. 2019). Reportedly, drought stress reduces the number of meristems due to slowed cell division resulting in reduced flowering capacity and pod formation (Ayub et al. 2020).

The present study revealed positive correlations between the number of pods per plant with pod yield per plant under drought-stressed conditions, suggesting that direct selection of a higher pod number can improve yield gains in water-limited environments. Okra genotypes such LS15 and LS17 with a higher number of fresh pods per plant and pod yield under drought-stressed conditions are recommended for production in dry environments. Also, these are recommended as parental genotypes to develop progenies with enhanced yield potential targeting cultivation in water-limited environments. There were non-significant variations in the number of seeds per plant and number of pods per plant in the glasshouse environment attributed to the difference in the genetic makeup of the studied okra genotypes. The longest pods were recorded in genotypes LS15 (15cm) and LS26 (17.07cm) in the glasshouse and field environments, respectively. According to Ezeh and Adejumo (2020), longer pods are more desirable as they allow for ovule development and accommodate more ovules inside the ovary, hence higher numbers of seed production. The positive correlation between fresh pod length and pod yield per plant under -stressed conditions indicates that pod characteristics are useful for direct selection to improve the fresh pod yield in okra.

Fresh pod yield in okra is a complex character that depends on the involvement of many yield components traits (dos Santos Fariasa et al. 2019; Shi et al. 2019). The present study revealed that secondary traits such as the number of branches, stem diameter, plant height, dry pod weight, dry pod length, the number of seeds per plant and the number of pods per plant were essential to improve pod yield per plant under drought stress condition (Tables 2.5 and 2.6). Further, the low correlations between yield and yield components traits suggested relatively small trade-offs on pod productivity. Thus, selecting secondary traits in a breeding program is possible to develop okra populations with enhanced drought stress tolerance. Okra genotypes such as LS01, LS09, LS15, LS17, LS18, LS21 and LS24 with higher fresh pod length, number of pods per plant and pod yield per plant under drought stress conditions were identified in the

present study. The identified okra genotypes possessing suitable phenotypic traits that promote the development of high yield potential will be crossed to develop populations for further testing and selection under drought stress conditions.

2.5 Conclusions

The present study determined the phenotypic response of various okra genotypes to identify yield components and guide selection and genetic improvement for yield gains under drought stress condition. High levels of phenotypic variation were recorded for the number of branches, stem diameter, plant height, fresh pod length, dry pod weight, dry pod length, the number of seeds per plant, the number of pods per plant and pod yield per plant. The assessed okra genotypes were phenotypically differentiated, and hence genetic improvement is possible through directional selections. Pod yield per plant was positively correlated with yield-related traits, including fresh pod length, dry pod weight and the number of pods per plant in drought-stressed conditions suggesting the value of secondary traits in okra improvement. The identified phenotypically desirable, and complementary okra genotypes are useful genetic resources for future crosses and selection of promising progenies based on combining ability analysis and heritability under water-limited environments.

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Chapter 3. Characterization of okra (*Abelmoschus esculentus* [L.] Moench) accessions with variable drought tolerance through simple sequence repeat markers and phenotypic traits

Abstract

Genetic diversity analysis of crop genetic resources is a prerequisite for parental selection with suitable and complementary profiles for breeding. The objectives of this study were to determine genetic diversity present among okra accessions using simple sequence repeat (SSR) and complementary phenotypic markers and to select genetically divergent and superior parental accessions for pre-breeding. Twenty-six preliminarily selected okra accessions were assessed using nine highly polymorphic SSR markers and phenotyped under drought-stressed (DS) and non-stressed (NS) environmental conditions using a 13 × 2 alpha lattice design with two replications. Data were collected on the following eleven phenotypic traits: plant height (PH), days to 50% maturity (DTM), fresh pod length (FPL), dry pod weight (DPW), dry pod length (DPL), number of pods per plant (NPPP), pod yield per plant (PYPP), total above-ground biomass (AGB), harvest index (HI), root weight (RW), and root to shoot ratio (RSR). The SSR markers revealed an expected mean heterozygosity value of 0.54, indicating moderate genetic diversity among the tested okra accessions. Cluster analysis based on phenotypic and SSR markers differentiated the accessions into three distinct genetic groups. PYPP was positively and significantly correlated with FPL (r = 0.81), ABG (r = 0.69), and HI (r = 0.67) under DS conditions, and FPL (r = 0.83) and AGB (r = 0.60) under NS conditions. Genetically complementary accessions such as LS04, LS05, LS06, LS07, LS08, LS10, LS11, LS15, LS18, LS23, LS24, and LS26 were identified for their high yield potential and related yield-improving traits under DS conditions. The identified accessions are recommended as parents for hybridization and selection programs to improve the yield potential of okra under drought-stressed environments.

Keywords: abiotic stress; genotyping; okra; phenotyping; molecular markers; SSR

3.1 Introduction

Okra (*Abelmoschus esculentus* L., 2*n* = 130) is an allotetraploid derived from the natural hybridization of a wild progenitor *A. tuberculatus* (2*n* = 58), with another yet unidentified

species with $2n = 72$ chromosomes. Okra is a vegetable crop that is widely cultivated for its fresh and succulent pods [1,2]. Okra is an autogamous species and predominantly a self-pollinating crop. However, varying levels of cross-pollination have also been reported depending on the activity of insect pollinators and the growing environment [1]. The tender green pod is the most economical and vital source of vitamins A, B_1, B_3, B_6, folic acid, C, and K, essential for the human diet [3]. Potassium, magnesium, phosphorus, and calcium are the principal and essential mineral elements present in the green and immature pods of okra [4]. In addition, the pod contains 9.7% carbohydrate, 2.2 % protein, and 1% fibre [5]. Okra grains contain 22.14% protein, rare amino acids (such as lysine and tryptophan), fat, and fibre. The seed oil content varies from 20–40%, and the major fatty acids of the seed are linoleic acid (49.54%), palmitic acid (28.60%), and oleic acid (20.38%)[6]. These nutritional attributes make okra an important food security crop, especially in sub-Saharan Africa (SSA), where malnutrition is the highest. Africa accounts for 32.8% of the world's okra production. West and Central African countries contribute to over 75% of total okra production in SSA [7]. Despite the significant contribution by SSA toward global okra production, the average crop yields are low and variable in the region due to a lack of improved and modern varieties.

Okra is drought-tolerant crop and can successfully grow under water-limited conditions with minimal supplemental irrigation. Despite being relatively drought-tolerant, the crop fails to reach its maximum yield potential, resulting in low marketable pod yields, primarily when drought stress occurs at the flowering and pod development stages. For example, 37 to 83% yield losses attributed to drought stress occurred during the reproductive stage [8,9]. The low yield performance is related with the cultivation of low-yielding and drought-sensitive varieties [9].

The crop exhibits extensive morphological variation for traits such as plant height, fresh pod length, number of days to 50% flowering and maturity, number of branches, number of pods per plant, and pod yield [1,2]. Phenotypic traits such as plant height, number of branches, fresh pod length, number of pods per plant, total biomass, and seed yield exhibit positive associations with pod yield [4,9–10]. Hence, these traits could serve as useful product profiles for breeding of improved okra accessions for high yield and related traits. Therefore, rigorous phenotypic assessment of okra genetic resources will identify beneficial traits for future breeding. However, phenotypic traits are influenced by the genotype, environment, and genotype-by-environment interaction, confounding trait heritability and genotype performances [10,11]. To complement phenotypic assessment and for detailed genetic analysis, molecular markers are

eminent genetic tools [1;6,12]. Molecular markers improve selection efficiency through phenotypic traits and accelerate genetic gains for desired traits.

Markers such as random amplified polymorphic DNA (RAPD) [10,13] inter-simple sequence repeat (ISSR) [12], amplified fragment length polymorphism (AFLP) [14,15], sequence-related amplified polymorphism (SRAP), and simple sequence repeats (SSR) [16,17] have been successfully used. The marker systems were applied to explore genetic diversity and relatedness among okra genetic resources. These allowed for delineating heterotic groups among core collections of the crop and assisted in the selection and variety design. Among the molecular markers, the SSRs are highly polymorphic and reproducible markers useful for effective genotyping and selection programs [9,18,19].

Improved varieties of okra are yet to be developed and marketed for food security, better nutrition, and economic gains. There are limited drought-adapted varieties in SSA, which necessitates developing high-yielding and drought-tolerant okra genotypes adapted to the region. Therefore, the objectives of this study were to determine the genetic diversity present among okra accessions using simple sequence repeat and complementary phenotypic markers and to identify heterotic groups to select genetically divergent and superior parental accessions for pre-breeding.

3.2 Materials and methods

3.2.1 Plant materials

The present study used 25 okra landrace accessions sourced from the Agricultural Research Council, Vegetable, Industrial and Medicinal Plants (ARC-VIMP)/South Africa. Locally adapted and grown okra variety "Clemson Spineless" was also included as a comparative control. The code and number and geographical origin of the okra accessions used in the study are presented in Table 3.1.

Table 3.1: Accession code and number, and geographical origin of the okra accessions used in the study

Accession code	Accession number	Geographical origin
LS01	VI033775	Malaysia

LS02	VI033797	Malaysia
LS03	VI056457	Yugoslavia
LS04	VI039651	Bangladesh
LS05	VI046561	Thailand
LS06	VI047672	Bangladesh
LS07	VI050150	Taiwan
LS08	VI050957	Zambia
LS09	VI050960	Zambia
LS10	VI055110	Malaysia
LS11	VI055119	Myanmar
LS12	VI055219	Malaysia
LS13	VI055220	Malaysia
LS14	VI055421	Viet Nam
LS15	VI056069	Cambodia
LS16	VI056079	Cambodia
LS17	VI056081	Cambodia
LS18	VI056449	United States of America
LS19	VI060131	Mali
LS20	VI060313	Tanzania
LS21	VI060679	India
LS22	VI060803	Turkey
LS23	VI060817	Brazil
LS24	VI060822	Nigeria
LS25	VI060823	Nigeria
LS26	Clemson Spineless	South Africa

ARC-VIMP = Agricultural Research Council Vegetable, Industrial and Medicinal Plants.

3.2.2. DNA extraction, purification, and quantification

Okra seeds were sent to SciCorp Laboratories (SciCorp-lab, SA Pty Ltd., Pietermaritzburg, South Africa) for SSR analysis. Genomic DNA was extracted from 20 seeds per genotype using modified CTAB method [20]. The quantity and quality of total genomic DNA were determined by 0.7% Tris-Borate-EDTA (TBE) agarose gel electrophoresis and spectrophotometer, respectively. A working concentration of 20 ngμL^{-1} was standardized for all extracted DNA.

3.2.3 Polymerase chain reaction (PCR) and SSR analysis

Okra seeds were found to be better for DNA sampling and analysis due to the mucilaginous material present in the leaves. Bulked DNA was used for amplification and analysis. SSR sequences were amplified through PCR using 9 selected diagnostic polymorphic SSR markers developed for okra (Table 3.2). These markers were selected based on their high polymorphic information content (PIC) and that they were developed and recommended for okra genetic diversity studies [18,21,22,23]. PCR amplification reaction contained 20 µL of PCR mix. The mix contained 1x PCR buffer, 3 mM MgCl, 1.25 U Taq polymerase, 0.2 mM dNTPs, 4pM each

primer, and 5 ng genomic DNA (Bioline, Meridian, MI, USA). A PCR profile of initial denaturation for 2 min at 94 °C and 33 cycles of denaturation for 1 min at 94 °C, the annealing temperature of 63 °C for 2 min, and extension for 2 min at 72 °C was used. PCR products were fluorescently labelled and separated by capillary electrophoresis on ABI 3130 automatic sequencer (Applied Biosystems Johannesburg, South Africa) and analysis was performed using GeneMapper 4.1 (Applied Biosynthesis, Johannesburg, South Africa). A 36 cm capillary and 3130 POP-7 polymer (Applied Biosystem, Johannesburg, South Africa) were used.

Table 3.2: Description of the SSR primers used for genotyping of 26 okra landrace accessions

Marker Name	Forward Primer Sequence	Reverse Primer Sequence	PIC
Okra 111	GATGGAATTGAGAAACCAGA	TGTGTTCTTCACTCTCGTCA	0.89
Okra 152	GCTCTATTGATGGCGAGTAA	AAAGTCATCCAAGGTGACAA	0.81
Okra 166	TTCCAGTTGGAGAGGTAAGA	CTTCCATTTCATCGACTTTC	0.82
AVRDC-Okra17	ACGAGAGTGAAGTGGAACTG	CTCCTCTTTCCTTTTTCCAT	0.81
AVRDC-Okra70	GTAGCTGAACCCTTTGCTTA	CTATCATGGCGGATTCTTTA	0.98
AVRDC Okra39	TGAGGTGATGATGTGAGAGA	TTGTAGATGAGGTTTGAACG	0.99
AVRDC-Okra64	AAGGAGGAGAAAGAGAAGGA	ATTTACTTGAGCAGCAGCAG	0.87
AVRDC-Okra9	ACCTTGAACACCAGGTACAG	TTGCTCTTATGAAGCAGTGA	0.85
AVRDC-Okra 57	CGAGGAGACCATGGAAGAAG	ATGAGGAGGACGAGCAAGAA	0.78
Okra137	GAGAGAGATTGCTTCGACTG	TAAACTTTAAACTCAGCGGC	0.80

SSR = simple sequence repeats, PIC = polymorphic information content.

3.2.4 Marker data analysis

3.2.4.1 Computation of Principal Coordinate Analysis (PCoA) and genetic parameters

The GenAlex software version 6.5 [24] was used for data analyses and to summarize PCoA and genetic diversity parameters. Two approaches were adopted to investigate the genetic diversity and structure among the accessions. The first approach treated DNA polymorphisms as binary data (presence or absence). To determine the genetic structure within and among landraces, a second approach was adopted based on the co-dominant nature of the marker. Genetic parameters such as number of alleles per locus (Na), number of effective alleles per locus (Ne), allelic richness (Ar), Shannon's information index (I), observed heterozygosity (Ho), and expected heterozygosity (He) were calculated using GenAlex version 6.5 according to Nei and Li [25]. Polymorphic information content (PIC) was calculated using the formula $PIC = 1 - \Sigma Pij^2$, where Pij is the frequency of the jth allele of the ith locus [25]. The number of polymorphic loci was estimated for each pre-determined group based on pedigree information. Online-based ClustVis (https://biit.cs.ut.ee/clustvis_large/, accessed on 22 May 2022) was used to visualize the heatmap, and plots of total genetic variation were analysed using pairwise genetic distance for haploid and co-dominant SSR markers [26].

3.2.4.2 Cluster analysis

The binary data were used to obtain a dissimilarity matrix using the Jaccard index. The matrix was used to perform cluster analysis based on the unweighted pair group method using the arithmetic mean algorithm (UPGMA) in DARwin 5.0 software [27]. A dendrogram was then generated on the dissimilarity matrix to determine genetic relationships among the tested accessions. Bootstrap analysis was performed for node construction using 10,000 bootstrap values to estimate the reliability of the clustering pattern. A joint hierarchical cluster was generated to determine the association between genotypic and phenotypic data for both stressed and non-stressed conditions. The clusters were constructed using the "Cluster" package in R software [28].

3.2.5 Phenotyping okra accessions

3.2.5.1 Experimental design and crop establishment

Two seeds of each genotype were grown in 5 L capacity plastic pots filled with composted pine bark growing media. Two plants were established per pot for each genotype. The day/night temperatures in the greenhouse (GH) were 30 °C/20 °C and the relative humidity ranged between 45 and 55% during the study. Inorganic fertilizers consisting of nitrogen (N), phosphorus (P), and potassium (K) were applied at a rate of 120, 30, and 30 kg ha^{-1}, based on soil fertility recommendations using urea (46-0-0), phosphorus pentoxide (P_2O_5), and potassium oxide (P_2O), respectively. The okra accessions were evaluated using a 13 × 2 alpha lattice design under drought-stressed (DS) and non-stressed (NS) conditions with two replications. DS was imposed at 50% flowering until physiological maturity to mimic terminal drought stress by withholding irrigation until the soil water content reached 30% field capacity. In addition, pots were irrigated at field capacity to allow for continued plant growth and development. The NS conditions on the different second pots involved maintaining soil moisture content at field capacity by supplying water through the dripper irrigation system until physiological maturity under GH environment. Tensiometers (Spectrum Technologies, Inc, IL, Aurora, Illinois, USA) were used to monitor soil moisture status during the experiment.

3.2.5.2 Phenotypic data collection

Data were collected from three randomly selected and tagged plants for each genotype. At physiological maturity, data were collected on the following phenotypic traits. Plant height

(PH) was measured in cm from the ground level to the apex of the plant on the main stem. Pods were harvested when 50% of the pods were 3–5 cm long, which is regarded as a marketable size [9]. Harvesting was conducted every third day by hand. At each harvest, the number of pods per plant (NPPP) were counted, and fresh pod length (FPL) was measured in cm. At the end of the experiment, data were computed on the number of pods per plant (NPPP), fresh pod length (FPL), and pod yield per plant (PYPP). Plants from the second pots were left until maturity to collect data on dry pod length (DPL) which was measured in cm, and mature dry pod weight (DPW) was determined by weighing dry pods harvested per plant and expressed in grams. Yield per plant was determined by weighing fresh pods harvested per plant and expressed in grams. The plants were cut at the soil surface to separate shoots and roots biomass. Total above-ground biomass (AGB) was determined in grams by weighing the stem and the pod of the plants per pot. Root weight (RW) was determined in grams by weighing all roots. Root to shoot ratio (RSR) was calculated as the ratio of shoot to root biomass. Harvest index (%) was calculated as HI = (pod weight/total above-ground biomass) × 100.

3.2.5.3 Phenotypic data analysis

Phenotypic data were subjected to analysis of variance (ANOVA) using a lattice procedure with GenStat 18th Edition (VSN International, Hempstead, UK). Treatment means were separated using the least significant difference (LSD) at the 5% significance level. Pearson's correlation coefficients were calculated using IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL, USA, 2008) to determine the magnitude of the relationship among phenotypic traits. Principal component analysis (PCA) was used to identify influential traits under NS and DS conditions using R Studio version 4.0, ggplot2(R Core Team, 2020, Vienna, Austria). Biplots were constructed using R version 4.0, ggplot2 (R Core Team, 2020) to determine relationships between the accessions and the assessed phenotypic traits. Hierarchical clusters were generated using phenotypic data based on the Gower method [29], using the Cluster package in R software [28].

3.3 Results and discussion

3.3.1 Marker characterization

Understanding the genetic diversity present among diverse okra accessions is useful for identifying and selecting suitable and contrasting parental genotypes for breeding, leading to the accelerated development of improved varieties. The estimated genetic parameters derived using SSR markers are presented in Table 3.3 . The SSR markers amplified 24 putative alleles among the tested okra accessions, ranging from 2 for the markers AVRDC-OKRA64, Okra 152, Okra 162, Okra 111, and AVRDC-Okra57 to 5 for the marker AVRDC-Okra 9 with a mean value of 2.70 alleles per locus. Out of 10 selected SSRs used in this study, 9 SSR primers could amplify successfully. The level of amplification in the present study was higher compared to the 75% reported in okra by Kumar et al. [30]. This indicates the suitability of the sampled SSR markers for the analysis of genetic variation and relationship in okra. A total of 24 alleles were amplified, with an average of 2.70 alleles per locus (Table 3.3), and this average number of alleles indicates that this genetic diversity would be relatively moderate [31]. This was lower than the value of 71 alleles per locus reported by Mohammed et al. [18] when assessing 32 okra accessions genotypes with 16 SSR markers. The variability in the number of alleles observed could be attributed to the genetic differences in the tested lines and the difference in the sampled SSR markers. Effective allele number (Ne) ranged from 2 for markers AVRDC-OKRA64, Okra 152, Okra 162, Okra 111, and AVRDC-Okra57 to 2.76 for marker AVRDC-Okra9 with a mean of 2.24, indicating that this genetic diversity would be relatively small. However, greater diversity has been reported [16], indicating a mean number of 4.8 effective alleles after evaluating 20 okra accessions using SSR markers. This corroborates with the results of two to seven (mean = four) alleles per locus reported by Kpodo et al. [15]. The mean Shannon information index value of the test population was 0.83, ranging from 0.69 for markers AVRDC-OKRA64, Okra 152, Okra 162, Okra 111, and AVRDC-Okra57 to 1.16 for marker AVRDC-Okra9. The observed heterozygosity value was 1, suggesting that all the accessions reached 100% heterozygosity. The expected heterozygosity ranged from 0.50 to 0.64 with a mean value of 0.54. Marker AVRDC-Okra9 had the highest He of 0.64. The inbreeding coefficient varied from −0.57 to −1.00, with a mean of −0.85. Nine markers (100%) were highly polymorphic with PIC > 0.50, indicating their high discriminating ability and their utility for genetic analysis studies in okra. PIC values ranged from 0.50 to 0.64 with a mean of 0.55, which is relatively higher than the mean PIC value of 0.51 reported by Kpodo et al. [15] and lower than the PIC value of 0.81 reported by Mohammed et al. [18] in okra. The average

PIC value of 0.55 indicates that these markers are informative for genetic diversity analysis [22]. High polymorphism values suggest that the selected markers are suitable for distinguishing the genetic diversity among the tested accessions. The high polymorphism values observed when using the sampled SSR markers may be due to the amphipolyploid nature of *Abelmoschus* species. In addition, there is a higher frequency of mutations in polyploids, such as in okra, than diploids [21], leading to increased genetic diversity and genetic plasticity [10].

Table 3.3: Genetic diversity parameters generated by SSR markers among 26 okra accessions

Marker	Genetic Parameters						
	Na	Ne	I	Ho	He	F_{IS}	PIC
AVRDC-Okra70	3	2.47	0.97	1.00	0.60	−0.68	0.60
AVRDC-Okra64	2	2.00	0.69	1.00	0.50	−1.00	0.50
Okra 152	2	2.00	0.69	1.00	0.50	−1.00	0.50
Okra 166	2	2.00	0.69	1.00	0.50	−1.00	0.50
AVRDC-Okra9	5	2.76	1.16	1.00	0.64	−0.57	0.64
AVRDC-Okra39	3	2.31	0.91	1.00	0.57	−0.76	0.57
Okra 111	2	2.00	0.69	1.00	0.50	−1.00	0.50
Okra137	3	2.58	1.01	1.00	0.61	−0.63	0.61
AVRDC-Okra57	2	2.00	0.69	1.00	0.50	−1.00	0.50
Average	2.70	2.24	0.83	1.00	0.54	−0.85	0.55
Standard deviation	1.00	0.30	0.18	0.00	0.06	0.19	0.06
Standard error	0.34	0.15	0.10	0.10	0.06	0.06	0.02

N_a = total number of alleles per locus; N_e = number of effective alleles per locus; I = Shannon information index; H_o = observed heterozygosity; H_e = expected heterozygosity; F_{IS} = inbreeding coefficient; PIC = polymorphic information content.

3.3.2 Principal Coordinate Analysis (PCoA) of 26 okra accessions genotyped using 9 SSR markers

The genetic structure of the assessed accessions was inferred with the PCoA based on the genetic matrix (Figure 3.1). The first principal coordinate (PC) accounted for 25.19% of variation present among accessions. The coordinate analysis indicated higher genetic diversity among the two accessions LS02 and LS11 compared to other genotypes, due to their inherent genetic variation. The second principal component suggests a further separation between LS02, LS11, and LS13, accounting for 18.24% of the total variation. The grouping of LS01, LS03, LS04, LS09, and LS26 into the same cluster may indicate the genetic similarity among these accessions. Hence, the genetic information generated can be useful to design crosses and exploit genetic diversity through selection programs.

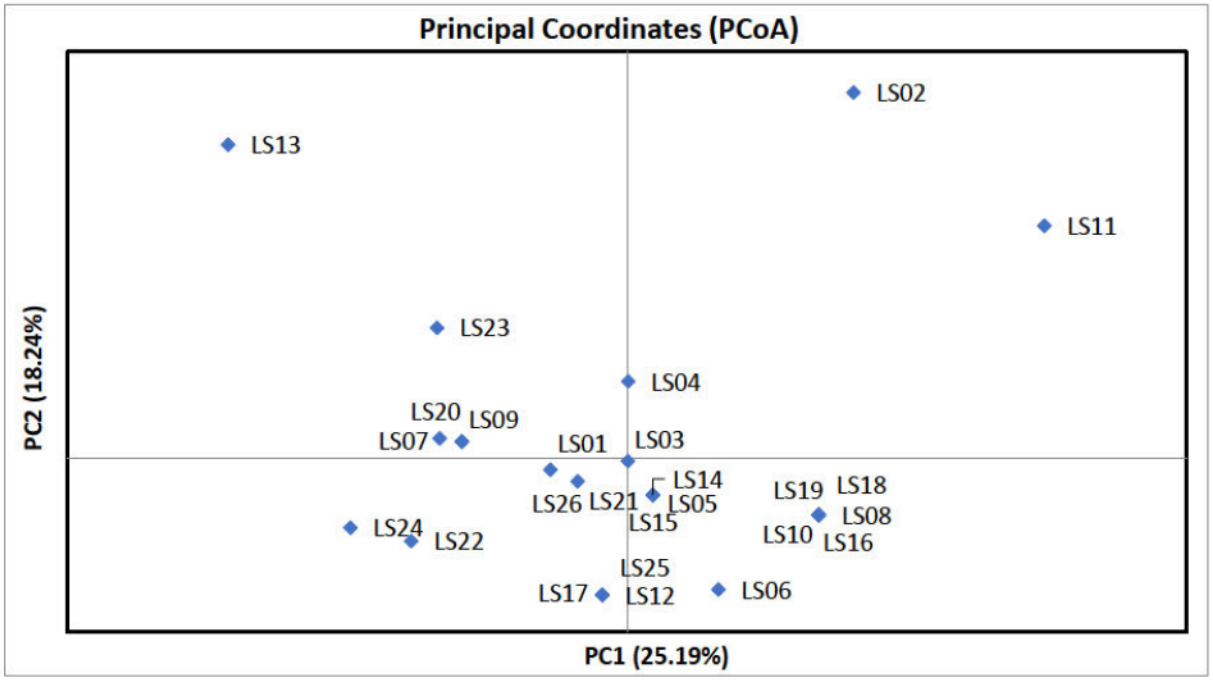

Figure 3.1: Principal coordinate analysis (PCoA) based on 9 polymorphic SSR markers and 26 okra accessions (coded LS01 to LS26, Table 3.1). Note PC1 denotes the first principal coordinate and PC2 the second principal coordinate.

3.3.3 . Heatmap cluster

A heatmap based on SSR marker transcription was constructed using the hierarchical clustering method discerning the genetic relationship of 26 okra accessions based on Jaccard's coefficient (Figure 3.2). The assayed okra accessions and SSR markers each were grouped into two main clusters. The first cluster had one subcluster consisting of eight accessions, of which four were collected from Malaysia (LS02 and LS10), Myanmar (LS11), and Cambodia (LS16) in Asia and two from Mali (LS19) and Zambia in Africa (LS08). The second cluster contained two subclusters with seven accessions, including LS24, LS13, LS07, LS22, LS23, LS09, and LS20 on the first subcluster, which was dominated by accessions collected from Nigeria, Malaysia, Taiwan, Turkey, Brazil, Zambia, and Tanzania, respectively, and eleven accessions LS06, LS01, LS26, LS25, LS12, LS17, LS04, LS21, LS15, LS05, and LS14 on the second subcluster, of which nine were sampled from Asia and one each from South Africa (LS26) and Nigeria (LS25). The observed genetic dissimilarity indicates that these accessions are related to different geographic locations and most of the cultivated accessions in each geographic region were uniquely differentiated. This may be due to the limited outcrossing rate among the geographic regions of the evaluated okra accessions. Genetic variability among okra accessions was also reported by Massucato et al. [12]. Information on the genetic grouping of the

accessions is essential in selecting contrasting parents based on the breeding history and genetic relationship of the assessed population and test environment.

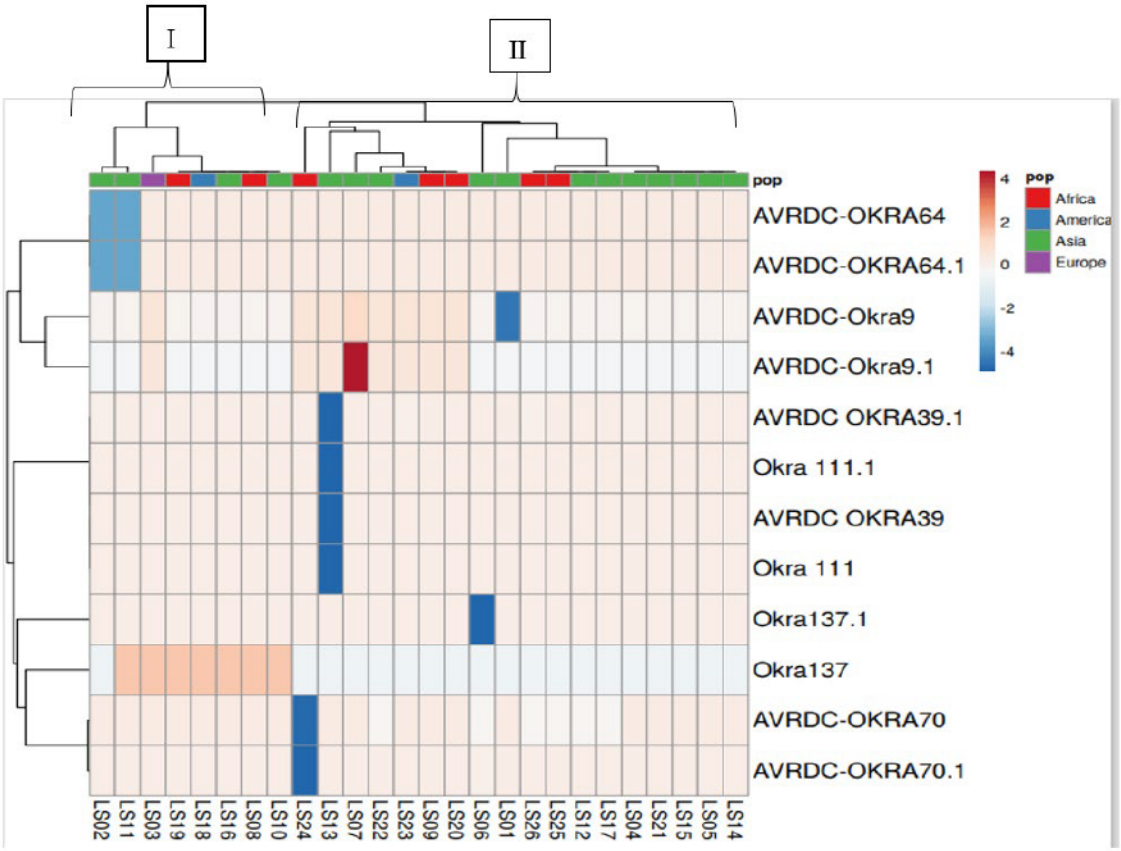

Figure 3.2: Heatmap showing the genetic relationship among 26 okra accessions using 9 SSR markers. Annotations in the heatmap show grouping of accessions and SSR marker clusters.

3.3.4 Cluster Analysis

Figure 3.3 summarizes the unweighted pair group method with the arithmetic mean method using Jaccard's dissimilarity matrix, showing the genetic inter-relationships among the studied okra accessions. Clustering is a multivariate technique that assists in indicating the pattern of genetic relationships among accessions. The accessions were grouped into three distinct major clusters, namely cluster I, consisting of eight accessions, and clusters II and III, consisting of nine accessions each, indicating the presence of a wide genetic variation among the studied okra accessions. This corroborates with the findings of Reddy et al. [7], Pradip et al. [32], and Ravishankar et al. [33], who presented a dendrogram that classified the tested accessions into three major groups. Accessions allocated in different clusters are genetically divergent and may serve as prospective parents for a breeding programme. Most accessions maintained their positions on the dendrogram compared to the heatmap cluster analysis, except for the

accessions LS01, LS03, and LS06. These clustering patterns indicate that accessions from different regions were genetically diverse. The high diversity among the accessions makes the assessed genotypes unique genetic resources to develop new breeding populations.

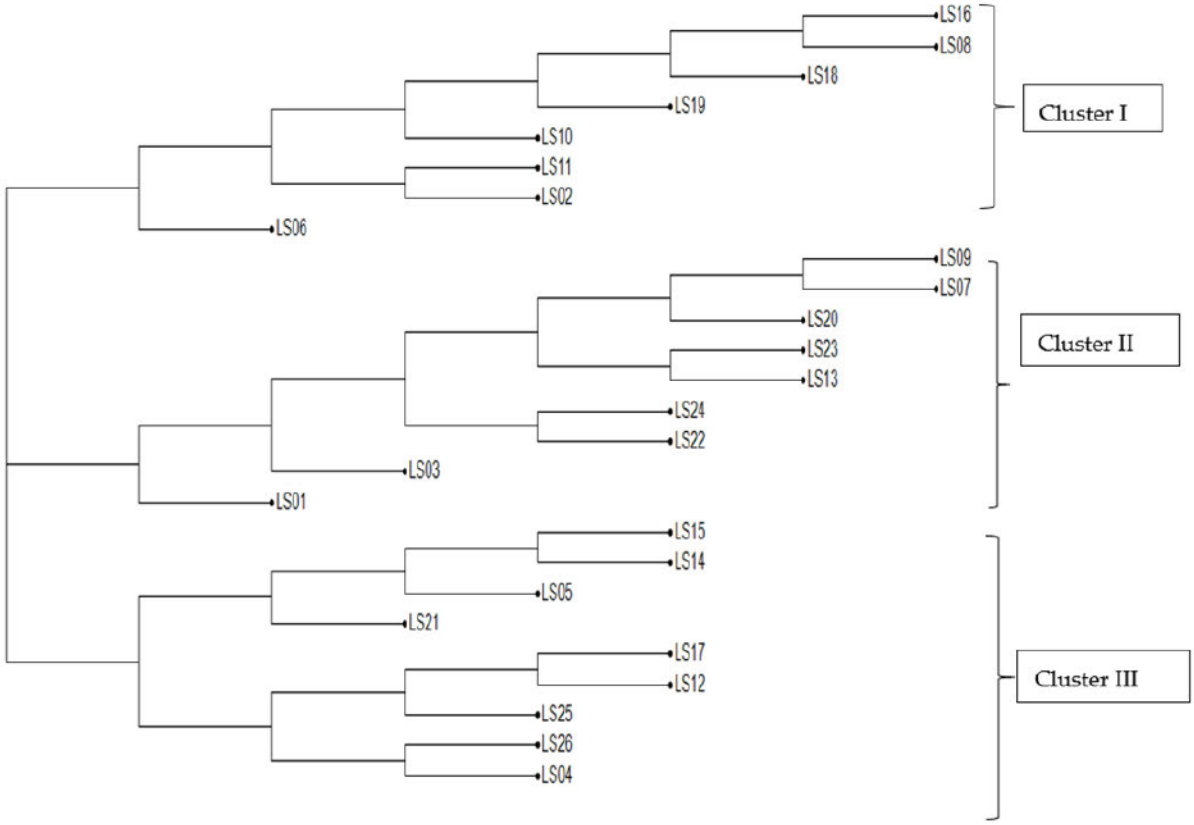

Figure 3.3: Dendrogram showing genetic relationships among 26 okra accessions assessed via 9 polymorphic SSR markers.

3.3.5 Accession and environmental effects on phenotypic traits

Analysis of variance indicated significant ($p < 0.05$) differences among the test accessions under different water treatments and their interactions for the assessed phenotypic traits (Table 3.4). Accessions had significant ($p < 0.05$) difference for PH, DTM, NPPP, PYPP, AGB, RW, and FPL. The water treatments had a significant ($p < 0.05$) effect on PH, FPL, and PYPP. The genotype × water treatment interaction exerted a significant ($p < 0.05$) effect on DTM, FPL, and PYPP (Table 3.4).

Table 3.4: Analysis of variance showing mean square values and significant tests of 26 okra accessions assessed for phenotypic responses in glasshouse environment under drought-stressed (DS) and non-stressed (NS) conditions

S.O.V.	df	PH	DTM	FPL	DPL	DPW	NPPP	PYPP	AGB	HI	RW	RSR
Replications	1	23.80 ^{ns}	11.44 ^{ns}	21.61 *	35.41 ^{ns}	81.39 *	0.01 ^{ns}	55.20 *	1423.50 **	1153 ^{ns}	1.50 ^{ns}	1.09 **
Incomplete blocks	1	2063.50 **	0.08 ^{ns}	9.99 ^{ns}	11.22 ^{ns}	13.89 ^{ns}	3.47 ^{ns}	1.11 ^{ns}	104.56 ^{ns}	790.10 ^{ns}	85.87 *	0.09 ^{ns}
Genotype (G)	26	336.40 *	225.57 *	15.15**	13.76 ^{ns}	7.54 ^{ns}	7.92 *	15.97 *	136.00 *	664.10 ^{ns}	17.97 *	0.14 ^{ns}
Water regime (WC)	1	2231.00 **	75.84 ^{ns}	77.13 **	13.18 ^{ns}	10.93 ^{ns}	16.56 *	229.47 **	578.52 ^{ns}	4736.10 *	82.41 *	0.04 ^{ns}
G × WC	25	234.60 ^{ns}	89.58 *	6.82 *	10.15 ^{ns}	6.99 ^{ns}	4.43 ^{ns}	12.01 *	55.27 ^{ns}	714.90 ^{ns}	8.91 ^{ns}	0.07 ^{ns}
Residual	49	139.80	48.05	3.96	11.88	8.26	3.98	6.96	75.76	429.40	10.19	0.09

S.O.V: source of variation, PH: plant height, DTM: days to maturity, FPL: fresh pod length, DPL: dry pod length, DPW: dry pod weight, NPPP: number of pods per plant, PYPP: pod yield per plant, AGB: above-ground biomass, HI: harvest index, RW: root weight, RSR: root: shoot ratio, * significant at 5% level of significance, ** significant at 1% level of significance, ^{ns} non-significant.

3.3.5.1 Performance of okra accessions for phenotypic traits under drought-stressed and non-stressed conditions

Phenotypic traits provide useful selection criteria for genotype selection and breeding. Mean values of phenotypic traits recorded among the tested 26 okra accessions evaluated under DS and NS treatments are presented in Table 3.5. The present study revealed a significant genotype × water regime effect interaction for several traits, including days to maturity, fresh pod length, and fresh pod yield. This allowed the identification and selection of ideal accessions suited for irrigated and drought-prone environments. Highly significant genotypic differences ($p < 0.001$) were observed for PH under NS conditions. Plant height is an important agronomic trait that reflects the vegetative growth behaviour of crop plants in response to drought-stressed conditions. In the present study, drought stress reduced plant height (Table 3.5), with accessions LS01, LS02, LS08, LS09, LS11, LS13, and LS18 being the tallest under DS conditions. According to Eshiet and Brisibe [34], the height of an okra plant can potentially affect yield, as taller plants are more prone to lodging, thus resulting in a reduced number of pods and yield. Taller plant height was observed for accessions LS01, LS02, LS08, LS09, LS11, LS13, and LS18 under DS than NS that could be attributed to higher biomass portioning in okra as a means for higher yield and drought tolerance [35]. Days to maturity is an important trait in

evaluating and selecting drought-tolerant okra genotypes. Under water-limited conditions, plants synthesize phytohormones, which synchronize the transition from vegetative to reproductive phases. Hence, the synthesis allows plants to regulate flowering, reproduction, and maturity periods [36]. Accessions LS01, LS03, LS06, LS08, and LS13 were early maturing under DS conditions (<85 days to maturity). Early maturing accessions could be selected as parents when breeding for drought escape through early maturity. Significant ($p < 0.05$) genotypic differences were recorded for FPL under both DS and NS conditions. Accessions LS06, LS07, LS10 and LS21 recorded the highest FPL (>8 cm) under DS conditions, whereas accessions LS10 and LS21 had the highest FLP (>10 cm) under NS conditions. Dry weight showed a reduction from 2.58 to 1.92 g under DS conditions. Chaturvedi et al. [9] reported that the reduction in dry weight is associated with the suppression of cell expansion and cell growth due to lower turgor pressure that occurs when plants are experiencing water shortages. Another record of reduced plant dry weight under drought stress was reported by Komolafe et al. [3]. Significant ($p < 0.05$) genotypic differences were recorded for NPPP under DS conditions. Accessions LS03, LS04, LS11, LS12, LS15, LS17, LS18, and LS21 recorded the highest NPPP (≥ 5), while LS13, LS19, and LS25 recorded the lowest NPPP (< 2) under DS conditions. Drought stress reduced NPPP in okra accessions due to the disturbance in photosynthesis and low carbohydrate production caused by limited water availability [37].

Significant ($p <0.05$) genotypic differences were produced for PYPP under DS conditions. Accessions LS05, LS07, LS10 and LS11 recorded the highest PYPP (≥6 g). Under drought stress conditions, okra plants can accumulate sufficient photo-assimilates, resulting in higher YPPP [9]. In addition, Komolafe et al. [3], reported that pod yield could be improved with selection of a higher number of pods per plant and heavier pods as breeding parents. Genotypes LS19, LS20, and LS25 recorded the lowest PYPP (<1 g) under DS conditions. The reduction in PYPP in DS is attributed to low water availability, which reduces cell division, resulting in lower dry matter and pod yield [37]. Significant ($p < 0.05$) genotypic differences were recorded for AGB under both DS and NS conditions. There were significant ($p < 0.05$) differences among accessions for HI under NS conditions only. The highest HI (>70%) was observed for accessions LS12, LS21, and LS26, whereas the lowest HI (<10%) was recorded for LS08 and LS19 under NS conditions. Non-significant differences were recorded for RW under both DS and NS conditions. Significant ($p < 0.05$) genotypic differences were recorded for RSR under DS condition. Genotype LS19 recorded the highest RSR (>1) compared to all other test accessions under DS conditions. Under water-limited conditions, the productivity of a plant

depends on some essential processes, such as temporal biomass distribution and dry matter partitioning [9]. Hence, the high fresh and dry weight of plants under restricted water supply is desirable and relates to high conversion efficiency. In the present study, accessions LS08, LS10, LS17, and LS23 produced higher biomass . Selecting parents with high biomass expression can help improve genetic gains. Based on this study, it can be indicated that reductions in most studied traits were highly associated with drought stress. These traits can effectively assess the drought tolerance potential of okra accessions and genotype variability for the studied traits and can be used to improve okra through selection.

Table 3.5: Mean values for phenotypic traits among 26 okra accessions evaluated under drought-stressed (DS) and non-stressed (NS) conditions

Accession Code	PH (cm)		DTM		FPL (cm)		DPL (cm)		DPW (g Per Plant)		NPPP		PYPP (g Per Plant)		AGB (g Per Plant)		HI (%)		RW (g Per Plant)		RSR	
	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS
LS01	71.50	64.25	80.25	95.17	3.92	7.96	11.59	7.78	6.50	2.00	3.50	8.00	3.92	7.02	13.92	13.02	17.15	54.91	5.25	7.75	0.58	0.59
LS02	65.37	61.62	101.00	95.67	5.19	7.13	9.13	1.75	4.00	0.00	3.50	3.50	2.58	7.83	7.08	17.83	36.67	44.47	6.00	5.25	0.83	0.30
LS03	60.50	68.38	83.00	83.50	3.03	5.96	5.15	6.83	3.50	3.50	8.00	7.00	2.50	8.79	17.50	17.29	13.96	51.86	5.25	6.25	0.33	0.37
LS04	49.87	52.12	86.25	86.75	6.21	7.50	8.29	8.54	1.00	2.50	6.00	5.50	4.19	6.09	9.19	12.09	47.52	56.69	0.75	3.00	0.09	0.29
LS05	54.50	72.50	89.00	80.25	5.73	7.25	6.50	7.08	2.00	8.00	4.00	5.50	6.17	7.33	16.17	18.33	38.30	44.78	5.25	3.75	0.3 2	0.27
LS06	61.50	82.88	77.00	77.00	8.23	9.06	4.50	8.85	2.50	5.50	4.00	5.00	5.05	8.00	16.55	29.50	62.42	30.64	4.00	7.50	0.50	0.25
LS07	58.38	70.38	86.25	89.00	8.58	4.96	7.60	9.00	3.00	6.50	4.00	3.50	7.92	2.92	16.92	14.92	65.16	29.01	5.00	4.50	0.34	0.37
LS08	73.50	64.00	77.00	95.00	6.00	1.50	3.50	4.67	1.50	1.00	4.50	2.50	6.58	2.63	21.58	17.12	41.57	8.97	8.75	12.25	0.49	0.96
LS09	82.25	69.75	89.67	83.50	3.80	7.83	7.50	4.21	1.00	1.00	3.00	3.00	4.60	7.17	17.10	16.67	21.83	48.07	10.50	7.75	0.85	0.50
LS10	68.75	88.25	95.17	90.00	10.17	11.50	0.00	8.38	0.00	1.50	3.00	3.50	14.00	13.25	32.00	37.75	43.75	35.80	4.00	7.25	0.13	0.20
LS11	79.00	66.50	95.50	86.00	6.51	9.75	8.33	3.63	3.00	0.00	6.00	4.00	6.76	9.23	16.76	19.23	40.34	47.33	7.75	9.00	0.46	0.47
LS12	65.25	82.38	101.00	83.17	3.99	5.51	3.75	5.08	2.50	4.00	6.50	6.50	2.85	7.68	19.85	21.68	19.12	46.84	5.00	7.25	0.33	0.40
LS13	74.25	49.75	80.25	98.25	2.50	7.46	5.71	6.19	2.50	0.50	1.00	5.50	2.00	6.13	17.00	9.13	10.98	79.55	6.50	4.00	0.40	0.48
LS14	65.50	87.25	86.42	83.17	5.93	8.09	6.44	7.38	0.50	3.00	4.50	6.00	4.48	8.56	18.97	23.06	23.56	60.68	8.00	8.25	0.42	0.49
LS15	52.50	57.88	89.00	92.42	6.71	5.47	6.40	6.40	1.50	1.50	5.00	6.00	4.71	4.82	10.71	8.32	47.71	57.66	3.50	3.75	0.40	0.45
LS16	53.50	78.12	95.17	92.25	3.95	7.75	7.40	6.92	3.50	3.00	2.50	4.50	2.63	11.55	13.62	24.55	18.99	47.05	4.25	8.75	0.29	0.36
LS17	67.00	79.00	98.00	83.50	4.23	3.88	4.75	8.08	1.00	5.00	5.50	3.50	3.69	6.00	21.19	20.00	23.54	30.08	7.00	9.25	0.49	0.46
LS18	83.62	75.00	98.25	86.75	7.21	7.33	7.75	10.38	1.50	1.50	6.00	3.50	5.42	6.10	16.42	25.10	33.94	24.68	10.75	6.25	0.74	0.25
LS19	54.12	72.00	95.50	83.50	1.00	0.00	4.00	5.25	0.50	0.00	1.00	1.00	0.50	0.00	6.50	12.50	16.67	0.00	5.50	9.25	1.43	0.73
LS20	60.25	67.12	95.16	89.67	1.81	8.46	1.88	7.63	0.00	2.50	2.50	4.00	0.75	8.08	11.25	18.08	7.89	48.95	4.50	6.25	0.45	0.42
LS21	54.12	66.25	92.17	92.25	8.50	10.08	7.58	7.58	4.00	1.50	6.00	6.00	4.17	9.58	10.17	12.08	41.96	84.21	2.00	2.75	0.27	0.24
LS22	63.50	119.25	89.67	92.75	5.52	8.04	5.83	7.63	0.00	6.00	3.00	6.00	1.75	11.44	10.75	37.94	12.96	30.57	6.25	11.75	0.62	0.31
LS23	69.75	86.75	92.17	89.50	4.94	7.29	6.13	8.79	2.50	5.00	2.50	7.00	5.88	8.00	20.87	34.50	30.75	23.72	6.50	10.00	0.35	0.28
LS24	59.12	86.25	95.17	77.00	4.83	5.31	1.50	3.38	0.00	0.50	3.00	2.50	4.17	6.88	17.17	25.87	27.86	19.23	1.00	9.50	0.11	0.42
LS25	59.62	83.62	101.00	90.00	0.00	6.92	0.00	3.50	0.00	0.00	1.00	3.50	0.00	7.04	12.50	26.54	0.00	35.18	1.50	10.75	0.04	0.41
LS26	46.12	52.62	86.75	83.50	4.67	5.63	5.45	3.05	2.00	1.50	2.00	6.00	4.00	5.24	6.00	7.24	33.33	82.28	1.75	2.50	0.15	0.22
Mean	63.59	73.23	90.61	87.67	5.12	6.83	5.64	6.46	1.92	2.58	3.90	4.71	4.28	7.21	15.30	20.01	29.92	43.20	5.25	7.10	0.44	0.40
p –value	ns	**	*	ns	*	*	ns	ns	*	*	*	ns	*	ns	*	*	ns	*	ns	ns	*	ns
SED	11.73	12.84	6.29	7.49	1.73	2.13	3.19	3.77	2.41	3.25	1.85	2.17	2.29	2.95	7.63	9.81	21.16	20.58	3.10	3.29	0.37	0.17
LSD (5%)	34.24	26.44	12.96	15.97	5.05	4.39	9.32	7.77	7.05	6.69	5.41	4.48	6.68	6.08	22.26	20.21	43.67	42.38	9.04	6.77	1.09	0.35
CV (%)	18.48	17.53	6.94	8.54	34.77	31.22	55.83	58.42	65.12	56.10	47.68	46.12	56.79	40.93	50.20	49.02	69.23	47.63	58.87	46.35	63.74	42.66

PH: plant height, DTM: days to maturity, FPL: fresh pod length, DPL: dry pod length, DPW: dry pod weight, NPPP: number of pods per plant, PYPP: pod yield per plant, AGB: above-ground biomass, HI: harvest index. RW: root weight, RSR: root: shoot ratio; NS: non-stressed, DS: drought-stressed, SED: standard deviation, LSD: least significant different, CV: coefficient of variation, * at 5% level of significance, ** significant at 1% level of significance, ^{ns} non-significant.

3.3.5.2 Associations among phenotypic traits under drought-stressed and non-stressed conditions

Understanding the associations between phenotypic traits provides a useful guide for the selection and improvement of desired traits. The levels of associations of the assessed phenotypic traits among accessions under DS and NS conditions in a GH environment are presented in Table 3.6. Correlation analysis provides a measure of associations among traits for effective selection. The poor associations recorded between DTM with DPL and NPPP suggest small trade-offs in pod yield. The high correlations between PYPP and DTM under drought stress suggest large trade-offs in yield responses. Under DS conditions, significant and positive associations were observed between PYPP with FPL ($r = 0.81$, $p \leq 0.001$). AGB had higher significant association with PYPP ($r = 0.69$, $p \leq 0.001$). HI positively and significantly correlated with FPL ($r = 0.85$, $p \leq 0.001$) and PYPP ($r = 0.67$, $p \leq 0.001$) under DS conditions, indicating that harvest index has a direct influence on pod yield. According to Kyriakopoulou et al. [10], crops under water-limited conditions show a significantly reduced harvest index. This is attributable to reduced photosynthesis, which could change pod yield. Positive and significant correlations were observed between RW and PH ($r = 0.81$, $p \leq 0.001$). RSR significantly and positively correlated with DPW ($r = 0.58$, $p \leq 0.001$) under DS conditions.

FPL positively and significantly correlated with PYPP ($r = 0.83$, $p \leq 0.001$) and HI ($r = 0.85$, $p \leq 0.001$) under NS condition. A positive and significant correlation between FPL and PYPP has been reported in okra [19,37], indicating that fresh pod length is vital for direct selection to improve the fresh pod yield in okra. Positive and highly significant correlations were recorded between AGB with PH ($r = 0.88$, $p \leq 0.001$) and PYPP ($r = 0.60$, $p \leq 0.001$) under NS conditions. There was a high positive association between above-ground biomass and plant height under drought stress conditions. This indicates that drought stress has a maximum impact on plant height due to the declined cell enlargement and cell growth due to low turgor pressure and more leaf senescence. Hence, more leaf senescence and reduced photosynthesis result in low biomass production in crops grown under water-limited conditions [7,12]. A suppression in dry biomass production in response to abiotic stress has been reported by Kaur et al. [12]. RW was positively and significantly correlated with PH ($r = 0.70$, $p \leq 0.001$) and AGB ($r = 0.62$, $p \leq 0.001$) and negatively correlated with HI ($r = -0.69$, $p \leq 0.001$). RSR was negatively and significantly correlated with FPL ($r = -0.56$, $p \leq 0.001$) and PYPP ($r = -0.57$, $p \leq 0.001$) but positively and significantly correlated with RW ($r = 0.49$, $p \leq 0.05$). The strong

associations between the assessed phenotypic traits in the present study allow effective genotype selection and genetic advancement.

Table 3.6: Pearson correlation coefficients showing the magnitude of associations of phenotypic traits among okra accessions under drought-stressed (upper diagonal) and non-stressed (lower diagonal) conditions

Traits	PH	DTM	FPL	DPL	DPW	NPPP	PYPP	AGB	HI	RW	RSR
PH		0.01 ^{ns}	0.06 ^{ns}	0.14 ^{ns}	0.07 ^{ns}	0.10 ^{ns}	0.24 ^{ns}	0.52 **	−0.19 ^{ns}	0.81 **	0.32 ^{ns}
DTM	−0.22 ^{ns}		−0.20 ^{ns}	−0.24 ^{ns}	−0.27 ^{ns}	−0.05 ^{ns}	0.52 **	−0.06 ^{ns}	−0.29 ^{ns}	−0.05 ^{ns}	0.07 ^{ns}
FPL	0.16 ^{ns}	0.12 ^{ns}		0.21 ^{ns}	0.12 ^{ns}	0.43 *	0.81 **	0.36 ^{ns}	0.85 **	0.03 ^{ns}	−0.23 ^{ns}
DPL	0.24 ^{ns}	0.03 ^{ns}	0.26 ^{ns}		0.71 **	0.30 ^{ns}	−0.03 ^{ns}	−0.35 ^{ns}	0.25 ^{ns}	0.29 ^{ns}	0.28 ^{ns}
DPW	0.41 *	−0.29 ^{ns}	0.06 ^{ns}	0.58 **		0.27 ^{ns}	0.04 ^{ns}	−0.14 ^{ns}	0.17 ^{ns}	0.03 ^{ns}	0.05 ^{ns}
NPPP	−0.03 ^{ns}	0.15 ^{ns}	0.39 **	0.24 ^{ns}	0.38 ^{ns}		0.26 ^{ns}	0.19 ^{ns}	0.34 ^{ns}	0.14 ^{ns}	−0.15 ^{ns}
PYPP	0.46 **	0.08 ^{ns}	0.83 **	0.16 ^{ns}	0.15 ^{ns}	0.37 ^{ns}		0.69 **	0.67 **	0.13 ^{ns}	−0.27 ^{ns}
AGB	0.88 **	−0.14 ^{ns}	0.36 ^{ns}	0.27 ^{ns}	0.30 ^{ns}	−0.06 ^{ns}	0.60 **		0.13 ^{ns}	0.34 ^{ns}	0.27 ^{ns}
HI	−0.47 *	0.22 ^{ns}	0.50 **	−0.07 ^{ns}	−0.11 ^{ns}	0.61 **	0.31 ^{ns}	−0.48 *		−0.09 ^{ns}	−0.13 ^{ns}
RW	0.70 **	−0.02 ^{ns}	−0.24 ^{ns}	−0.12 ^{ns}	−0.05 ^{ns}	−0.32 ^{ns}	−0.08 ^{ns}	0.62 **	−0.69 **		0.58 **
RSR	−0.18 ^{ns}	0.20 ^{ns}	−0.65 **	−0.29 ^{ns}	−0.33 ^{ns}	−0.35 ^{ns}	−0.57 **	−0.31 ^{ns}	−0.37 ^{ns}	0.49 *	

PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPL: dry pod length, DPW: dry pod weight, NPPP: number of pods per plant, PYPP: yield per plant, AGB: above-ground biomass, HI: harvest index, RW: root weight, RSR: root: shoot weight, * significant at 5% level of significance, ** significant at 1% level of significance, ^{ns} non-significant.

3.3.5.3 Principal component analysis (PCA)

PCA showing the loading scores and cumulative variations for phenotypic traits under DS and NS conditions are presented in Table 3.7. PCA is the most frequently used multivariate statistical analysis [21]. Three and four principal components (PCs) were identified for assessed traits under DS and NS conditions, accounting for a cumulative variance of 70.07% and 85.34%, respectively. Under DS conditions, PC1 was positively correlated with FPL, NPPP, PYPP, and HI, which accounted for 29.69% of the total variation. The results indicated that the tested okra accessions were genetically diverse. PH, RW, and RSR were positively correlated with PC2, accounting for 21.64% of the total variation under DS conditions. DPL and DPW were negatively correlated while AGB was positively associated with PC3, which accounted for 19.37% of the total variation. Under NS conditions, PC1 was positively associated with FPL, DPL, NPPP, and PYPP and negatively correlated with RSR, which accounted for 32.24%

of the total variation. PC2 was positively associated with PH, AGB, and RW and negatively correlated with HI, which accounted for 28.99% of the total variation among the test accessions. PC3 was negatively correlated with DPW, while PC4 was positively associated with DTM, accounting for 14.22% and 9.89% of the total variation, respectively. The current PCA results successfully identified variables that contribute most to the response of okra accessions against drought stress.

Table 3.7: Principal component loading scores explained and cumulative variances of phenotypic traits among 26 okra accessions under drought-stressed and non-stressed conditions

Traits	Drought-Stressed			Non-Stressed			
	PC1	PC2	PC3	PC1	PC2	PC3	PC4
PH	0.32	**0.74**	0.46	0.55	**0.76**	0.05	0.01
DTM	−0.33	−0.03	0.32	−0.09	−0.27	0.48	**0.74**
FPL	**0.89**	−0.23	−0.07	**0.79**	−0.31	0.37	−0.09
DPL	0.29	0.50	**−0.73**	**0.51**	0.05	−0.53	0.41
DPW	0.28	0.30	**−0.69**	0.53	0.17	**−0.71**	0.17
NPPP	**0.53**	0.07	−0.22	**0.53**	−0.49	−0.15	0.37
PYPP	**0.87**	−0.20	0.30	**0.85**	−0.01	0.46	−0.05
AGB	0.58	0.01	**0.70**	0.64	**0.71**	0.19	−0.02
HI	**0.78**	−0.29	−0.27	0.24	**−0.90**	0.16	−0.01
RW	0.26	**0.87**	0.30	−0.06	**0.88**	0.31	0.21
RSR	−0.20	**0.74**	−0.07	**−0.76**	0.30	0.10	0.39
Explained variance (eigenvalue)	3.27	2.38	2.13	3.55	3.19	1.56	1.09
Proportion of total variance (%)	29.69	21.64	19.37	32.24	28.99	14.22	9.89
Cumulative variance (%)	29.69	51.33	70.70	32.24	61.23	75.45	85.34

PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPL: dry pod length, DPW: dry pod weight, NPPP: number of pods per plant, PYPP: pod yield per plant, AGB: above-ground biomass, HI: harvest index, RW: root weight, RSR: root: shoot ratio, PCs with ≥ 0.5 loading scores are boldfaced.

The relationship between the accessions and the studied phenotypic traits is illustrated using principal component biplots (Figure 3.4). Angles less than 45 ^{0}C between the dimensions of two variables indicate high trait associations, whereas longer vectors show the discriminating ability of a particular trait. As a result, accessions excelling in a particular trait were plotted to the vector line. Accessions LS17 and LS02 were grouped together based on the high values for RSR under DS conditions. Under NS conditions, accessions LS14, LS16, LS18, LS23, and LS01 were grouped together based on high values of PH, FPL, DPW, DPL, NPPP, and PYPP.

Accessions LS21, LS01, LS12, LS14, and LS09 were grouped together based on high values of PH, FPL, DPW, DPL, NPPP, and PYPP under DS conditions. Accessions LS17, LS12, LS09, LS19, and LS09 were grouped together based on the high values of PH, FPL, DPW, DPL, NPPP, and PYPP under NS conditions.

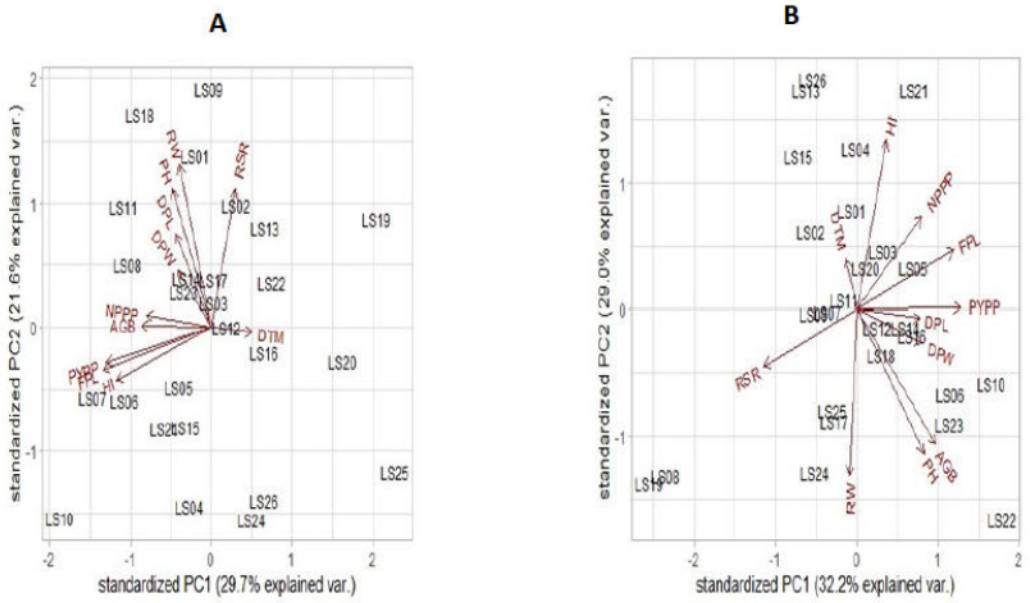

Figure 3.4: Principal component biplot of PC1 vs. PC2 showing groupings of 26 okra accessions based on phenotypic traits under drought-stressed (**A**) and non-stressed (**B**) conditions. PH: plant height, DTM: number of days to maturity, FPL: fresh pod length, DPL: dry pod length, DPW: dry pod weight, NPPP: number of pods per plant, PYPP: pod yield per plant, AGB: above-ground biomass, HI: harvest index, RW: root weight, RSR: root: shoot ratio.

3.3.5.4 Phenotypic hierarchical clustering

Hierarchical cluster analysis using phenotypic data allocated the okra accessions into three groups under drought-stressed conditions (Figure 3.5). The largest cluster (cluster I) consisted of 11 accessions, followed by cluster III with 8 accessions and cluster I with 7 accessions. High-yielding accessions (e.g., LS05 and LS07) were grouped in cluster II. Cluster I consisted of accessions which were characterized by taller plant height and with the highest number of pods per plant. Cluster III contained accessions with low pod yield. Accessions LS10 and LS18, which were grouped in cluster I under drought-stressed conditions, can be selected to develop breeding populations for enhanced pod yield. This cluster also contained taller accessions, which usually have higher biomass than shorter plants and contribute to carbon sequestration for better soil health [37]. The test accessions were also grouped into three clusters under non-

stressed conditions (Figure 3.6). The largest cluster (cluster I) contained 12 accessions, while the second largest cluster (cluster II) contained 8 accessions, and the smallest cluster III consisted of only 6 accessions. Cluster I comprised accessions with a higher number of pods, whereas cluster III had accessions with higher harvest index and early maturity, critical attributes for drought escape due to accelerated growth and development. Similar results were reported by [18] in a study of genetic divergence and population structure of okra through SSR markers. Three main clusters with four subclusters were observed, suggesting most of the studied accessions were unique and isolated.

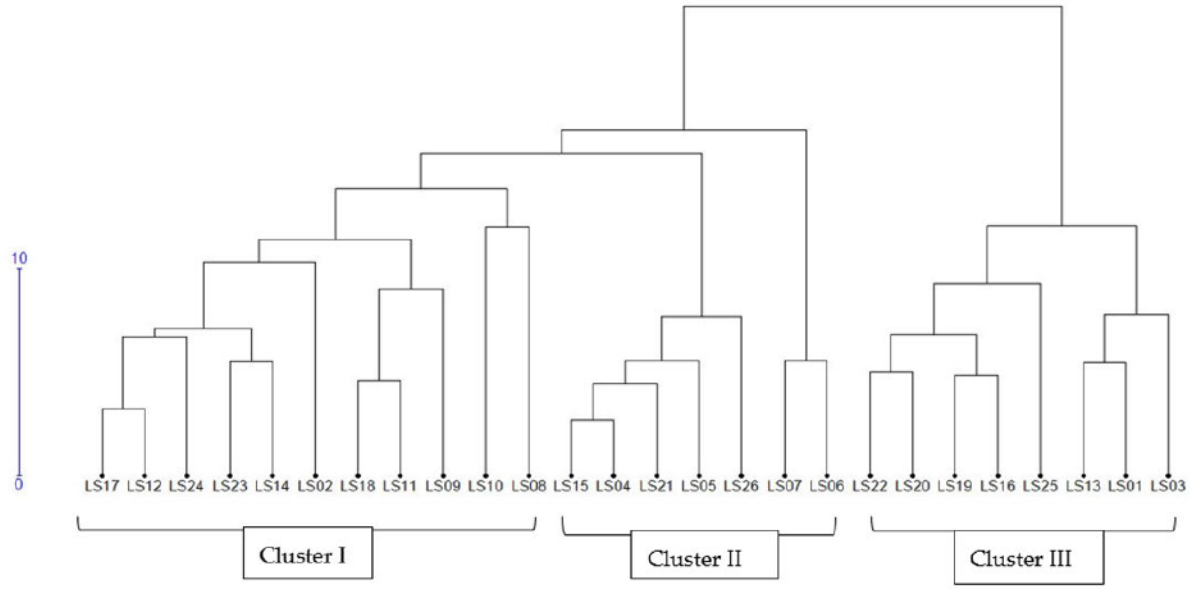

Figure 3.5: Hierarchical clustering of 26 okra accessions based on phenotypic traits evaluated under drought-stressed conditions.

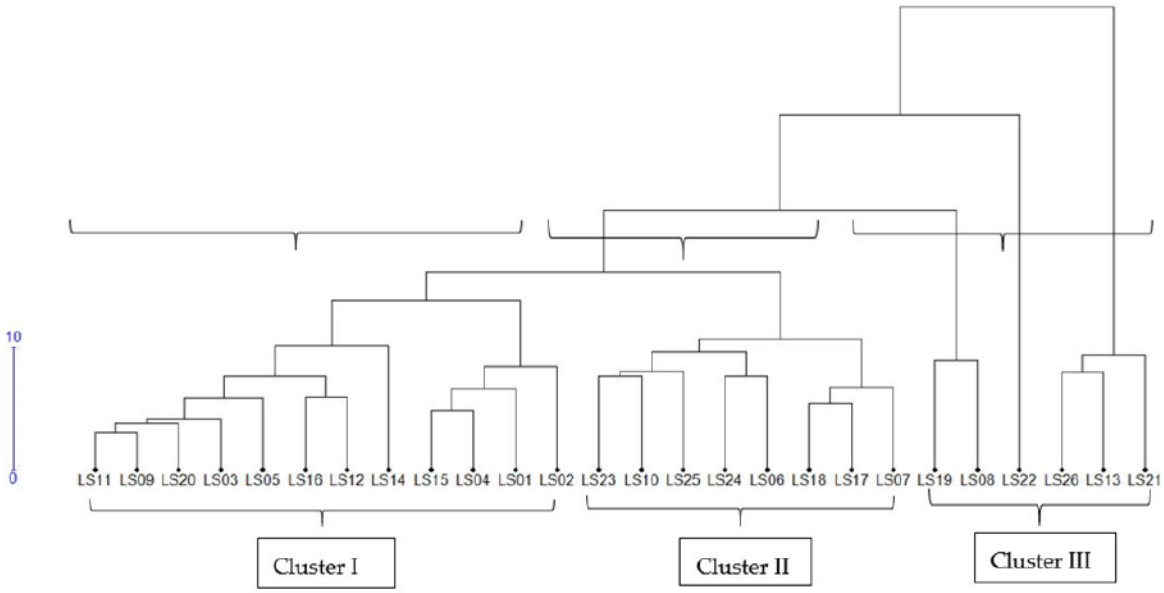

Figure 3.6: Hierarchical clustering of 26 okra accessions based on phenotypic traits under non-stressed conditions.

3.3.6 Comparison of phenotypic and genotypic hierarchical clusters

Genetic markers have proven to be a powerful tool for assessing genetic variation and elucidating genetic relationships within and among okra species, while phenotypic traits are essential indicators of genotypes in a given environment. A comparison of phenotypic and genotypic clusters was conducted to establish genotype compatibility among different dendrograms. None of the accessions maintained their positions when phenotypic hierarchical clusters were compared to genotypic hierarchical clustering under drought-stressed conditions (Figure 3.7). Similarly, under non-stressed conditions (Figure 3.8), the phenotypic clustering was opposite to the genotypic cluster. The tanglegram comparison indicated that 42% of the accessions under drought-stressed conditions maintained their cluster membership in the phenotypic and genotypic hierarchical clustering (Figure 3.7). Under non-stressed conditions, 69% of the accessions maintained their membership in the phenotypic and genotypic hierarchical clustering (Figure 3.8). The phenotype and genotype clusters under drought and non-stressed conditions were inconsistent due to the genotype-by-environment interactions, resulting in variation in the phenotypic expression of the phenotypic traits [36]. Lower consistency in the phenotypic and genotypic clustering under drought-stressed conditions compared to non-stressed conditions is attributable to the selection pressure exerted by the drought treatment.

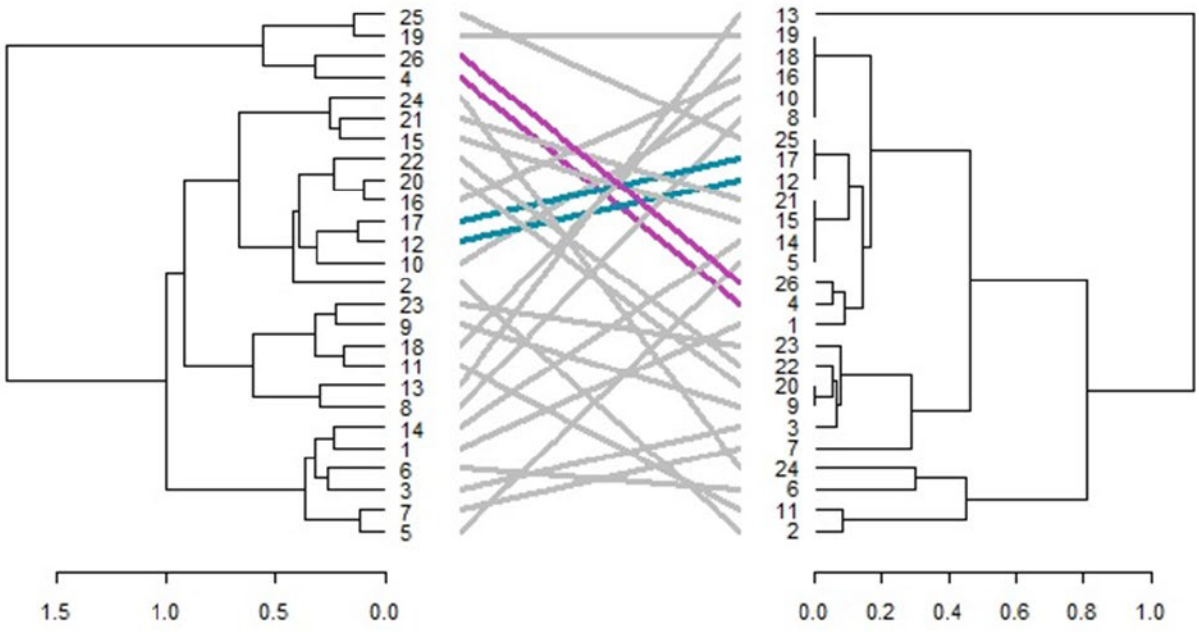

Figure 3.7: Tanglegram comparison of phenotypic and genotypic hierarchical clusters of 26 okra accessions based on 9 SSR markers and phenotypic data measured under drought-stressed conditions.

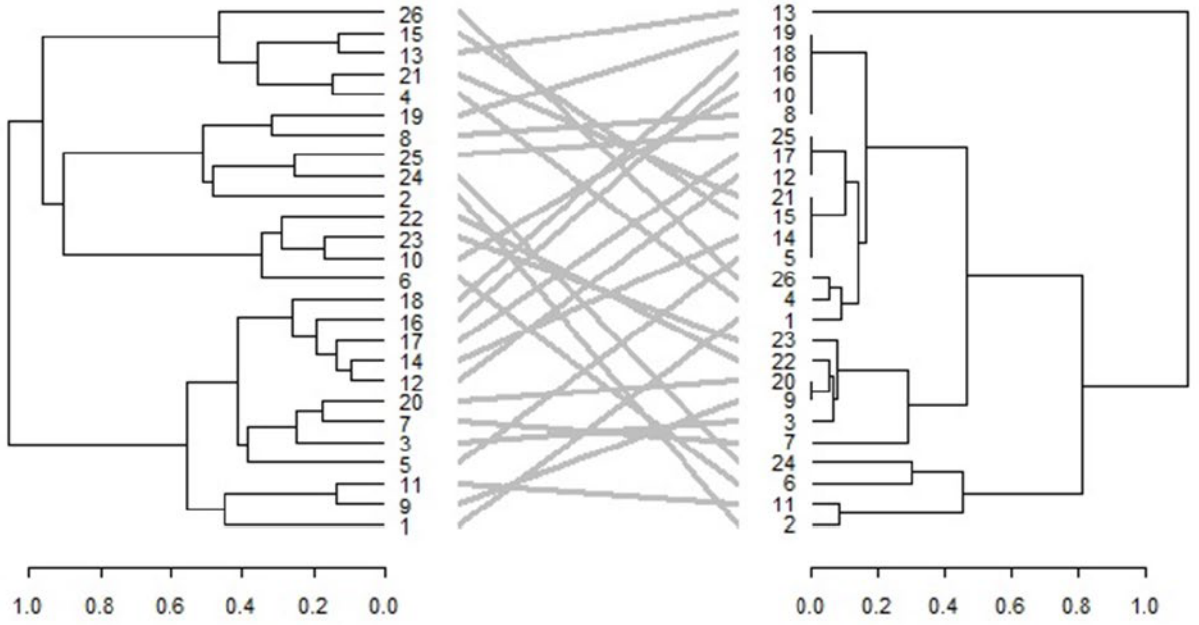

Figure 3.8: Tanglegram comparison of phenotypic and genotypic hierarchical clusters of 26 okra accessions based on 9 SSR markers and phenotypic data measured under non-stressed conditions.

3.4 Conclusions

The present study evaluated the genetic and phenotypic diversity and relationships among selected okra accessions as a guide for selecting parental accessions for breeding. SSR-assisted phenotypic and genotype evaluation and classification in the present study suggest sufficient genetic diversity in okra accessions to initiate a trait-based pre-breeding program. Genetically unrelated accessions such as LS04, LS05, LS06, LS07, LS08, LS10, LS11, LS15, LS18, LS23, LS24, and LS26 were selected based on their high yield potential and related yield-improving traits under drought stress conditions. The identified accessions are recommended as suitable breeding parents for hybridization and selection programs to improve the yield potential of okra under drought-stressed environments.

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Chapter 4. Drought tolerance assessment of okra (*Abelmoschus esculentus* [L.] Moench) accessions based on leaf gas exchange and chlorophyll fluorescence

Abstract

Physiological and complementary phenotypic traits are essential in the selection of drought-adapted crop genotypes. Understanding the physiological response of diverse okra genotypes under drought stress conditions is critical to the selection of drought-tolerant accessions for production or breeding. The objective of this study was to assess the levels of drought tolerance in preliminarily selected okra accessions based on leaf gas exchange and chlorophyll fluorescence to determine best-performing genotypes for drought-tolerance breeding. Twenty-six genetically diverse okra accessions were screened under non-stressed (NS) and drought-stressed (DS) conditions under a controlled glasshouse environment using a 13 × 2 alpha lattice design in three replicates, in two growing seasons. Data were subjected to statistical analyses using various procedures. A significant genotype × water condition interaction effect was recorded for transpiration rate (T), net CO_2 assimilation (A), intrinsic water use efficiency (WUE*i*), instantaneous water use efficiency (WUE*ins*), minimum fluorescence (*Fo′*), maximum fluorescence (*Fm′*), maximum quantum efficiency of photosystem II photochemistry (*Fv′/Fm′*), the effective quantum efficiency of *PSII* photochemistry (*ϕPSII)*, photochemical quenching (*qP*), nonphotochemical quenching (*qN*) and relative measure of electron transport to oxygen molecules (ETR/A). The results suggested variable drought tolerance of the studied okra accessions for selection. Seven principal components (PCs) contributing to 82% of the total variation for assessed physiological traits were identified under DS conditions. Leaf gas exchange parameters, T, A and WUE*i*, and chlorophyll fluorescence parameters such as the *ϕPSII*, *Fv′/Fm′*, *qP*, *qN*, ETR and ETR/A had high loading scores and correlated with WUE*i*, the *ϕPSII*, *qP* and ETR under DS conditions. The study found that optimal gas exchange and photoprotection enhance drought adaptation in the assessed okra genotypes and tested water regimes. Using the physiological variables, the study identified drought-tolerant accessions, namely LS05, LS06, LS07 and LS08 based on high A, T, *Fm′*, *Fv′/Fm′* and ETR, and LS10, LS11, LS18 and LS23 based on high *AES*, *Ci*, *Ci/Ca*, *WUEi*, *WUEins*, *ϕPSII* and AES. The selected genotypes are high-yielding (≥5 g/plant) under drought stress conditions and will complement phenotypic data and guide breeding for water-limited agro-ecologies.

Keywords: abiotic stress; chlorophyll fluorescence; drought tolerance; leaf gas exchange; physiological traits

4.1 Introduction

Okra (*Abelmoschus esculentus* [L.] Moench), belonging to the *Malvaceae* family, is an important crop mainly cultivated as pods , vegetables, and seed oil. It is extensively grown in tropic and subtropic regions [1] and arid and semi-arid regions with limited and erratic rainfall conditions [2]. The tender and immature pods of okra are consumed as cooked vegetables [3]. The pods are rich in protein content (25 %) and amino acids, notably lysine and tryptophan [4], fat, fibre, vitamins (A, C and K), vital mineral elements such as calcium, potassium, sodium, magnesium, iron, zinc and manganese [5], and soluble sugars such as sucrose (110.4 g/100 g FW), fructose (34.8 g/100 g FW and glucose (30.9 g/100 g FW [6]. In addition, minor quantities of organic acids, including citric, oxalic, and malic acid, are present in the succulent pods [6]. The mature and dry seeds are a vital source of edible oils. The seed oil content ranges from 20–40%, consisting of the following major fatty acids: linoleic, palmitic, oleic, diacylglycerols and triacylglycerols acids [7].

Continental Asia accounts for a total annual okra production of 6 million tons from 592,375 million hectares of cultivated land, whereas Africa is the second major producer, with 3 million tons per annum from approximately 1.9 million ha of cultivated land [8]. Commercial and small-scale farmers produce okra. In sub-Saharan Africa (SSA), the crop is mainly grown in marginal conditions characterised by low and erratic rainfall, with minimal agricultural inputs and production technologies. In SSA, okra is mainly cultivated under rainfed conditions, and these agro-ecologies face moderate to severe droughts during the growing season [9]. Drought stress significantly reduces growth, biomass, and yield [10]. Drought alone accounts for yield losses ranging between 30 and 100% in okra, primarily when the stress occurs during the flowering and pod-filling stages [3]. Breeding okra cultivars with drought adaptation is the major objective in improvement programs. Physiological and complementary phenotypic traits are critical in the selection of drought-adapted crop genotypes.

Phenotyping of plants using gas exchange and chlorophyll fluorescence traits has been reported as a preferred approach for selecting drought-tolerant okra accessions [11]. Some gaseous exchange traits used to assess drought tolerance include photosynthesis rate, stomatal conductance, chlorophyll content and transpiration rate. Further, chlorophyll fluorescence parameters (e.g., minimum fluorescence, maximum fluorescence, effective quantum efficiency of *PSII* photochemistry, photochemical quenching and non-photochemical quenching) have been used in phenotyping for drought tolerance [11-13]. Drought stress affects okra growth and

productivity, disrupting physiological functions and the photosynthetic rate, resulting in yield losses [11,13,14]. Mkhabela et al. [3] reported that okra yield loss under drought stress could be significantly minimized by breeding drought-tolerant ideotypes with intrinsic water use efficiency. Hence, understanding the physiological response of diverse okra genotypes under drought stress conditions is essential for the selection of drought-tolerant accessions for production or breeding.

There has been limited progress in the breeding of okra for drought tolerance. This could be due to limited accessions identified as sources of drought and heat tolerance and insect and disease resistance genes [15]. Some unique accessions, including Sabz Pari [16], NHAe 47-4 [17], Pusa Sawari, Iraq P, Hala [1] and Xianzhi [18], were identified as useful sources of genes for enhancing drought tolerance under water-limited conditions. Compared to the highest genetic diversity reported in the cultivated okra [19], the identified accessions with tolerance to drought are relatively few. Therefore, there is a need for concerted research and development in okra to develop market-led and improved varieties for water-limited conditions.

In South Africa, okra is an important but under-researched and under-utilised crop. It is grown under rainfed conditions using local and unimproved accessions with poor adaptation and low yield potential. Genetically unique okra accessions could be sourced from different geographical regions to enhance okra pre-breeding programs [3]. Morphological traits associated with drought tolerance in okra include the number of pods per plant, fresh pod length, number of seeds per pod, hundred seed weight, number of branches per plant, plant height and total pod production [1,19]. Reportedly, a higher number of branches, pod length and number of pods per plant, plant height between 150 and 170 cm and pod weight have a direct influence on pod yield [19]. Drought tolerance assessment of okra accessions using the combination of morphological and physiological traits could increase the efficiency of identifying and selecting drought-tolerant accessions for cultivar development under dry environments. Therefore, this study aimed to assess the levels of drought tolerance in preliminarily selected okra accessions based on leaf gas exchange and chlorophyll fluorescence to determine best-performing genotypes for drought-tolerance breeding.

4.2 Materials and methods

4.2.1 Plant materials and study site

Twenty-five genetically distinct okra accessions were used for the study. The accessions were sourced from the Agricultural Research Council, Vegetable, Industrial and Medicinal Plants (ARC-VIMP) gene bank, and one local variety was included. The accessions were previously studied for their morphological responses to drought stress under field and glasshouse environments [3]. Detailed information on their geographical origin and drought resistance index are presented in Table 4.1. The experiment was conducted under glasshouse conditions at the Controlled Environment Facility (CEF) of the University of KwaZulu-Natal during the 2020/2021 and 2021/2022 growing seasons. The first experiment was conducted from September 2020 to December 2020, and the second from February 2021 to May 2021. The accessions were evaluated under non-stressed (NS) and drought-stressed (DS) conditions in the glasshouse environment. Drought tolerance index was calculated as DTI = (Ys/Yn)/(Ms/Mn), where Ys and Yn are the genotype yields under stress and non-stress, and Ms and Mn are the mean yields of the accessions under stressed and non-stressed conditions, respectively [20].

Table 4.1: Accession code, accession number, database, geographical origin, drought tolerance index and stem colour of the okra accessions evaluated in the study.

Accession Code	Accession Number	Database Name	Geographical Origin	DTI	Stem Colour
LS01	VI033775	ARC/South Africa	Malaysia	0.02	Red
LS02	VI033797	ARC/South Africa	Malaysia	1.16	Green
LS03	VI056457	ARC/South Africa	Yugoslavia	1.46	Red
LS04	VI039651	ARC/South Africa	Bangladesh	0.67	Green
LS05	VI046561	ARC/South Africa	Thailand	1.80	Red
LS06	VI047672	ARC/South Africa	Bangladesh	1.00	Green
LS07	VI050150	ARC/South Africa	Taiwan	0.13	Green
LS08	VI050957	ARC/South Africa	Zambia	0.04	Green
LS09	VI050960	ARC/South Africa	Zambia	0.31	Green
LS10	VI055110	ARC/South Africa	Malaysia	0.15	Red
LS11	VI055119	ARC/South Africa	Myanmar	0.73	Red
LS12	VI055219	ARC/South Africa	Malaysia	0.99	Red
LS13	VI055220	ARC/South Africa	Malaysia	4.67	Green
LS14	VI055421	ARC/South Africa	Viet Nam	1.02	Green
LS15	VI056069	ARC/South Africa	Cambodia	0.14	Red
LS16	VI056079	ARC/South Africa	Cambodia	3.15	Green
LS17	VI056081	ARC/South Africa	Cambodia	0.53	Red
LS18	VI056449	ARC/South Africa	United States of America	0.43	Red
LS19	VI060131	ARC/South Africa	Mali	0.00	Green
LS20	VI060313	ARC/South Africa	Tanzania	6.49	Green
LS21	VI060679	ARC/South Africa	India	0.61	Green
LS22	VI060803	ARC/South Africa	Turkey	8.64	Green
LS23	VI060817	ARC/South Africa	Brazil	0.45	Green
LS24	VI060822	ARC/South Africa	Nigeria	0.31	Green
LS25	VI060823	ARC/South Africa	Nigeria	0.00	Green
LS26	Clemson Spineless	ARC/South Africa	South Africa	0.23	Green

ARC = Agricultural Research Council, DTI = drought tolerance index.

4.2.2 Experimental Design and Crop Establishment

Five seeds were initially planted in 5 L capacity plastic pots filled with composted pine bark growing media. Later, two plants were established per pot for each genotype. The day and night temperatures in the greenhouse (GH) were 30 °C and 20 °C, respectively, and the relative humidity ranged between 45 and 55% during the study. Inorganic fertilizers consisting of nitrogen (N), phosphorus (P) and potassium (K) were applied at a rate of 120, 30 and 30 kg ha^{-1}, based on soil fertility recommendations using urea (46-0-0), phosphorus pentoxide (P_2O_5) and potassium oxide (P_2O), respectively.

The trials were established using a 13 × 2 alpha lattice design under drought-stressed and non-stressed conditions with three replications. Drought stress was imposed at 50% flowering until physiological maturity by withholding irrigation until the soil water content reached 30% field capacity for plants under DS. The duration of stress was seven days before sampling. Plants under NS conditions were irrigated regularly to maintain soil moisture content at field capacity until physiological maturity. To determine pod yield, plants reached maturity, and pods were harvested sequentially at the soft, most digestible, and immature stage. Tensiometers, moisture monitors (Spectrum Technologies, Inc, Chicago, IL, USA), were used to detect soil moisture levels at the root zone. Agronomic performance of the test genotypes was reported in Mkhabela et al. [19].

4.2.3 Data Collection

Gas exchange and chlorophyll fluorescence parameters were measured using an LI-6400 XT Portable Photosynthesis system (Licor Bioscience, Inc. Lincoln, NE, USA) integrated with an infrared gas analyser (IGRA) attached to a leaf chamber fluorometer (LCF) (640040B, 2 cm^2 leaf area, Licor Bioscience, Inc, Lincoln, NE, USA). External leaf CO_2 concentration (C_a) and artificial saturating photosynthetic active radiation (PAR) were set at 400 µmol mol^{-1} and 1000 µmol m^{-2} s^{-1}, respectively. Water flow rate and relative humidity were maintained at 500 µmol and 43%, respectively. The leaf-to-air vapour pressure deficit in the cuvette was maintained at 1.7 kPa to avoid stomatal closure due to low air humidity. Gas exchange and chlorophyll fluorescence measurements were taken on the third half fully formed leaf inside the sensor head. Under both NS and DS conditions, measurements were taken from five plants of each accession.

The following gas exchange parameters were determined: stomatal conductance (gs), net CO_2 assimilation rate (A), transpiration rate (T), intercellular CO_2 concentration (C_i) and the ratio of intercellular and ambient CO_2 (C_i/C_a) concentrations. The ratio of net CO_2 assimilation rate to intercellular CO_2 concentration (A/C_i) was computed according to Kitao et al. [21]. The ratio of A and gs was used to compute intrinsic water use efficiency [22] and the ratio of A and T was used to calculate instantaneous water use efficiency) [23].

To estimate chlorophyll fluorescence variables, a saturation flash intensity of 1300 µmol m^{-2} s^{-1} was applied. The following parameters were recorded. The minimum (Fo') and maximum fluorescence (F_m') of light-adapted leaves under natural glasshouse conditions. The steady-state fluorescence (F_s) was also determined in light-adapted photosynthesis. Equation (1) was used

to determine the variable fluorescence in light-adapted leaves, while Equation (2) calculated fluorescence changes [24].

$$Fv' = Fm' F_0' \quad (1)$$

$$\Delta F = Fm' - Fs \quad (2)$$

Additional chlorophyll fluorescence parameters were estimated according to Evans [25], Fv'/Fm', the maximum quantum efficiency of photosystem II photochemistry, the effective quantum efficiency of photosystem II photochemistry ($\phi PSII$), photochemical quenching (qP), non-photochemical quenching (qN) and electron transport rate (ETR). The ratio of ETR and A was used to calculate a relative measure of electron transport to oxygen molecules. The alternative electron sink (AES) was calculated as the ratio of photosystem II effective quantum efficiency to net CO_2 assimilation (A) [26]. Chlorophyll fluorescence was measured using a pulse-amplitude modulated (PAM) fluorometer, which applies a short pulse of light to the sample and measures the resulting fluorescence emitted by the chlorophyll. This measurement provided information on the photosynthetic efficiency and health of the crop. Gas exchange and chlorophyll fluorescence parameters were measured on fully expanded leaves. At the end of the second experiment, yield per plant (YPP) was determined by harvesting fresh pods when 50% of the pods were 3–5 cm long by hand every third day.

4.2.4 Statistical Analysis

Data were subjected to analysis of variance using Genstat 20th edition (VSN International, Hempstead, UK). The mean data for the two seasons were combined for analysis. Means were separated using Fisher's protected least significant difference (LSD) test at the 5% significance level. Pearson's correlation coefficients were calculated using IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL, USA) to determine the magnitude of the relationship among physiological traits. Principal component analysis (PCA) based on a correlation matrix was used to identify influential traits under NS and DS conditions using R Studio version 4.0, ggplot2 (R Core Team, 2018). Biplots were built using XLSTAT to determine relationships among the accessions and response variables (physiological traits). Principal component biplot diagrams were used to identify drought-tolerant and drought-susceptible okra accessions using XLSTAT. ClustVis (https://biit.cs.ut.ee/clustvis_large (accessed on 23 November 2022)) was used to visualise the heatmap analysis of physiological traits.

4.3 Results

4.3.1 Leaf gas exchange and chlorophyll fluorescence parameters in response to drought

The effects of genotype, water regime and interaction of genotype × water regime were significantly different for most evaluated traits of leaf gas exchange and chlorophyll fluorescence (Table 4.2). Drought stress significantly reduced gs, A and A/C*i* among the evaluated accessions (Tables 4.3 and 4.4). Accessions LS02, LS09, LS10, LS17, LS19 and LS26 recorded *gs* values of >0.3 mmol m^{-2} s^{-1} under NS conditions. Under DS, accessions LS04, LS11, LS13 and LS20 recorded *gs* values <0.1 µmol m^{-2} s^{-1}. Regarding T, accessions LS03, LS13, LS15, LS19, LS23 and LS24 recorded values ≥ 7.01 mmol H_2O m^{-2} s^{-1} under NS conditions, while, under DS conditions, genotypes LS01, LS03, LS04, LS08, LS09, LS11, LS12, LS14, LS19 and LS22 recorded T values ≤ 1.00 mmol H_2O m^{-1} s^{-1}. Under NS conditions, A values of ≥ 30 µmol CO_2 m^{-2} s^{-1} were observed from accessions LS08, LS10 and LS21, while values ≤ 20 µmol CO_2 m^{-2} s^{-1} were recorded for accessions LS03 and LS06.

. Okra genotypes LS02 and LS21 exhibited high A/C_i values of 0.23 and 0.28 µmol. mol^{-1}, respectively, under DS conditions compared to other accessions. Significant ($p < 0.05$) differences were observed in C_i/C_a values among accessions under both NS and DS conditions. Intrinsic water use efficiency and instantaneous water use efficiency were increased by drought stress (Table 4.4). Accessions LS13 and LS20 had the highest WUE_i under drought-stress conditions, with 1438.80 and 1256.10 µmol CO_2 m^{-2}, respectively. The highest WUE_{ins} values under drought stress were recorded for accessions LS04 (2164.70 $\mu mol \cdot mol^{-1}$) and LS22 (2161.00 $\mu molmol^{-1}$).

The effect of drought stress on chlorophyll fluorescence parameters among the tested okra accessions are highlighted in Table 4.2. Chlorophyll fluorescence parameters indicated significant differences for genotype, water regime and genotype x water regime interaction, showing that the evaluated genotypes responded differently under non-stress and drought-stress conditions. Non-significant differences were observed for *Fo′* under non-stress, while significant ($p < 0.001$) differences were recorded under drought-stress conditions (Tables 4.3 and 4.4). Genotypic variability ($p < 0.001$) with respect to F_m' was observed under non-stress and drought-stress conditions. Drought stress decreased *Fv′/Fm′*, from 0.51 under non-stressed to 0.35 under drought-stressed conditions. The $\phi PSII$ varied significantly among the tested genotypes under non-stress and drought-stress conditions. LS07, LS12 and LS19 revealed

considerably higher values for $\phi PSII \geq 0.40$ compared to other genotypes under non-stress conditions.

Photochemical quenching was significantly reduced from 0.32 to 0.13 by drought stress among the evaluated genotypes, of which LS04, LS12 and LS13 had the highest values of $qP > 0.40$. A variable genotypic response was observed with respect to *qN* under non-stress and drought-stress conditions. The mean for *qN* was higher under drought-stress (1.96) than non-stress conditions (1.39). The *qN* values ranged from 0.68 to 2.80 under non-stress (Table 4.3) and from 0.66 to 3.75 under drought-stress conditions (Table 4.4). LS02, LS03 and LS11 revealed *qN* values ≥ 2 under non-stress conditions. Genotypes LS01, LS02, LS10, LS11 and LS18 showed *qN* values ≥ 3 under drought-stress conditions. Non-significant differences were observed for ETR under non-stress conditions, while genotypic variation was observed for ETR under drought-stress conditions. LS08, LS09 and LS17 revealed the highest ETR value of ≥34,541 µmol e^{-1} m^{-1} s^{-1}, whereas LS16, LS22 and LS26 showed the lowest ETR ≤ 8071 under DS conditions. Drought stress significantly increased ETR/A (Table 4.4). The highest ETR/A (≥1542 µmol e µmol-1 CO_2) was recorded from LS03, LS08, LS09 and LS17 under drought-stress conditions. Drought stress significantly increased AES (154.72) compared to NS (26.98). AES ranged from 12.77 to 61.12 under non-stress and from 64.90 to 562.80 under drought-stress conditions. Yield per plant was significantly reduced, from 7.20 g/plant to 4.31 g/plant, by drought stress among the evaluated genotypes. Accessions LS11, LS19, LS21, LS22 and LS24 had the highest yield (>9 g/plant) under NS conditions, whereas LS05, LS06, LS07, LS08, LS10, LS11, LS18 and LS23 exhibited the highest yield (>5 g/plant) under DS conditions.

Table 4.2: Analysis of variance indicating mean squares and significant tests of leaf gas exchange and chlorophyll fluorescence parameters of 26 okra genotypes evaluated under non-stress and drought-stress conditions averaged across two seasons

Source of Variation	d.f.	Leaf Gas Exchange Parameters							
		gs	*T*	*A*	*Ci*	A/C_i	C_i/C_a	WUE_i	WUE_{ins}
Replications	1	0.07 *	3.19 ns	34.76 ns	806.541 *	0.03 ns	7.30 *	324.534 *	3675 ns
Incomplete Blocks	1	0.01 ns	1.92 ns	54.88 ns	15.807 ns	0.05 ns	1.48 ns	4185 ns	685 ns
Genotype (G)	25	0.13 **	13.45 **	65.50 ns	165,972 ns	0.06 ns	1.13 ns	140,060 *	652.347 **
Water Regime (WR)	1	0.38 **	75.62 **	448.16 **	1,830.530 *	0.04 ns	20.94 **	3.444.897 **	20.180.480 **
G × WR	25	0.01 ns	14.46 **	30.41 *	100.917 ns	0.06 ns	1.03 ns	140,099 *	644.150 **
Residual	50	0.11	4.33	31.35	174.990	0.07	1	56,471	205.739

Source of Variation	d.f.	Chlorophyll Fluorescence Parameters									YPP
		F_o'	F_m'	F_v'/F_m'	*ϕPSII*	*qP*	*qN*	ETR	ETR/A	AES	
Replications	1	8892 ns	32.989 ns	0.22 *	0.08 ns	0.11 ns	0.25 ns	3.72 ns	586.500 ns	2518 ns	1186.7*
Incomplete Blocks	1	8535 ns	468.996 *	0.02 ns	0.06 ns	0.24 *	1.69 *	1.40 ns	164.477 ns	15.882 ns	127.6 ns
Genotype (G)	25	28.927 **	292.297 *	0.17 *	0.17 **	0.25**	1.94 **	2.86 *	356.680 ns	14.198 ns	1023.2*
Water Regime (WR)	1	844.279 **	20.220.415 **	0.66 **	1.35 **	1.20 **	8.68 **	4.16 ns	101.913 **	424.290 **	6913.0**
G × W	25	19.264 *	472.144 **	0.05 *	0.16 **	0.20 **	1.69**	1.86 ns	301.433 *	12.027 ns	194.9*
Residual	50	9080	115.681	0.08	0.03	0.14	0.38	1.20	156.859	11,175	183.9

d.f.: degree of freedom, *gs*: stomatal conductance, *T*: transpiration rate, *A*: net CO_2 assimilation, *Ci*: intercellular CO_2 concentration, *A*/*Ci*: CO_2 assimilation rate/intercellular CO_2 concentration, *Ci*/*Ca*: ratio of intercellular and atmospheric CO_2, WUE_i: intrinsic water use efficiency, WUE_{ins}: instantaneous water use efficiency, F_0': minimum fluorescence, *Fm'*: maximum fluorescence, *Fv'*/*Fm'*: maximum quantum efficiency of photosystem II photochemistry, *ϕPSII*: the effective quantum efficiency of PSII photochemistry, *qP*: photochemical quenching, *qN*: non-photochemical quenching, ETR: electron transport rate, ETR/A: relative measure of electron transport to oxygen molecules, AES: alternative electron sinks, YPP: yield per plant, * and ** denote significance at 5 and 1% probability levels, respectively, ns: non-significant.

Table 4.3: Means of leaf gas exchange and chlorophyll fluorescence parameters of okra accessions under non-stressed conditions

Genotype	Leaf Gas Exchange Parameters								Chlorophyll Fluorescence Parameters								
	gs	T	A	*Ci*	A/*Ci*	*Ci*/*Ca*	WUE*i*	WUE*ins*	Fo′	Fm′	*Fv′/Fm′*	*ϕPSII*	*qP*	*qN*	ETR	ETR/A	AES	YPP
LS01	0.19	1.52	21.85	1.33	0.12	1.33	171.30	17.77	388.40	828.10	0.75	0.33	0.14	1.24	22026	1129.9	56.59	7.02
LS02	0.30	1.06	27.91	0.74	0.15	0.74	162.40	26.39	302.90	787.70	0.41	0.19	0.19	2.23	30068	1082.6	26.09	7.83
LS03	0.26	7.01	16.24	0.84	0.14	0.84	62.00	2.32	311.60	871.80	0.57	0.30	0.35	2.78	13186	801.6	17.98	8.79
LS04	0.24	2.52	24.93	0.78	0.17	0.78	102.20	10.58	168.80	860.00	0.28	0.29	0.48	0.75	21020	807.40	20.85	6.09
LS05	0.20	2.01	21.05	1.37	0.07	1.37	164.90	10.45	186.80	421.40	0.61	0.04	0.02	1.23	2815	137.10	33.70	7.33
LS06	0.23	6.56	26.95	0.68	0.15	0.68	195.90	4.18	179.70	252.3	0.37	0.37	0.25	0.74	8148	306.70	18.36	8.00
LS07	0.22	1.56	29.82	0.62	0.18	0.62	136.60	28.44	166.30	678.00	0.44	0.24	0.15	0.68	24945	739.40	18.34	2.92
LS08	0.29	1.52	30.01	0.75	0.11	0.75	109.70	20.87	444.40	552.30	0.40	0.24	0.16	0.76	37921	1226.80	26.13	2.63
LS09	0.39	4.02	21.02	1.65	0.14	1.65	55.10	11.61	227.40	822.00	0.64	0.40	0.14	1.24	23498	1113.60	26.52	7.17
LS10	0.34	1.26	35.54	1.66	0.15	1.66	108	28.26	434.00	811.10	0.80	0.30	0.33	1.23	52313	1464.40	18.63	6.88
LS11	0.27	1.01	28.85	0.78	0.11	0.78	107.40	28.44	207.10	139.60	0.53	0.37	0.14	2.31	23806	776.30	14.05	9.23
LS12	0.29	5.52	23.9	0.59	0.16	0.59	80.40	5.90	263.60	769.00	0.35	0.29	0.83	1.22	24860	1047.40	37.65	7.68
LS13	0.28	9.02	25.05	0.78	0.18	0.78	92.80	2.78	193.70	864.70	0.51	0.24	0.48	0.77	9184	378.90	15.30	6.13
LS14	0.19	2.02	29.08	1.90	0.17	1.90	173	14.42	391.30	845.40	0.44	0.28	0.13	2.76	26106	852.30	15.70	8.56
LS15	0.50	7.56	29.91	0.66	0.13	0.66	61.30	4.39	370.70	472.90	0.93	0.37	0.26	1.76	37168	1223.10	30.08	4.82
LS16	0.14	5.51	16.5	0.83	0.06	0.83	303.10	10.20	174.80	824.00	0.13	0.03	0.16	0.75	26910	1553.60	24.12	0.01
LS17	0.53	2.51	22.08	0.71	0.12	0.71	41.40	12.74	289.80	794.50	0.74	0.31	0.36	1.24	21657	961.70	33.09	6.00
LS18	0.22	6.41	27.08	0.69	0.18	0.69	142.80	4.43	355.90	903.20	0.45	0.43	0.15	1.77	18416	690.20	22.91	6.10
LS19	0.32	8.02	28.84	0.61	0.18	0.61	88.70	3.60	260.50	692.70	0.36	0.40	0.12	1.69	26298	918.80	18.44	11.55
LS20	0.14	1.22	21.77	1.00	0.12	1.00	163.30	57.12	79.60	909.80	0.59	0.00	0.10	2.80	6732	323.60	12.72	8.08
LS21	0.17	1.26	38.69	2.27	0.13	2.27	238.50	30.68	146.10	790.50	0.51	0.33	2.33	0.73	59155	1528.80	61.12	9.58

LS22	0.24	1.11	22.39	0.98	0.10	0.98	98.00	55.81	321.00	229.10	0.48	0.17	0.33	1.78	17721	763.20	12.77	11.44
LS23	0.16	7.51	21.38	1.73	0.18	1.73	168.70	3.25	124.20	155.40	0.49	0.27	0.20	0.82	20206	939.50	32.53	8.00
LS24	0.27	9.26	27.77	1.34	0.10	1.34	102.60	3.07	229.50	500.20	0.50	0.25	0.10	0.80	35469	1381.90	30.68	13.25
LS25	0.28	1.12	23.3	0.76	0.10	0.76	86.40	26.53	111.60	927.50	0.33	0.36	0.39	0.78	28312	1215.7	59.69	7.04
LS26	0.33	3.57	23.68	0.69	0.11	0.69	73.10	7.91	200.60	495.20	0.57	0.09	0.13	1.25	28203	1191.7	17.33	5.24
Mean	0.27	3.91	25.6	1.03	0.14	1.03	126.52	16.62	251.17	661.48	0.51	0.26	0.32	1.39	24852	944.47	26.98	7.20
p-value	*	*	*	ns	ns	*	*	*	ns	**	*	**	**	**	ns	ns	ns	**
SED	0.09	2.23	5.50	201	0.44	0.51	52.13	19.36	122	191.10	0.16	0.08	0.23	0.43	13977	459.90	15.50	4.35
LSD (5%)	0.18	4.59	11.35	415	0.09	1.05	107.40	29.87	251.3	393.6	0.33	0.16	0.47	0.89	28786	947	32.06	5.55
CV (%)	32.2	55.97	25.52	51.74	32.79	39.54	41.20	48.47	48.58	28.89	31.66	30.25	71.11	31.04	56.24	48.69	47.7	33.78

gs: stomatal conductance (mmol m^{-2} s^{-1}), T: transpiration rate (mmol H_20 m^{-1} s^{-1}), A: net CO_2 assimilation (µmol CO_2 m^{-1} s^{-1}), A/Ci: CO_2 assimilation rate/intercellular CO_2 concentration (µmol.mol^{-1}), Ci: intercellular CO_2 concentration (µmol.mol^{-1}), Ci/Ca: ratio of intercellular and atmospheric CO_2, WUEi: intrinsic water use efficiency ((µmol $(CO_2)m^{-2}$), WUEins: instantaneous water use efficiency (µmol.mol^{-1}), F_0': minimum fluorescence, Fm′: maximum fluorescence, *Fv′/Fm′*: maximum quantum efficiency of photosystem II photochemistry (ratio), *ϕPSII*: the effective quantum efficiency of *PSII* photochemistry, *qP*: photochemical quenching , *qN*: non-photochemical quenching, ETR: electron transport rate (µmol e^{-1} m^{-2} $s^{-1)}$, ETR/A: relative measure of electron transport to oxygen molecules (µmol e μmol^{-1} CO_2), AES: alternative electron sinks, SED: standard deviation, YPP: yield per plant (g/plant), LSD: least significant difference, CV: coefficient of variation, * and ** denote significance at 5 and 1% probability levels, respectively, ns: non-significant.

Table 4.4: Means of leaf gas exchange and chlorophyll fluorescence parameters of okra accessions under drought-stressed conditions

Genotype	Leaf Gas Exchange Parameters								Chlorophyll Fluorescence Parameters								
	gs	T	A	*Ci*	A/*Ci*	*Ci*/*Ca*	WUE*i*	WUE*ins*	*Fo'*	*Fm'*	*Fv'*/*Fm'*	*ϕPSII*	*qP*	*qN*	ETR	ETR/A	AES	YPP
LS01	0.16	0.01	24.6	425.1	0.12	1.11	847.90	1881.90	442.20	1826	0.36	0.06	0.11	3.75	27140	1111	263	3.92
LS02	0.31	1.01	29.03	316.1	0.23	0.80	193.30	1212.90	420.80	1733	0.40	0.05	0.11	3.72	19975	682	116.60	2.58
LS03	0.16	0.01	11.11	216.9	0.14	3.55	72.60	1196.30	465.60	1775	0.33	0.05	0.13	0.72	19196	1782	98.00	2.50
LS04	0.09	0.01	19.9	1064	0.07	1.97	225.80	2164.70	489.80	282	0.24	0.04	0.21	2.80	16373	791	66.80	4.19
LS05	0.13	4.51	15.11	763.3	0.15	0.66	178.50	372.80	443.50	1890	0.41	0.00	0.05	1.78	10688	683	562.80	6.17
LS06	0.12	2.51	15.27	920.7	0.05	2.86	312.20	696.50	54.80	1775	0.36	0.03	0.09	2.74	13797	898	113.70	5.05
LS07	0.17	4.51	16.03	728.6	0.13	0.83	198.90	312.60	104.80	1746	0.49	0.04	0.06	0.71	18534	1156	104.20	7.92
LS08	0.10	0.01	16.82	225.8	0.15	1.08	252.70	1354.60	509.10	1774	0.34	0.06	0.13	1.72	34541	2044	86.00	6.58
LS09	0.29	0.01	24.22	881.6	0.05	2.79	98.60	1933.30	483.00	1598	0.38	0.10	0.11	0.66	39986	1902	199.90	4.60
LS10	0.27	6.12	30.16	671.2	0.13	3.07	565.20	522.10	449.50	2867	0.37	0.04	0.03	3.7	16452	551	263.50	14.00
LS11	0.03	0.01	20.65	1205.9	0.12	1.83	923.40	1986.30	506.80	1809	0.24	0.05	0.12	3.74	20909	1007	70.40	6.76
LS12	0.21	0.01	22.17	221.40	0.14	0.57	155.90	1918.90	461.20	344	0.42	0.05	0.34	1.75	23095	1057	111.50	2.85
LS13	0.02	2.01	25.32	1058	0.05	2.74	1438.8	734.60	505.70	640	0.32	0.05	0.10	2.67	23537	871	112.80	2.00
LS14	0.17	0.51	24.54	290.40	0.10	1.25	311.4	761.80	519.20	963	0.34	0.06	0.16	0.69	24880	1041	114.40	4.48
LS15	0.34	1.01	22.62	598.30	0.13	2.06	565.6	995.90	497.90	1805	0.35	0.06	0.16	1.74	29353	1316	170.70	4.71
LS16	0.15	2.01	17.14	234.40	0.15	1.09	122.9	330.50	539.00	1533	0.37	0.02	0.09	1.32	8937	516	67.60	2.63

LS17	0.28	9.01	26.83	959.40	0.10	2.49	901.1	1048	429.90	806	0.38	0.09	0.19	2.71	41445	1542	89.20	3.69
LS18	0.25	3.67	23.51	1167.80	0.09	1.71	696.1	601.70	478.40	1709	0.33	0.04	0.19	3.69	19453	842	311.90	5.42
LS19	0.14	0.01	16.48	641.60	0.04	1.65	764.1	1392.50	373.80	1803	0.47	0.05	0.2	1.75	20599	1228	91.50	0.50
LS20	0.02	3.51	24.42	909	0.05	2.36	1256.1	1039.60	449.40	1714	0.26	0.05	0.11	2.7	19890	849	163.10	0.75
LS21	0.16	0.62	24.17	211.60	0.28	0.54	836.5	97.70	498.80	762	0.39	0.06	0.12	0.72	25724	1072	82.20	4.17
LS22	0.10	0.01	21.33	718.40	0.08	1.85	476.4	2161	307	1826	0.33	0.03	0.05	0.8	8129	381	64.90	1.75
LS23	0.10	4.51	24.23	234.80	0.12	3.60	511.8	375.60	505.90	1651	0.23	0.05	0.09	0.67	10832	485	154.50	5.88
LS24	0.26	3.66	24.96	863.40	0.10	3.23	297.5	257.80	518.20	1848	0.26	0.04	0.13	2.25	18797	772	102.9	4.17
LS25	0.11	4.01	19.88	697.70	0.06	2.30	366.5	706.30	364.20	1774	0.30	0.05	0.00	0.78	21796	1140	360.60	0.01
LS26	0.10	4.01	17.15	815.40	0.06	2.09	184.6	1379.50	397.10	1874	0.34	0.04	0.26	0.79	8071	466	80.00	4.00
Mean	0.16	2.20	21.45	644	0.11	1.93	490.55	1055.21	431.37	1543.35	0.35	0.05	0.13	1.96	20851.1	1007.12	154.72	4.31
p-value	*	**	*	ns	ns	*	*	**	**	**	ns	*	ns	**	*	**	ns	*
SED	0.07	1.91	4.5	503	0.08	1.21	317.1	641.5	57.65	442.6	0.1	0.02	0.09	0.61	6837	304.6	148.1	2.25
LSD (5%)	0.14	3.92	9.3	1036	0.18	2.5	653.1	1321	118.7	911.6	0.21	0.04	0.19	1.23	14081	627.3	305	4.21
CV (%)	42.44	46.4	20.98	76.77	74.92	62.98	64.65	71.47	13.36	28.68	28.96	39.83	69.85	30.87	32.79	30.25	95.71	25.76

gs: stomatal conductance (mmol m $^{-2}$ s^{-1}), T: transpiration rate (mmol H_20 m $^{-1}$ s^{-1}), A: net CO_2 assimilation (µmol CO_2 m^{-1} s^{-1}), A/*Ci*: CO_2 assimilation rate/intercellular CO_2 concentration (µmol·mol $^{-1}$), *Ci*: intercellular CO_2 concentration (µmol·mol $^{-1}$), *Ci*/*Ca*: ratio of intercellular and atmospheric CO_2, WUE*i*: intrinsic water use efficiency ((µmol $(CO_2)m^{-2}$), WUE*ins*: instantaneous water use efficiency (µmol·mol^{-1}), F_0': minimum fluorescence, *Fm'*: maximum fluorescence, *Fv'/Fm'*: maximum quantum efficiency of photosystem II photochemistry (ratio), *ϕPSII*: the effective quantum efficiency of *PSII* photochemistry, *qP*: photochemical quenching, *qN*: non-photochemical quenching, ETR: electron transport

rate (µmol e^{-1} m^{-2} $s^{-1)}$, ETR/A: relative measure of electron transport to oxygen molecules (µmol e µmol^{-1} CO_2), AES: alternative electron sinks, YPP: yield per plant (g/plant), SED: standard deviation, LSD: least significant difference, CV: coefficient of variation, * and ** denote significance at 5 and 1% probability levels, respectively, ns: non-significant.

4.3.2. Correlation between leaf gas exchange and chlorophyll fluorescence parameters under non-stressed and drought-stressed conditions

Pearson correlation coefficients showing relationships among leaf gas exchange and chlorophyll fluorescence parameters among the tested okra accessions under NS and DS conditions are presented in Table 4.5. Under NS conditions, Ci/Ca was highly and significantly correlated with Ci ($r = 1$, $p < 0.001$), WUE*i* with gs ($r = -0.75$, $p < 0.001$), WUE*ins* with T (r = −0.75, $p < 0.001$) and *ϕPSII* with A/Ci ($r = 0.61$, $p < 0.001$). In addition, *qP* was positively and significantly correlated with A ($r = 0.55$, $p < 0.05$), C*i* ($r = 0.48$, $p < 0.05$) and C*i*/C*a* ($r = 0.48$, $p < 0.05$). ETR was positively and highly significantly correlated with A ($r = 0.71$, $p < 0.001$) and *qP* ($r = 0.52$, $p < 0.001$). Positive and highly significant correlation was observed between ERT/A and ETR ($r = 0.86$, $p < 0.001$) and AES and q*P* ($r = 0.52$, $p < 0.001$), while a negative and highly significant association was observed between YPP and A ($r = -0.69$, $p < 0.001$). A significant positive correlation was observed between YPP and ETR/A (r = 0.49, $p < 0.05$), YPP and Ci ($r = 0.34$, $p < 0.05$) and YPP and C*i*/C*a* (r = 0.45, $p < 0.05$), while a negative significant correlation was observed between YPP and qN ($r = -0.45$, $p < 0.05$) under NS conditions.

Under DS conditions, a significant positive correlation was detected between A and gs ($r = 0.57$, $p < 0.05$), while A/C*i* was negatively and highly significantly correlated with C*i* ($r = -0.61$, $p < 0.001$). A highly significant negative association was found between C*i*/C*a* and A/Ci ($r = -0.57$, $p < 0.001$). WUE*i* was positively and significantly correlated with A ($r = 0.48$, $p < 0.05$), while WUE*ins* was negatively and highly significantly correlated with T ($r = -0.55$, $p < 0.001$). *Fv′/Fm′* was positively correlated with gs ($r = 0.47$, $p < 0.05$). *ϕPSII* was positively and highly significantly correlated with gs ($r = 0.54$, $p < 0.001$), while significantly associated with A ($r = 0.42$, $p < 0.05$) and *Fv′/Fm′* ($r = 0.46$, $p < 0.05$). *qP* was positively correlated with WUEins ($r = 0.39$, $p < 0.05$) and highly significantly correlated with *Fm′* (r = 0.55, $p < 0.001$). Positive correlations were observed between *qN* and A ($r = 0.48$, $p < 0.05$) and C*i* ($r = 0.48$, $p < 0.05$) and WUE*i* ($r = 0.43$, $p < 0.05$). ETR was positively correlated with gs ($r = 0.45$, $p < 0.05$), A ($r = 0.45$, $p < 0.05$), *Fv′/Fm′* ($r = 0.53$, $p < 0.001$) and *ϕPSII* (r = 0.82, $p < 0.001$). Relative measure of electron transport to oxygen molecules was positively and significantly correlated with WUE*i* ($r = 0.68$, $p < 0.001$) and ETR ($r = 0.82$, $p < 0.001$), while AES was positively correlated with T ($r = 0.45$, $p < 0.05$) and *qP* ($r = 0.48$, $p < 0.05$). YPP was highly positively correlated with C*i* ($r = 0.66$, $p < 0.001$), *Fo′* ($r = 0.83$, $p < 0.001$) and Ci/Ca ($r = 0.67$, $p < 0.001$), while significantly associated with WUE*i* ($r = 0.48$, $p < 0.05$) and Fm′ ($r =$

0.40, $p < 0.05$) and negatively correlated with ETR/A ($r = -0.60$, $p < 0.001$) under DS conditions.

Table 4.5: Correlation coefficients for gas exchange and chlorophyll fluorescence parameters under non-stressed (bottom diagonal) and drought-stressed (top diagonal) conditions

Traits	*gs*	T	A	C_i	A/C_i	C_i/C_a	WUE_i	WUE_{ins}	F_o'	F_m'	F_v'/F_m'	*ϕPSII*	*qP*	*qN*	ETR	ETR/A	AES	YPP
gs	1.00	0.17 ^{ns}	0.57 *	−0.16 ^{ns}	0.31 ^{ns}	−0.18 ^{ns}	−0.33 ^{ns}	−0.13 ^{ns}	0.19 ^{ns}	0.23 ^{ns}	0.47 *	0.54 **	0.14 ^{ns}	0.11 ^{ns}	0.45 *	0.21 ^{ns}	0.18 ^{ns}	0.25 ^{ns}
T	0.13 ^{ns}	1.00	0.23 ^{ns}	0.30 ^{ns}	−0.12 ^{ns}	0.30 ^{ns}	0.14 ^{ns}	−0.55 **	−0.22 ^{ns}	0.28 ^{ns}	0.27 ^{ns}	−0.07 ^{ns}	−0.21 ^{ns}	0.11 ^{ns}	−0.16 ^{ns}	0.24 ^{ns}	0.45 *	0.31 ^{ns}
A	0.14 ^{ns}	−0.19 ^{ns}	1.00	0.12 ^{ns}	0.15 ^{ns}	0.09 ^{ns}	0.48 *	−0.34 ^{ns}	0.46 ^{ns}	−0.18 ^{ns}	0.37 ^{ns}	0.42 *	−0.03 ^{ns}	0.43 *	0.45 *	−0.26 ^{ns}	0.02 ^{ns}	0.18 ^{ns}
Ci	−0.31 ^{ns}	−0.29 ^{ns}	0.30 ^{ns}	1.00	−0.61 **	0.31 ^{ns}	0.38 ^{ns}	0.26 ^{ns}	−0.26 ^{ns}	0.03 ^{ns}	0.16 ^{ns}	−0.03 ^{ns}	−0.03 ^{ns}	0.43 *	0.04 ^{ns}	0.14 ^{ns}	0.14 ^{ns}	0.66 **
A/*Ci*	0.04 ^{ns}	0.29 ^{ns}	0.35 ^{ns}	−0.02 ^{ns}	1.00	−0.57 **	−0.28 ^{ns}	−0.29 ^{ns}	0.23 ^{ns}	−0.16 ^{ns}	0.03 ^{ns}	−0.06 ^{ns}	−0.07 ^{ns}	0.01 ^{ns}	0.15 ^{ns}	0.01 ^{ns}	0.03 ^{ns}	0.24 ^{ns}
Ci/Ca	−0.31 ^{ns}	−0.29 ^{ns}	0.38 ^{ns}	1.00 **	−0.02 ^{ns}	1.00	0.13 ^{ns}	0.07 ^{ns}	0.22 ^{ns}	0.36 ^{ns}	0.28 ^{ns}	−0.19 ^{ns}	−0.19 ^{ns}	0.01 ^{ns}	0.03 ^{ns}	0.03 ^{ns}	−0.19 ^{ns}	0.67 **
WUE*i*	−0.75 **	−0.14 ^{ns}	0.16 ^{ns}	0.45 ^{ns}	−0.20 ^{ns}	0.35 ^{ns}	1.00	−0.17 ^{ns}	0.24 ^{ns}	−0.12 ^{ns}	0.33 ^{ns}	0.22 ^{ns}	−0.07 ^{ns}	0.43 *	0.23 ^{ns}	0.68 **	−0.04 ^{ns}	0.48 *
WUE*ins*	−0.24 ^{ns}	−0.74 **	0.16 ^{ns}	0.13 ^{ns}	−0.35 ^{ns}	0.13 ^{ns}	0.14 ^{ns}	1.00	0.15 ^{ns}	−0.29 ^{ns}	0.03 ^{ns}	0.30 ^{ns}	0.39 *	0.16 ^{ns}	0.29 ^{ns}	0.23 ^{ns}	0.27 ^{ns}	−0.23 ^{ns}
Fo'	0.36 ^{ns}	−0.06 ^{ns}	0.39 ^{ns}	0.01 ^{ns}	0.12 ^{ns}	0.01 ^{ns}	−0.35 ^{ns}	−0.21 ^{ns}	1.00	−0.22 ^{ns}	0.14 ^{ns}	0.24 ^{ns}	0.24 ^{ns}	0.18 ^{ns}	0.24 ^{ns}	0.18 ^{ns}	0.03 ^{ns}	0.83 **
Fm'	0.01 ^{ns}	−0.12 ^{ns}	−0.15 ^{ns}	0.02 ^{ns}	0.26 ^{ns}	0.02 ^{ns}	−0.01 ^{ns}	−0.08 ^{ns}	0.13 ^{ns}	1.00	0.33 ^{ns}	−0.27 ^{ns}	0.55 **	0.13 ^{ns}	−0.27 ^{ns}	−0.12 ^{ns}	0.25 ^{ns}	0.40 *
Fv'/Fm'	0.21 ^{ns}	−0.17 ^{ns}	0.193 ^{ns}	−0.12 ^{ns}	0.23 ^{ns}	−0.12 ^{ns}	0.03 ^{ns}	−0.18 ^{ns}	00.21 ^{ns}	0.27 ^{ns}	1.00	0.46 *	00.08 ^{ns}	0.197 ^{ns}	0.53 **	0.19 ^{ns}	0.28 ^{ns}	0.36 ^{ns}
ϕPSII	0.38 ^{ns}	0.36 ^{ns}	0.42 ^{ns}	0.02 ^{ns}	0.51 **	0.02 ^{ns}	−0.36 ^{ns}	−0.44 ^{ns}	0.30 ^{ns}	0.04 ^{ns}	0.05 ^{ns}	1.00	0.23 ^{ns}	0.16 ^{ns}	0.87 **	0.67 **	0.29 ^{ns}	−0.09 ^{ns}
qP	−0.12 ^{ns}	−0.24 ^{ns}	0.55 *	0.48 *	0.08 ^{ns}	0.48 *	0.23 ^{ns}	0.13 ^{ns}	−0.19 ^{ns}	0.29 ^{ns}	0.13 ^{ns}	0.29 ^{ns}	1.00	0.15 ^{ns}	0.28 ^{ns}	0.13 ^{ns}	0.48 *	−0.21 ^{ns}
qN	−0.01 ^{ns}	−0.25 ^{ns}	−0.13 ^{ns}	−0.27 ^{ns}	0.17 ^{ns}	−0.27 ^{ns}	−0.14 ^{ns}	0.18 ^{ns}	0.35 ^{ns}	0.10 ^{ns}	−0.12 ^{ns}	−0.17 ^{ns}	−0.25 ^{ns}	1.00	0.08 ^{ns}	−0.17 ^{ns}	0.13 ^{ns}	0.25 ^{ns}
ETR	0.22 ^{ns}	−0.19 ^{ns}	0.71 *	0.38 ^{ns}	−0.25 ^{ns}	0.48 *	0.06 ^{ns}	0.11 ^{ns}	0.31 ^{ns}	0.12 ^{ns}	0.15 ^{ns}	0.37 ^{ns}	0.52 **	−0.24 ^{ns}	1.00	0.82 **	0.07 ^{ns}	−0.03 ^{ns}
ETR/A	0.22 ^{ns}	−0.16 ^{ns}	0.38 ^{ns}	0.25 ^{ns}	−0.22 ^{ns}	0.25 ^{ns}	0.16 ^{ns}	−0.15 ^{ns}	0.37 ^{ns}	0.23 ^{ns}	0.27 ^{ns}	0.27 ^{ns}	0.31 ^{ns}	−0.37 ^{ns}	0.86 **	1.00	0.29 ^{ns}	−0.60 **
AES	−0.03 ^{ns}	−0.20 ^{ns}	0.10 ^{ns}	0.33 ^{ns}	−0.33 ^{ns}	0.33 ^{ns}	0.27 ^{ns}	−0.14 ^{ns}	−0.10 ^{ns}	0.21 ^{ns}	0.12 ^{ns}	0.38 ^{ns}	0.52**	−0.45*	0.37 ^{ns}	0.49*	1.00	0.19 ^{ns}
YPP	−0.29 ^{ns}	−0.26 ^{ns}	−0.69 **	0.45*	00.32 ^{ns}	0.45*	0.37 ^{ns}	0.35 ^{ns}	0.35 ^{ns}	−0.35 ^{ns}	−0.04 ^{ns}	−0.29 ^{ns}	−0.23 ^{ns}	0.22 ^{ns}	0.16 ^{ns}	0.11 ^{ns}	0.16 ^{ns}	1.00

gs: stomatal conductance, T: transpiration rate, A: net CO_2 assimilation, A/*Ci*: CO_2 assimilation rate/intercellular CO_2 concentration, *Ci*: intercellular CO_2 concentration, *Ci*/*Ca*: ratio of intercellular and atmospheric CO_2, WUE*i*: intrinsic water use efficiency, WUE*ins*: instantaneous water use efficiency, F_0': minimum fluorescence, *Fm'*: maximum fluorescence, *Fv'/Fm'*: maximum quantum efficiency of photosystem II photochemistry (ratio), *ϕPSII*: the effective quantum efficiency of *PSII* photochemistry, *qP*: photochemical quenching, *qN*: non-photochemical quenching, ETR: electron transport rate, ETR/A: relative measure of electron transport to oxygen molecules, AES: alternative electron sinks, YPP: pod yield per plant. * and ** denote significance at 5 and 1% probability levels, respectively, ns: non-significant.

4.3.3 Principal component analysis (PCA) for leaf gas exchange and chlorophyll fluorescence traits

Values of PCA, eigenvalues, percent, and cumulative explained variances are summarised in Table 4.6. Under NS conditions, seven principal components exhibited eigenvalues > 1 and accounted for 82% of total phenotypic variation. Net CO_2 assimilation, C_i, C_i/C_a, *qP*, ETR, ETR /A, AES and YPP were positively correlated with PC1, which accounted for 22% of the total variation. PC2 was positively correlated with gs, *Fo′* and *ϕPSII*, whereas WUE_i and WU_{Eins} were negatively correlated with PC2, which accounted for 17% of the total variation. Transpiration rate was negatively correlated with PC3, whereas WUE_{ins}, *qP* and YPP were positively correlated with PC3, which contributed 11.42% of total variation. A/C*i* positively correlated with PC4 accounted for 10.67% of total variation. PC5 was positively correlated with *Fm′* and F_v'/F_m', contributing 8% of total variation, whereas PC6 was positively correlated with Fm′, contributing 7% of total variation.

Similarly, under DS conditions, seven PCs with eigenvalues > 1 were detected, which contributed 80% of the total phenotypic variability. Yield per plant was negatively correlated with PC1, whereas *ϕPSII*, ETR and ETR/A were positively correlated with PC1, which accounted for 20% of total variation. Transpiration rate, net CO_2 assimilation, C_i, WUE_i, F_v'/F_m', *qN* and YPP were positively associated with PC2, accounting for 18% of the total variation. Stomatal conductance and A/Ci were positively correlated with PC3, whereas Ci and WUE_{ins} negatively associated with PC3 contributed 14% of the total variation. Net CO_2 assimilation and *qN* were positively correlated with PC4, whereas ETR/A was negatively correlated with PC4, accounting for 10% of total variation. Instantaneous water use efficiency was positively correlated with PC5, which accounted for 7% of total variation, whereas stomatal conductance and photochemical quenching were positively correlated with PC6, which contributed 6% of total variation.

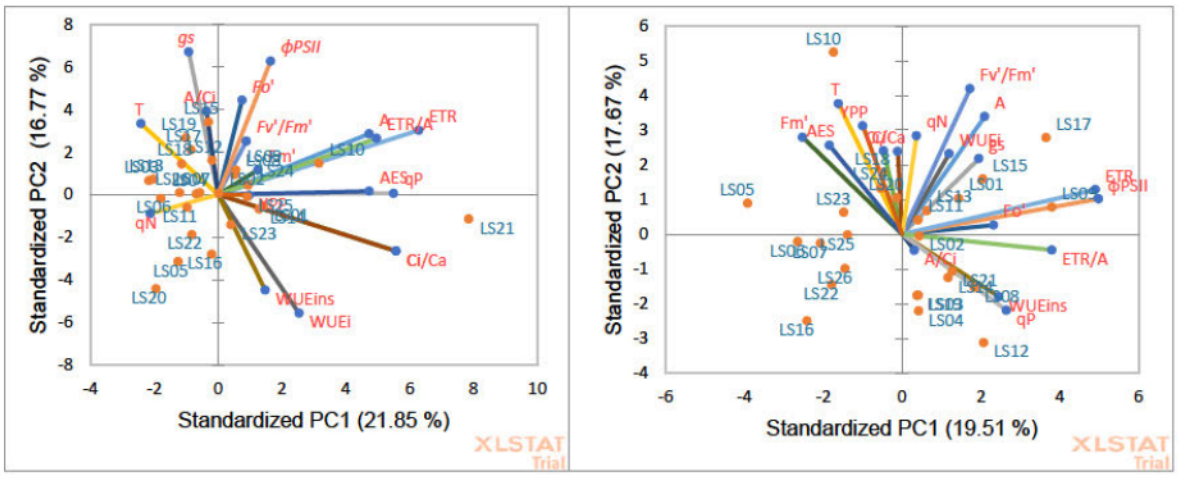
Principal component biplots based on PCA analysis were used to indicate the relationships among okra accessions for leaf gas exchange and chlorophyll fluorescence parameters under NS (Figure 4.1A) and DS (Figure 4.1B) conditions. Traits presented by parallel vectors or those close to each other revealed a strong positive association, and those located nearly opposite (at 180°) showed a highly negative association, while the vectors toward sides expressed a weak relationship. Under NS conditions, accessions LS06, LS11, LS22, LS05 and LS20 were grouped based on high *qN*. Accessions LS19, LS17 and LS18 were grouped together based on

high gs, T and A/Ci. LS02, LS10 and LS24 were grouped based on high $\phi PSII$, Fo', F_v'/F_m', A, ETR, ETR/A, AES and qP. Accessions LS25, LS01, LS23 and LS16 were grouped together based on high C_i/C_a, WUE_i and WUE_{ins}. Under DS conditions, accessions LS10, LS24, LS25, LS05 and LS06 were clustered together based on high Fm', AES, T, C_i and YPP. LS13, LS15, LS17 and LS09 were grouped together based on high C_i/Ca, F_v'/F_m', WUE_i and $\phi PSII$. Accessions LS02, LS19, LS21, LS08 and LS12 were grouped based on high Fo', ETR/A, WUE_{ins} and qP.

Table 4.6: Principal component analysis showing eigenvalue, percent and cumulative variation of leaf gas exchange and chlorophyll fluorescence traits of 26 okra accessions under non-stressed and drought-stressed conditions

Traits	Non-stressed							Drought-stressed						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC1	PC2	PC3	PC4	PC5	PC6	PC7
gs	-0.12	**0.77**	0.27	-0.26	-0.2	-0.03	-0.18	0.41	0.19	**0.61**	-0.08	0.2	**0.54**	0.00
T	-0.33	0.38	**-0.69**	0.12	-0.18	0.12	0.19	-0.2	**0.67**	0.18	-0.08	-0.36	0.31	-0.33
A	**0.62**	0.33	0.20	0.29	0.13	-0.48	-0.19	0.45	**0.52**	0.17	**0.52**	-0.13	0.12	0.16
Ci	**0.72**	-0.31	0.10	0.40	-0.28	-0.22	-0.03	-0.04	**0.56**	**-0.63**	-0.03	0.16	0.13	-0.26
A/C_i	-0.06	0.45	-0.20	**0.68**	0.38	-0.09	-0.04	0.07	-0.27	**0.78**	0.39	0.02	-0.15	-0.02
C_i/C_a	**0.72**	-0.31	-0.10	0.40	-0.28	0.22	-0.03	0.02	0.49	-0.29	-0.42	-0.37	0.15	0.48
WUE_i	0.34	**-0.64**	-0.26	0.02	0.21	0.05	0.45	0.25	**0.51**	-0.38	0.39	-0.22	-0.46	-0.10
WUE_{ins}	0.19	-0.52	**0.68**	0.00	0.13	-0.27	-0.2	0.39	-0.29	**-0.53**	-0.02	**0.53**	0.04	0.26
F_o'	0.1	**0.51**	0.49	0.15	-0.19	0.32	0.36	0.42	0.03	0.15	0.43	-0.13	-0.17	0.40
F_m'	0.18	0.13	0.09	0.04	**0.57**	**0.64**	-0.24	-0.38	0.46	0.28	-0.36	0.35	-0.09	0.37
F_v'/F_m'	0.13	0.29	0.12	0.19	**0.61**	0.09	0.42	0.41	**0.62**	0.22	-0.14	0.19	-0.06	0.18
ϕ_{PSII}	0.21	**0.72**	0.16	0.30	-0.02	-0.09	-0.26	**0.91**	0.07	-0.02	-0.23	-0.18	-0.02	0.02
qP	**0.72**	0.01	-0.18	0.00	0.29	-0.19	-0.27	0.42	-0.41	-0.28	0.25	0.44	**0.52**	-0.08
qN	-0.3	-0.1	**0.56**	0.47	-0.06	0.32	-0.1	0.13	**0.50**	-0.19	**0.50**	0.46	0.04	-0.08
ETR	**0.83**	0.35	0.19	-0.14	-0.11	-0.10	0.16	**0.91**	0.09	0.10	-0.20	0.01	-0.15	-0.27
ETR/A	**0.66**	0.3	0.07	-0.05	-0.25	0.19	0.15	**0.67**	-0.20	0.08	-0.56	0.08	-0.25	-0.20
AES	**0.63**	0.02	-0.27	-0.39	0.07	0.15	-0.35	-0.27	0.44	0.34	-0.08	0.36	-0.2	-0.24
YPP	**0.97**	-0.03	**0.87**	-0.06	-0.23	-0.29	0.21	**-0.65**	**0.54**	0.45	-0.02	0.32	0.21	0.08
Eigenvalue	3.93	3.02	2.06	1.92	1.42	1.18	1.11	3.51	3.18	2.44	1.83	1.29	1.14	1.03
Variability (%)	21.85	16.77	11.42	10.67	7.87	6.56	6.16	19.51	17.67	13.55	10.17	7.16	6.30	5.70
Cumulative (%)	21.85	38.62	50.04	60.70	68.58	75.14	81.30	19.51	37.18	50.73	60.90	68.06	74.36	80.06

gs: stomatal conductance, T: transpiration rate, A: net CO_2 assimilation, A/Ci: CO_2 assimilation rate/intercellular CO_2 concentration , Ci: intercellular CO_2 concentration, Ci/Ca: ratio of intercellular and atmospheric CO_2, WUE*i*: intrinsic water use efficiency, WUE*ins*: instantaneous water-use efficiency, F_0': minimum fluorescence, Fm': maximum fluorescence, *Fv'/Fm'*: maximum quantum efficiency of photosystem II photochemistry (ratio), *ϕPSII*: the effective quantum efficiency of *PSII* photochemistry, *qP*: photochemical quenching , *qN*: non-photochemical quenching, ETR: electron transport rate, ETR/A: relative measure of electron transport to oxygen molecules , AES: alternative electron sinks, YPP: yield per plant. Note: boldface values denote values with high loading scores.

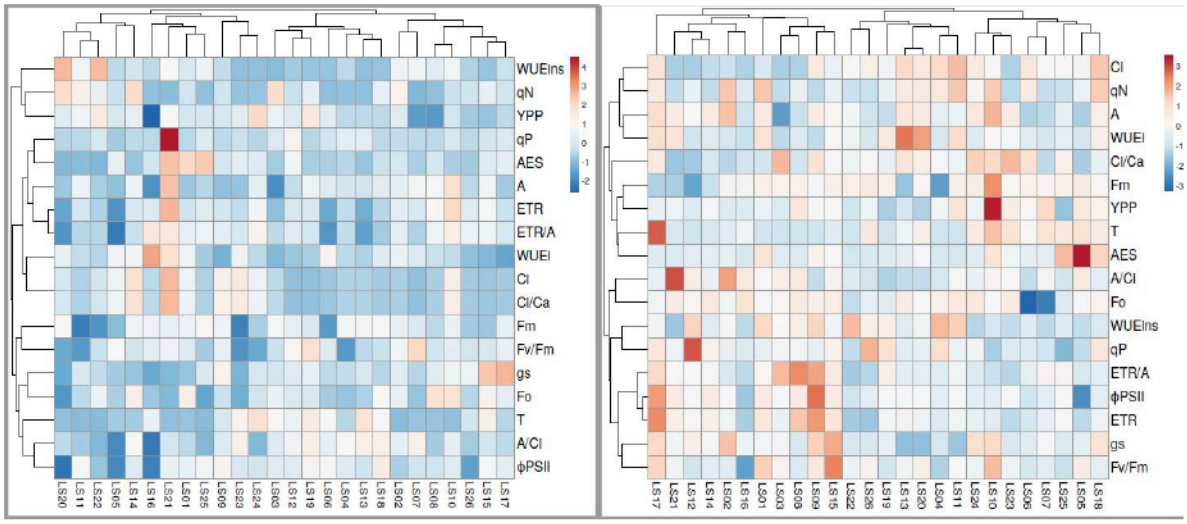


(A) **(B)**

Figure 4.1: Principal component (PC) biplot of PC1 vs. PC2 depicting the relationships among physiological traits among 26 okra accessions evaluated under non-stressed (**A**) and drought-stressed **(B)** conditions.

4.3.4 Heatmap analysis for leaf gas exchange and chlorophyll fluorescence traits

A heatmap based on leaf gas exchange and chlorophyll fluorescence traits under NS and DS conditions was constructed using a hierarchical clustering method to discern the relationship of 26 okra accessions based on Jaccard's coefficient (Figure 4.2). Under NS (Figure 4.2A) conditions, physiological traits were grouped into four main clusters. The first cluster consists of two subclusters, dominated by eight accessions, including LS19, LS12, LS06, LS18, LS13, LS07 and LS02, which were grouped based on high negative correlations with WUE*ins*, *qN* and YPP. The second subcluster consisted of accessions LS22, LS11, LS1, LS08, LS20 and LS14, which were negatively correlated with A/Ci and T. LS25, LS01, LS21, LS16, LS24 and LS23 dominated the fourth subcluster under NS conditions and positively correlated with *qP*. Under DS (Figure 4.2B), physiological traits were grouped into three main clusters and six subclusters. The first cluster is dominated by accessions LS19, LS09, LS03, LS15, LS14 and LS17, based on their positive correlations with ETR and ETR/A. LS26, LS22, LS04, LS20, LS13 and LS11 dominated the second cluster under DS conditions, with positive correlations with WUE*i*, WUE*ins*, *qN* and YPP. AES was positively correlated with LS25 and LS05 in the third cluster under DS conditions.



(A) **(B)**

Figure 4.2: Heatmap showing the relationship among physiological traits among 26 okra accessions evaluated under non-stressed (**A**) and drought-stressed (**B**) conditions.

4.4 Discussion

Okra is one of the most important commercial vegetable crops grown for its fresh pods and dry seeds. Drought is the major impediment to okra production in dry regions. To adapt to drought stress, plants have undergone many biochemical, molecular, and physiological changes. These changes increase the plants' tolerance to drought stress. Drought stress influences plant performance by reducing gas exchange and altering chlorophyll fluorescence formation. Gas exchange and chlorophyll fluorescence confer drought tolerance in okra [14,27]. Plants alter gene expression, disrupting the production of photosynthetic pigments and regulating stomatal function to adapt to and tolerate stress conditions [27]. Developing new strategies for maintaining high yield under drought-stress conditions is one of the major challenges in the current crop production system.

In this study, various physiological drought responses were assessed in okra accessions. Reductions in okra's stomatal conductance and transpiration rates have been associated with water conservation that allows plants to tolerate drought stress and the loss of physiological functions [9]. Stomatal closure leads to a reduction in CO_2 assimilation and minimises the rate of water loss through transpiration. This role of drought-induced stomatal closure limits CO_2 uptake by the leaves and possibly leads to increased susceptibility to photodamage [14]. Similar findings were reported for okra accessions under water shortages [14,27]. These physiological

changes increase the plants' resistance to drought stress, enabling the crop to survive in environments with limited water availability.

Drought tolerance should be considered as a comprehensive evaluation of carbon assimilation during global climate change challenges [28]. In the current study, okra accessions exhibited a reduction in net CO_2 assimilation under drought-stressed conditions (Table 4.4). The decrease in net CO_2 assimilation during water-stressed conditions might be reversible initially. However, drought at the pod-filling stage might cause irreversible damage to the photosynthetic pathway, thereby affecting carbon assimilation [29]. Further, utilisation of assimilates is relevant in addition to the photosynthetic performance of leaves. The evaluated okra accessions revealed high water use efficiency under drought-stressed conditions (Table 4.4). Enhancing water use efficiency to sustain okra production under water-limited conditions remains the most important task for water management. Hence, specific responses for enhancing water use efficiency could be achieved with more precise data on crop stress detection [14]. Drought-tolerant accessions exhibited high WUE_i and WUE_{ins} compared to drought-susceptible accessions (Table 4.4). This indicates that the evaluated accessions use water efficiently, attributed to drought escape mechanisms such as the transpiration rate. Drought-tolerant accessions use water efficiently, maintain tissue water status, reduce water loss and produce stable yield during water shortages [30].

Chlorophyll fluorescence is a non-invasive measurement detecting the authenticity of photosystem II [31]. Chlorophyll fluorescence parameters, including photosystem II photochemistry, minimum fluorescence, maximum fluorescence, photochemical quenching and electron transport rate are useful for detecting drought-stress severity, genetic variation and determining damage to *PSII* [32]. F_v'/F_m' is considered the most important parameter of chlorophyll fluorescence, widely used to evaluate drought-stress response. In this study, a reduced F_v'/F_m' value was recorded under drought-stress conditions, corroborating with results reported by Ahmed and El-Sayed, [27]. According to Paknejad et al. [33], reduced F_v'/F_m' under drought-stress conditions indicate the presence of a protective mechanism of light absorption in response to water shortages. Hence, the F_v'/F_m' parameter can be applied to determine the potential efficiency of *PSII*.

In the present study, drought-tolerant okra accessions showed an efficient photosynthetic affinity compared to sensitive accessions. Photosystem *II* is highly drought tolerant. However, under drought-stress conditions, photosynthetic electron transport through *PSII* is inhibited

[24]. The decrease in *PSII* might be due to the photo-protective increase in thermal energy dissipation induced by the excess of absorbed light [34]. However, there are contradictory reports on the direct effect of *PSII* functionality under drought-stress conditions. A study reported that, under mild water stress, *PSII* is not affected [35], while another study reported that, under drought-stress conditions, damage occurs to both photosystem I and photosystem II [36]. The current study found that *PSII* was significantly affected by drought stress. Under drought-stress conditions, the *PSII* thermal energy dissipation was strongly limited due to damage to *PSII* structure and functionality. A decrease in photochemical quenching was observed in the studied okra accessions under drought-stress conditions. Similar results were reported by Ashraf et al. [37] in the study of gas exchange characteristics and water relations in some elite okra cultivars under water-deficit conditions. The decrease in *qP* is attributable to either a decrease in the rate of consumption of reductants and ATP produced from non-cyclic electron transport relative to the rate of excitation of open *PSII* reaction centres or damage to *PSII* reaction centres [24].

Positive correlations were observed between non-photochemical quenching and intrinsic water use efficiency under drought-stress conditions, indicating a protective mechanism by the plants against reactive oxygen species that harm antenna pigments and closing reactions in the photosystem. Drought stress also affects the electron transport rate (ETR) and alternative electron sink (AES) [38]. An increase in alternative electron sink was observed among the studied okra accessions under drought-stress conditions. Drought-tolerant accessions indicated higher AES values. An increase in AES was reported as an indicator of drought stress [39]. Alternative electron sink is the second most important mechanism after photosynthesis used to remove electrons, which occurs at high rates in the leaves under drought stress conditions [40].

4.5 Conclusions

Drought is one of the most important factors affecting physiological traits and yield in crop plants, including okra. In the present study, it was observed that drought stress affected physiological processes such as reduced stomatal conductance, transpiration rate, net carbon dioxide assimilation, maximum quantum efficiency, effective quantum efficiency of *PSII* photochemistry, photochemical quenching and electron transport rate among the studied okra accessions. These physiological traits could be useful for drought-tolerance breeding in okra. Principal component analysis-based biplots allowed the identification of drought-tolerant accessions such as LS05, LS06, LS07 and LS08 based on high A, T, *Fm'*, *Fv'/Fm'* and ETR,

and LS10, LS11, LS18 and LS23 based on high *AES*, *Ci*, *Ci/Ca*,*WUEi*, *WUEins*, *ϕPSII* and AES. The selected genotypes are high yielding (≥5 g/plant) under drought-stress conditions. These accessions are suitable candidates for parental genotypes for drought-tolerance breeding in okra to enhance water use efficiency under water-limited conditions.

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Chapter 5. Combining ability and heterosis analyses of selected okra (*Abelmoschus esculentus*) accessions for agronomic traits under non-stressed and drought-stressed conditions

Abstract

Okra (*Abelmoschus esculentus* [L.] Moench) is a multi-purpose crop cultivated globally in dry environments. Sustainable okra cultivation in water-scarce production environments requires breeding and adaptation of high-yielding and drought-tolerant genotypes. Therefore, the objective of this study was to determine the combining ability and heterosis of selected okra accessions for yield and yield-related traits to identify superior parents and progenies for breeding. Eight selected parental genotypes with variable drought tolerance and contrasting agronomic traits were crossed using an 8 × 8 half-diallel mating design to generate 28 crosses. The crosses and their parents were evaluated under non-stressed and drought-stressed conditions across two sites using a 12 × 3 lattice design with three replications. Data were subjected to analysis of variance, combining ability and heterosis analyses. GCA and SCA effects were significant ($P<0.05$) for most traits, indicating the role of additive and non-additive gene action underlying the inheritance of the assessed traits. Parental genotypes LS09, LS10 and L24 showed positive GCA effects for pod yield under drought conditions and were selected to be valuable germplasm for variety design to widen genetic variability for drought tolerance and yield-related traits. Crosses LS01 × LS17, LS01 × LS18, LS09 × LS10, LS09 × LS18, LS09 × LS24, LS15 × LS18, LS15 × LS21, LS15 × LS24 and LS17 × LS21 expressed positive SCA effects for pod yield under drought condition and are recommended for genetic advancement, production, and commercialization in water-scarce environments of South Africa.

Keywords: Combining ability analysis, diallel analysis, drought tolerance, heterosis, okra

5.1 Introduction

Okra (*Abelmoschus esculentus* L. Moench; 2n = 8x = 72; 144) is a muti-purpose crop of the Malvaceae family. Tender and immature okra pods are used to prepare various dishes and

consumed as vegetables, whereas mature and dry seeds are processed for their edible oils. The seeds are rich in oil (~18%), fatty acids including linoleic acid (i.e., 23.6-50.65%), palmitic acid (i.e., 10.3-36.35%) and oleic acid (i.e., 19.22%) (Jarret et al. 2011, Moosavi et al. 2018). The pods, stems, and leaves consist of mucilaginous material that can be used as a plasma replacement (Kumar et al. 2013). Likewise, okra leaves are edible vegetables, traditionally boiled and consumed or to make tea in rural areas of Africa (Roy et al. 2014). The composition of leaves per 100 g edible portion includes water (81.5 g), energy (235 KJ), protein (4.4 g), fat (0.6 g), carbohydrates (11.3 g) and 2.1 g of fibre (Gemede et al. 2015). Okra products are a source of essential nutrients, including vitamins, protein, and mineral elements (Keerthana et al. 2021). These nutritional attributes make okra a vital food crop in sub-Saharan Africa (SSA) and Asia.

Okra production occurs predominately in Asian and African countries. Asia accounts for a total annual production of ~ 7 million tons from 606 440 ha of cultivated land, whereas Africa for about 3 million tons from 186 279 ha of land (FAOSTAT 2021). Okra is a relatively drought-tolerant crop that can grow successfully under limited water conditions with minimal supplemental irrigation. Despite being relatively drought-tolerant, the crop fails to reach its maximum yield potential, resulting in low marketable pod yields, primarily when drought stress occurs at the flowering and pod formation stages (Mkhabela et al. 2021). For example, over 70% of yield losses are attributed to drought stress during the reproductive stage (Alake 2020). The low yield performance is related to the cultivation of low-yielding and drought-sensitive varieties. There is a need to explore okra genetic resources to identify sources of unique traits for drought tolerance enhancement and breeding.

Characterisation of okra genetic resources aided the identification of potential genotypes for drought tolerance breeding. Some drought-tolerant varieties of okra have been identified, including Sabz Pari (Munir et al., 2016), Kano (Barzegar et al. 2016), NHAe 47-4 (Adejumo et al. 2019), Pusa Sawani, Iraqi and Hala (Abd El-Fattah et al., 2020) and LS10, LS 15, LS18 and LS24 (Mkhabela et al. 2022). The selected genetic stocks would serve as valuable germplasm for breeding with enhanced drought tolerance and yield components for targeted production in water-limited conditions. Improved okra varieties are yet to be bred and adopted by growers for targeted production areas in water-stressed environments. A dedicated okra breeding program is essential to develop high-yielding and drought-tolerant genotypes. Economic traits for selection in okra breeding include early maturity, relatively taller plants, pod prolificacy, seed yield and pod yield per plant. Moreover, traits such as fresh pod length

and number of fresh pods per plant positively correlated with pod yield under drought-stressed conditions, suggesting that direct selection of higher pod length and fresh pods can improve yield gains in water-limited environments (Mkhabela et al. 2022).

Combining ability analysis aims to discern the nature of gene action underlying yield expression and yield-related traits to guide the selection of breeding parents and high-performing progenies (Kumar and Reddy 2016). General combining ability (GCA) measures the mean performance of a genotype in a series of crosses. In contrast, specific combining ability (SCA) measures deviation from the predicted performance of a cross based on parents' performance (Reddy et al. 2013). GCA and SCA effects are attributed to additive and non-additive gene action, respectively. Additive and non-additive genes control pod yield and its component traits in okra (Reddy et al. 2011; Priyanka et al. 2018). In okra, parental lines with positive GCA effects for plant height, number of branches, fresh pod length, number of fresh pods per plant and pod yield have been identified (Reddy et al. 2013 , Abed et al. 2020). In addition, crosses with desirable SCA effects for the number of branches, number of fresh pods per plant and pod yield have been reported (Javiya et al. 2020 ; Kumar and Reddy 2016). The first breeding procedure is the selection of parents with good GCA effects through their progenies with high SCA effects for desirable traits. Combining ability studies are useful for implementing breeding strategies for cultivar design in crop improvement programmes (Javiya et al. 2020).

Okra is increasingly becoming a popular commercial crop in many parts of Africa. However, there are very few varieties developed in the region with farmer-and-consumer desired traits, including high yield potential and drought tolerance. To initiate an okra pre-breeding program for drought tolerance in South Africa, genetically diverse collections were characterised using agronomic and physiological traits and polymorphic simple sequence repeat (SSR) markers (Mkhabela et al. 2022 ; Mkhabela et al. 2023). These enabled the selection of potential and complementary parents with high yield potential and drought tolerance for breeding. There is a need to explore the breeding value of the identified genotypes. Therefore, the objective of this study was to determine the combining ability and heterosis of selected okra accessions for yield and yield-related traits to identify superior parents and progenies for breeding.

5.2 Materials and methods

5.2.1 Plant materials

Eight okra genotypes were selected and used in this study to generate new genetic combinations (Table 5.1). The parents were selected based on their high yield potential and tolerance to drought stress. The genotypes were sourced from the Agricultural Research Council-Vegetables, Industrial and Medicinal Plants (ARC-VIMP), South Africa, assembled from diverse regions of origin. The levels of drought tolerance in the test genotypes were assessed using drought tolerance index (DTI) (Bindinger et al. 1982).

Table 5.1: Description of okra parents used in an 8 × 8 half-diallel mating design

Accession code	Accession name	Origin	Drought tolerance index (DTI)[a]
LS01	VI033775	Malaysia	Moderate
LS09	VI050960	Zambia	High
LS10	VI055110	Zambia	High
LS15	VI056069	Cambodia	High
LS17	VI056081	Cambodia	Moderate
LS18	VI056449	United States of America	High
LS21	VI060679	India	Moderate
LS24	VI060822	Nigeria	Moderate

[a] Drought tolerance index (DTI) was computed as follows: = (Ys/Yn)/(Ms/Mn), where Ys and Yn are the genotype yields under stress and non-stress, and Ms and Mn are the mean yields of the accessions under stressed and non-stressed conditions, respectively. A drought index > 1 suggests relative drought resistance, and an index < 1 suggests relative drought susceptibility.

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5.2.2 Mating design and crosses

The selected eight parents were crossed using an 8 × 8 half diallel mating design during the 2021 cropping season. The parents were planted under field conditions at the ARC-VIMP ($25^0$59″ S, $28^0$35″ E) research station during the 2021/2022 growing season. The eight parents were stagger planted with a 2-week interval to synchronize flowering and pollen supply. The crosses were made by hand emasculation and subsequent pollination. Plants with flower buds near to open were tagged; each flower was hand-pollinated the next day. The pollinated flowers were labelled, tagged, and dried pods from successful crosses were regularly harvested. The crossing blocks were irrigated daily from October 2021 to January 2022. Weeding and other

agronomic practices were carried out to optimize flowering, seed setting and maturity based on the common scientific standards. Subsequently, 28 new generations were developed.

5.2.3 Study sites and experimental design

The crosses and eight parents were field evaluated using a 12 × 3 lattice design with three replications. The genotypes were evaluated under non-stressed (NS) and drought-stressed (DS) conditions at two locations, namely Agricultural Research Council – Loskop (25.1767^0 S, 29.3881^0 E) and the Agricultural Research Council – Brits (25.6100^0 S, 27.7960^0 E). The Loskop site is situated in the Limpopo Province and the Brits site in North-West Provinces of South Africa. The study sites are known for their high temperatures and relative humidity (South African Weather Service, 2022), useful for drought tolerance evaluations. The experimental plots consisted of a single 6 m long row with inter and intra spacing of 3 m and 1 m, respectively. Each row consisted of ten plants. Drought stress was imposed at 50% flowering until physiological maturity by withholding irrigation until the soil water content reached 30% field capacity to allow for continued plant growth and development. Soil moisture content was monitored using tensiometers (Spectrum Technologies, Inc, USA, Illinois) to detect soil moisture levels at the rootzone level. All other agronomic practices were done per the study sites' recommendations.

5.2.4 Data collection

Data were collected from five randomly selected and tagged plants from each plot for individual plant data collection. The following data were collected: days to 50% flowering (DTF) recorded as the number of days from sowing to when 50% of the plants flowered, plant height (PH, expressed in cm) measured from the base of the plant to the apex of the plant at maturity, number of branches per plant (NOB) and number of leaves per plant (NOL) were counted, stem diameter (SD, cm) measured using a digital vernier caliper. Fresh pod length (FPL) was measured in cm using a ruler. Fresh pods were harvested when 50% of the pods were 3– 5 cm long. Harvesting was conducted every third day by hand, and at each harvest, the number of fresh pods per plant (NFPP) were counted. Fresh pod yield per plant (PYP, g) measured as the total fresh pod weight per plant. The remaining five plants from the row were sampled, tagged, and left until maturity to collect data on mature, dry pod weight (DPW, g) by weighing dry pods harvested and expressed in grams and hundred seed weight (HSW, g) were measured by weighing a random sample of 100 seeds at 12% seed moisture content.

5.2.5 Data analysis

5.2.5.1 Analysis of variance

The collected data were subjected to a combined analysis of variance (ANOVA) using GenStat 20th edition (Payne et al. 2018) after using the Bartlet homogeneity of variance test. The least significant difference (LSD) was computed to compare treatment means at 5% probability level of significance.

5.2.5.2 Computation of the general combining ability (GCA) and specific combining ability (SCA) effects and heterosis

The mean performances data across replications were used to determine magnitudes of GCA and SCA effects across the two locations and separately for the NS and DS conditions, using Griffing's Method II (Parents and F_1's) (Griffing 1956). The parents and F_1's were considered fixed, whereas the test environments, replications and water regimes were treated as a random effect. Griffing's Model I (fixed effects) was employed to estimate GCA and SCA effects. The analysis was performed by using Analysis of Genetic Designs in R (AGD-R) v5.0 (Rodríguez et al. 2018), using the following fixed-effect model:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk}$$

where Y_{ijk} is the recorded value for the cross involving the i^{th} and j^{th} parents (ij^{th}) in the k^{th} replication, μ is the population mean, g_i and g_j are the GCA effects for the i^{th} and j^{th} parents, respectively; s_{ij} the SCA effect of the cross of the i^{th} and j^{th} parents and e_{ijk} the error term associated with the ij^{th} cross in the k^{th} replication.

The significance of the GCA and SCA effects for the assessed traits was computed by using a t-test at 5% level of significance.

The mid-parent heterosis (MPH) and better-parent heterosis (BPH), for each cross, were determined according to Falconer and Mackay (1996) using the following equations:

$$\text{MPH\%} = \frac{F1-MP}{MP} \times 100$$

$$\text{BPH\%} = \frac{F1-BP}{BP} \times 100$$

Where F1 = mean performance of the cross, MP = mean of two parents making the cross and BP = mean value of the better parent

5.2.5.3 Associations for agronomic traits

Pearson's correlation coefficients (r) were calculated using IBM SPSS Statistics 25.0 (SPSS Inc., Chicago, IL, USA, 2018) to determine the magnitude of the relationship among phenotypic traits. The significance of the correlations for the assessed traits was computed using a t-test at 5% probability level of significance, presented as supplementary results.

5.3 Results

5.3.1 Genotype, moisture regime, environments, and their interaction effects on agronomic trait

Analysis of variance showing the effects of genotypes, water regime, environment and their interactions for the studied traits is presented in Table 5.2. The experimental setup of the eight okra parental genotypes and their 28 F_1 progenies at the Loskop site in Limpopo province in South Africa is depicted in Figure 5.1. Significant ($P<0.05$) genotypic and site effects were computed for all traits. Water regime significantly affected all studied traits except for DPW and PYP. Significant ($P<0.05$) site × water regime interaction effects were recorded for DTF, NOB, PH, PYP, DPW and HSW. In addition, significant ($P<0.05$) genotype × water regime × site interaction effect was detected for DTF, NOB, SD, PH, NFPP, PYP and DPW.

Figure 5.1: The experimental setup involving the eight okra parental genotypes and their 28 F_1 progenies at the Loskop site in Limpopo Province in South Africa.

Table 5.2: Analysis of variance with the mean squares and significant tests for studied traits among 8 parental genotypes and their 28 F_1 progenies of okra assessed under non-stressed (NS) and drought-stressed (DS) conditions in the Loskop and Brits sites in South Africa

Source of variations	d.f.	Traits									
		DTF	NOB	NOL	SD	PH	FPL	NFPP	PYP	DPW	HSW
Incomplete blocks in replication	2	46.92^{ns}	9.34	119.41*	28.04*	811.60*	29.28^{ns}	44.14*	1032.01*	91.70^{ns}	0.6463
Replication (Rep) in sites	2	52.99^{ns}	19.44*	373.59*	6.85^{ns}	59.40^{ns}	105.74*	49.62^{ns}	148.20^{ns}	627.46**	0.6261^{ns}
Site	1	12008.15**	99.86**	39130.63**	19904.14**	60971.60**	1559.43**	91.76*	9051.50**	8072.16**	7.8846**
Water regime (WR)	1	4682.44**	218.60**	299.17*	708.24**	6435.50**	900.29**	1536.09**	360.80^{ns}	0.40^{ns}	21.3015**
Genotype (Gen)	35	94.71**	16.66**	105.85*	49.58**	1301.30**	35.83*	96.38**	9903.50**	80.35*	0.5545*
Site*WR	1	1615.71**	40.16*	23.78^{ns}	47.10^{ns}	5046.10**	11.75^{ns}	26.22	25349**	934.78**	2.3159*
Gen* Site	35	165.19**	14.22**	122.18**	46.55**	1267.20**	21.93^{ns}	55.44**	648.50^{ns}	66.22^{ns}	0.8727**
Gen*WR	35	135.93**	15.17**	58.65ns	21.31^{ns}	796.30*	40.23*	36.83*	66.30^{ns}	129.69**	0.7344*
Gen*WR*site	35	67.90*	11.69**	68.46ns	32.84*	753.70*	22.95^{ns}	74.09**	7702**	86.17*	0.3870^{ns}
Residual	282	39.54	5.28	60.31	18.54	441.01	24.22	20.28	702.9	52.97	0.42

d.f.: degree of freedom, DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight, *: significant at 5% probability level, **: significant at 1% probability level.

5.3.2 Performance of okra parents and crosses for agronomic traits

Mean performance among parents and crosses for the evaluated traits is presented in Tables 5.3 and 5.4 for the Loskop and Brits sites, respectively. The effect of different water conditions on okra pod formation is depicted in Figure 5.2. The DTF exceeding 50 days were recorded for crosses LS01 × LS24, LS09 × LS15, LS09 × LS18, LS09 × LS21, LS09 × LS24, LS10 × LS24, LS15 × LS17, LS17 × LS18 and LS18 × LS21 in Loskop under NS condition. Under DS condition, crosses LS01 × LS17 and LS15 × LS21 recorded the lowest DTF of 48 and 50 days, respectively. In Brits, DTF of 45 and 48 days were recorded for crosses LS01 × LS17 and LS15 × LS24, respectively. NOB varied from 2 to 15 for the newly developed F_1 crosses. Under NS condition, the cross LS01 × LS10 recorded the lowest NOB (2) at Loskop, and cross LS10 × LS17 recorded a NOB of 2 in Brits. Under DS, crosses LS10 × LS24 and LS01 × LS21 recorded the highest NOB (>7) in the Loskop site, whereas the highest NOB was recorded for crosses LS09 × LS24 and LS17 × LS18 in the Brits environment.

Regarding NOL, the tested okra genotypes recorded values ranging from 2 to 41 under DS condition. The highest NOL was recorded for crosses LS01 × LS21 and LS10 × LS24 under DS conditions at the Loskop site, whereas cross LS09 × LS24 recorded the highest NOL (11.5) under the Brits site. The NOL for LS09 × LS24 is higher than those recorded for the parental genotypes. For SD, the crosses LS01 × LS21 and LS10 × LS24 recorded the highest values of 23.84 and 23.68 cm, respectively, under DS condition at the Loskop site, whereas LS01 × LS21 recorded SD of 6.47 cm under DS condition in Brits site. PH of the newly developed F_1 crosses varied between 13.83 and 111.35 cm. Crosses LS21 × LS24 and LS15 × LS17 recorded the lowest PH of 29.46 and 18.83 cm for Loskop and Brits under NS condition. Compared to the parental genotypes, the PH of the crosses was lower (< 30 cm). FPL of the tested okra genotypes varied from 5.25 to 20.83 cm under DS conditions. The highest FPL was recorded for crosses LS09 × LS10 and LS09 × LS17 (>19 cm) under DS condition at the Loskop site, whereas crosses LS09 × LS15, LS10 × LS18 and LS15 × LS21 recorded FPL of >13 cm. Under DS condition, the highest NFPP values were recorded for crosses LS10 × LS24 and LS17 × LS18 (>13) for the Loskop site and crosses LS10 × LS17 and LS10 × LS24 (>20) for the Brits site. Crosses LS01 × LS10, LS10 × LS18, LS10 × LS21, LS15 × LS21 and LS18 × LS21 recorded the highest PYP of > 40 g/ plant under NS condition, whereas crosses LS01 × LS17, LS09 × LS21, LS09 × LS17, LS10 × LS21, LS15 ×LS17 and LS17 × LS18 recorded PYP > 25 g/plant in Loskop site. In addition, for Brits site, crosses LS01 × LS10, LS01 × LS15, LS09 × LS15, LS09 × LS21 and LS15 × LS21 recorded PYP of > 70 g/plant under NS condition, whereas

under DS condition crosses LS09 × LS24, LS10 × LS21, LS15 × LS18, LS18 × LS21 and LS21 × LS24 recorded PYP of > 40 g/plant. There was a reduction in DPW due to drought stress. DPW was reduced from 17.21 to 14.63 g for the Loskop site, whereas DS reduced DPW from 3.68 to 1.09 cm for the Brits site. Crosses LS01× LS24 and LS09 × LS10 recorded the highest DPW (>30 cm) under NS condition in Loskop. The crosses LS09 × LS17 and LS15 × LS24 recorded the highest SW of 0.97 and 0.84 g, respectively, whereas LS17 × LS21 and LS18 × LS21 recorded the highest HSW of 2.73 and 2.26 at the Brits site under DS condition. HSW of these crosses is higher than recorded for parental genotypes LS09, LS15, LS17, LS18, LS21 and LS24.

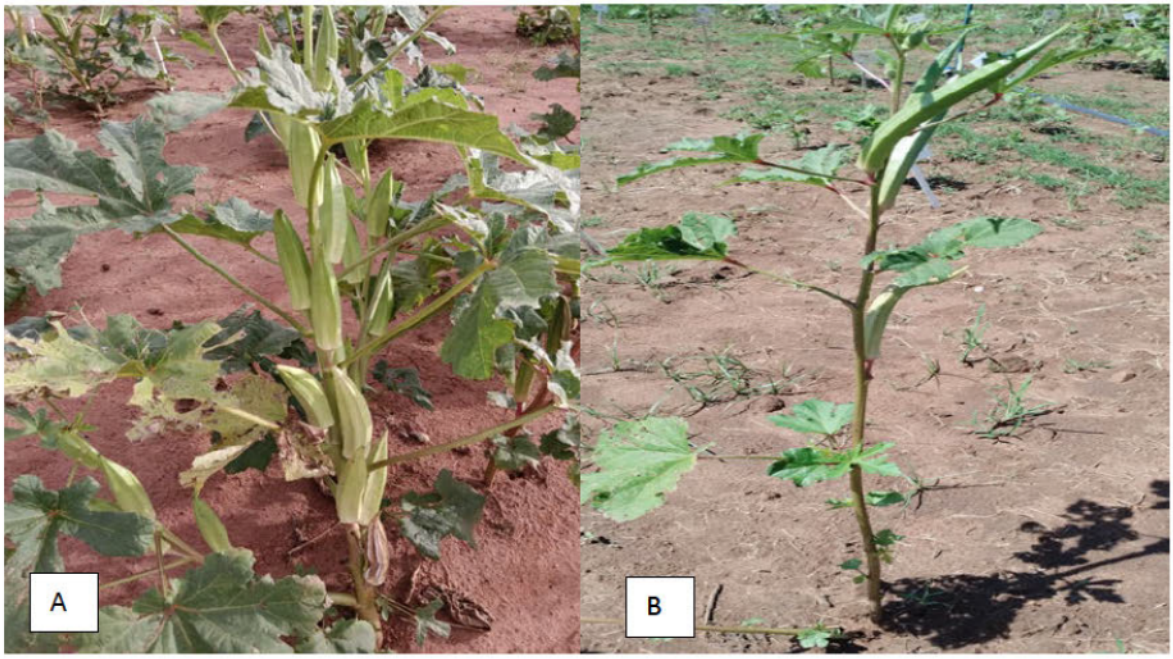

Figure 5.2: Pod formation of the cross LS15 × LS21 (A) under stress conditions and parent LS21 (B) under non-stress conditions at the Brits site in North-West Province in South Africa.

Table 5.3: Mean values for agronomic traits among 8 parental genotypes and 28 F_1 okra progenies evaluated under non-stressed (NS) and drought-stressed (DS) conditions at the Loskop site in Limpopo Province

Genotype	Traits																			
	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFPP		PYP (g/plant)		DPW (g/plant)		HSW (g)	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
Parents																				
LS01	47.69	55.01	2.67	3.67	46.33	34.00	28.79	20.92	106.90	105.33	22.64	11.67	19.50	5.00	24.17	12.17	21.86	23.43	0.89	0.27
LS09	62.70	62.51	6.00	2.50	19.17	20.33	12.85	25.22	47.03	59.50	10.23	18.58	5.17	9.33	58.43	27.45	21.49	22.00	0.27	0.43
LS10	47.04	51.89	3.50	3.00	25.17	19.00	25.69	21.78	79.48	75.83	19.85	15.00	13.83	11.83	47.24	17.30	21.12	11.63	0.75	0.29
LS15	54.84	55.20	6.00	5.00	20.67	24.83	21.24	16.42	63.26	48.83	18.58	10.83	7.50	4.67	30.54	23.99	15.81	15.37	0.78	0.49
LS17	50.38	54.52	2.17	2.33	28.33	24.83	17.26	16.34	75.85	67.50	20.07	19.33	11.17	4.17	33.49	14.89	20.77	9.00	1.44	0.27
LS18	54.75	50.56	5.00	6.00	22.83	21.67	16.47	17.38	73.85	52.50	8.92	16.67	10.67	10.00	33.50	12.85	20.72	10.73	1.40	0.18
LS21	45.50	55.24	6.67	3.00	44.33	39.50	27.79	19.91	98.00	117.83	17.73	10.33	11.50	8.00	33.35	12.61	14.47	6.92	1.25	0.49
LS24	55.46	57.93	3.17	4.33	36.17	36.83	21.96	20.30	70.41	41.50	17.17	10.67	16.33	14.17	19.91	11.60	14.06	13.42	0.10	0.07
Crosses																				
LS01XLS09	54.92	52.81	4.00	2.33	28.17	20.50	19.95	15.85	58.51	48.00	19.00	17.33	13.00	11.33	36.13	16.26	13.73	7.81	0.11	0.48
LS01XLS10	53.49	54.57	2.33	1.50	29.50	20.67	14.74	17.90	45.35	65.00	5.07	17.33	17.00	8.67	48.28	18.27	16.70	20.36	0.41	0.02
LS01XLS15	53.30	53.56	6.67	4.67	21.17	26.00	19.65	15.38	62.91	71.50	20.88	15.00	14.50	10.50	33.21	13.21	7.42	23.89	0.57	0.18
LS01XLS17	51.13	47.84	4.00	3.67	26.50	17.67	21.73	14.24	67.18	65.17	17.18	12.83	14.33	4.67	10.64	37.27	10.43	8.78	0.77	0.31
LS01XLS18	51.09	54.79	4.33	6.17	27.00	34.83	21.40	23.18	60.62	97.00	21.17	16.67	15.50	9.83	29.03	21.02	22.78	9.26	0.50	0.10
LS01XLS21	51.43	54.50	6.33	7.50	43.67	41.17	23.58	23.84	109.65	101.33	17.83	15.67	12.83	8.67	29.68	18.33	14.78	22.45	1.39	0.10
LS01XLS24	46.63	53.67	2.83	3.50	34.00	36.33	20.92	16.18	90.86	67.83	18.00	18.50	11.50	6.17	25.54	20.43	30.04	8.90	0.61	0.38
LS09XLS10	59.15	63.31	6.50	2.83	24.50	26.83	22.85	21.81	76.01	88.00	21.32	20.83	17.67	8.33	46.34	18.03	30.30	13.81	0.04	0.22
LS09XLS15	48.25	53.56	4.83	3.83	30.17	35.33	26.01	20.53	87.81	66.83	14.38	18.50	13.50	5.83	34.65	10.11	17.08	5.41	0.31	0.24
LS09XLS17	56.96	57.02	5.00	3.50	24.83	34.33	14.15	19.93	43.55	53.83	15.11	19.33	8.17	5.67	19.22	26.76	21.72	16.91	0.58	0.97
LS09XLS18	48.36	57.88	3.67	5.67	28.33	14.50	17.23	12.60	87.61	49.67	24.27	8.33	1 5.17	7.33	28.78	15.74	5.72	10.84	0.05	0.33
LS09XLS21	46.61	56.42	8.17	3.00	14.83	28.67	15.47	15.46	45.40	92.17	16.93	18.00	7.67	8.50	33.69	43.37	17.58	7.12	0.71	0.14

LS09XLS24	47.86	51.79	4.17	2.83	24.17	17.17	27.36	12.84	64.23	60.33	16.98	15.17	11.00	8.93	32.26	14.19	19.11	12.17	0.79	0.10
LS10XLS15	51.25	51.30	5.50	2.50	25.67	18.33	20.57	18.96	78.96	78.33	17.47	15.02	12.50	8.17	39.49	16.37	10.70	22.43	1.47	0.40
LS10XLS17	53.32	53.54	3.33	4.33	24.33	22.17	26.62	17.99	63.96	41.00	19.55	18.83	17.50	10.00	39.86	14.52	11.43	30.06	0.55	0.35
LS10XLS18	51.04	54.16	2.67	3.50	30.83	24.67	20.14	16.22	96.88	80.00	21.75	13.00	14.83	6.67	40.30	11.97	15.75	14.44	1.02	0.06
LS10XLS21	57.44	51.91	9.67	3.67	37.67	24.67	19.91	19.67	75.83	70.50	16.72	15.50	11.67	9.17	53.55	26.52	6.39	8.03	0.29	0.42
LS10XLS24	48.98	53.72	4.00	7.67	29.83	40.33	18.58	23.68	75.88	99.17	21.68	18.33	18.00	14.50	32.71	13.79	12.77	13.17	0.09	0.50
LS15XLS17	49.74	59.96	4.50	1.50	21.83	15.00	19.32	8.50	77.70	39.50	21.17	8.67	15.33	2.83	15.77	29.09	10.73	24.01	1.07	0.49
LS15XLS18	50.46	57.63	6.00	4.33	37.67	13.17	20.23	15.69	50.68	44.50	18.80	9.17	13.67	10.00	26.85	13.16	17.13	20.44	0.11	0.81
LS15XLS21	51.97	49.78	3.00	5.50	28.83	15.83	18.17	18.94	66.92	94.00	20.65	15.00	11.50	9.17	42.21	12.36	26.83	13.73	0.34	0.61
LS15XLS24	50.24	50.53	7.00	2.67	22.00	11.50	20.38	13.65	105.00	45.67	20.70	12.00	10.00	5.83	31.84	21.92	18.27	15.80	0.50	0.84
LS17XLS18	48.35	51.68	5.37	4.83	19.17	33.67	18.49	21.91	63.01	82.33	18.50	13.83	10.33	13.33	27.67	28.52	20.26	12.52	0.49	0.31
LS17XLS21	50.48	52.82	5.00	2.33	40.33	22.83	24.77	19.26	89.35	63.17	19.12	13.00	11.00	3.17	15.90	17.30	29.42	18.39	1.05	0.34
LS17XLS24	53.05	51.36	3.73	5.67	28.00	32.50	14.64	16.60	111.35	90.17	15.70	11.00	17.67	8.00	28.66	15.35	18.75	14.45	1.07	0.55
LS18XLS21	48.74	60.04	3.50	5.33	22.33	19.17	17.38	15.79	50.41	50.50	17.13	7.17	11.00	5.83	50.16	19.61	21.12	2.09	0.44	0.32
LS18XLS24	54.99	51.46	7.75	5.75	31.00	29.00	29.47	20.58	93.19	78.25	20.23	10.00	20.50	12.25	38.61	10.55	7.01	12.78	0.59	0.37
LS21XLS24	55.26	55.42	8.00	4.83	24.67	18.50	16.08	17.64	29.46	58.50	13.10	10.67	5.00	8.33	12.57	14.99	15.38	24.11	1.47	0.60
Mean	51.75	54.44	4.92	4.03	28.44	25.45	20.61	18.14	73.42	69.75	17.93	14.38	13.00	8.30	32.90	18.66	17.21	14.63	0.67	0.36
P. value	*	ns	ns	ns	*	*	ns	ns	*	*	*	*	*	ns	*	*	*	ns	ns	ns
S.E.	5.21	6.78	3.63	2.93	10.02	10.84	6.43	6.73	23.91	29.47	5.45	5.56	4.90	5.34	11.55	11.08	9.60	10.50	0.78	0.41
LSD (5%)	8.73	11.36	6.10	4.90	16.79	18.17	10.78	11.28	40.33	49.37	9.12	9.32	8.20	8.95	19.36	18.57	16.09	17.59	1.30	0.69
CV(%)	10.08	12.46	75.00	72.56	37.71	22.73	32.35	37.27	34.39	23.01	30.34	28.48	37.84	64.76	35.95	29.24	25.55	71.77	116.30	113.60

DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight, *: significant at 5% probability level, **: significant at 1% probability level, ns: non-significant.

Table 5.4: Mean values for agronomic traits among 8 parental genotypes and 28 F_1 progenies of okra evaluated under non-stressed (NS) and drought-stressed (DS) conditions in Brits site in North-West Province

Genotype	Traits																			
	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFPP		PYP (g/plant)		DPW (g/plant)		HSW(g)	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
Parents																				
LS01	56.00	77.67	14.67	1.17	7.50	1.50	6.60	0.85	44.17	68.50	26.03	11.29	5.16	1.70	46.37	6.80	6.00	0.50	1.29	0.24
LS09	63.67	45.00	6.17	5.67	6.67	10.33	7.65	5.30	37.67	85.33	21.24	12.42	20.50	8.57	24.23	50.23	11.00	5.50	0.66	2.83
LS10	62.67	81.67	11.33	2.67	15.83	5.00	5.77	3.41	53.33	33.00	14.34	7.65	13.75	7.09	56.03	10.50	6.50	6.50	0.83	0.20
LS15	62.11	44.00	7.17	5.67	18.00	7.17	8.50	5.38	49.33	38.67	12.05	7.65	8.54	4.24	75.67	75.87	4.50	1.50	1.27	0.56
LS17	60.56	46.00	11.83	2.00	17.17	2.67	8.62	2.50	72.33	21.67	15.24	13.00	12.64	5.27	36.83	9.90	7.50	2.00	1.69	0.17
LS18	59.89	79.00	7.33	4.83	18.83	4.67	9.45	3.37	61.00	36.67	33.97	11.06	25.54	19.32	93.53	13.30	5.50	6.00	1.71	0.17
LS21	63.33	71.00	3.33	2.83	7.83	4.50	7.62	3.20	56.33	96.50	14.07	11.00	7.73	16.75	79.00	7.90	8.50	3.00	1.34	0.14
LS24	54.56	71.67	5.33	3.50	6.50	7.17	7.48	5.57	36.50	75.00	9.01	12.26	7.04	24.26	43.90	21.87	7.00	5.00	0.67	1.45
Crosses																				
LS01XLS09	60.33	55.00	7.67	4.17	8.67	4.67	8.72	3.85	75.33	35.17	14.61	8.50	10.76	9.77	57.87	35.03	8.00	2.00	1.50	1.93
LS01XLS10	64.00	52.00	12.17	3.33	6.17	4.33	10.17	2.93	36.67	22.00	13.74	13.46	12.37	18.70	89.27	5.40	7.50	5.50	2.13	0.31
LS01XLS15	55.44	73.00	6.50	3.67	12.50	3.17	9.02	1.55	54.67	16.67	9.75	7.55	13.58	8.12	93.60	8.30	6.00	2.00	1.29	0.06
LS01XLS17	58.56	45.00	6.00	1.83	14.17	2.33	7.57	4.20	65.83	15.67	12.19	10.70	7.82	16.71	37.03	8.87	6.50	3.50	1.43	0.09
LS01XLS18	51.00	57.33	3.83	5.50	8.67	6.33	7.10	2.93	66.67	84.00	5.90	12.10	11.09	14.80	64.23	20.63	6.00	8.00	1.08	0.10
LS01XLS21	63.11	77.00	6.50	5.17	16.17	8.00	6.28	6.47	55.67	39.33	27.79	9.00	23.65	15.77	39.20	26.97	5.00	1.00	1.25	0.42
LS01XLS24	55.78	78.67	13.50	6.67	7.50	7.17	7.42	2.75	38.83	37.83	10.03	10.00	8.29	9.07	29.07	6.33	13.50	1.50	1.08	0.31
LS09XLS10	61.78	50.00	14.67	4.33	7.00	5.17	6.65	3.52	54.33	73.00	16.24	12.70	15.56	9.80	51.10	16.90	11.00	7.50	0.94	1.25

LS09XLS15	64.11	76.67	8.67	4.50	7.83	4.83	9.20	3.88	45.83	95.33	32.48	13.26	15.44	18.19	40.13	21.03	11.50	4.00	1.19	0.28
LS09XLS17	61.56	56.67	6.17	6.17	17.33	8.33	8.30	4.17	45.67	46.00	11.82	9.50	6.74	6.73	72.17	30.83	9.50	3.50	1.38	0.62
LS09XLS18	52.00	69.00	7.67	4.83	7.83	6.33	7.58	4.82	55.83	27.83	17.61	7.68	12.46	5.37	21.60	31.70	4.00	4.00	1.05	0.97
LS09XLS21	55.78	73.67	6.00	4.17	8.50	5.17	7.33	4.22	45.00	46.50	8.72	8.65	7.36	5.23	81.80	24.07	8.50	2.00	0.90	0.50
LS09XLS24	59.11	74.67	6.00	7.50	6.17	11.50	6.82	4.63	51.83	48.17	10.36	4.30	20.99	2.29	13.23	48.33	12.00	1.00	0.24	1.97
LS10XLS15	55.89	66.67	12.00	2.33	6.00	5.33	5.82	2.62	83.17	49.67	35.07	11.00	9.45	19.28	35.13	1.50	11.50	7.00	1.61	0.14
LS10XLS17	58.00	72.67	1.83	5.17	13.00	8.50	1.33	3.82	29.17	87.67	9.25	17.62	4.75	21.46	5.57	39.43	2.00	4.00	1.24	0.75
LS10XLS18	53.89	73.00	6.67	3.50	13.00	4.83	5.23	3.85	58.00	24.83	13.28	13.41	17.33	12.28	42.80	27.13	9.50	3.50	0.67	0.58
LS10XLS21	62.67	61.33	6.83	7.83	6.50	8.83	8.15	4.00	58.50	75.17	9.61	12.59	24.21	4.65	23.37	55.80	9.50	5.50	0.43	0.79
LS10XLS24	61.67	64.00	5.33	3.83	9.33	7.50	7.58	3.93	44.17	26.00	23.12	7.15	24.64	21.04	32.53	22.67	6.00	2.00	0.85	0.47
LS15XLS17	49.11	65.33	1.33	4.00	7.67	2.50	5.27	2.52	53.17	13.83	33.45	8.08	6.83	9.23	16.93	7.57	4.00	1.50	0.56	0.06
LS15XLS18	60.11	69.00	5.83	4.33	6.17	5.33	6.73	4.21	49.67	39.83	11.19	11.62	20.23	11.16	6.57	81.20	10.50	2.00	0.44	1.83
LS15XLS21	57.22	56.00	7.83	3.83	19.00	5.33	8.08	2.67	65.67	76.50	14.60	13.14	14.37	17.15	95.27	7.77	10.00	13.00	1.62	0.73
LS15XLS24	49.44	48.33	4.67	5.67	10.00	8.00	6.22	3.55	40.00	69.83	9.06	8.76	13.76	9.28	21.60	22.07	5.00	4.00	0.52	0.42
LS17XLS18	53.11	70.00	4.00	7.50	9.00	7.33	5.10	3.78	47.67	96.67	12.70	10.83	11.20	14.07	46.63	31.17	3.50	1.50	1.22	0.90
LS17XLS21	56.67	62.67	7.00	3.67	3.67	3.67	4.35	4.73	62.33	33.17	14.60	11.50	18.87	7.29	30.83	34.87	4.50	3.00	0.51	2.73
LS17XLS24	63.33	63.33	14.50	3.67	7.50	4.33	6.08	3.52	48.00	23.17	26.48	5.25	9.18	12.62	18.23	11.67	9.50	1.00	0.21	0.22
LS18XLS21	56.67	65.00	5.33	4.33	9.50	6.00	9.25	4.87	59.50	46.83	11.14	6.40	7.73	12.34	41.17	56.23	6.00	1.50	0.52	2.26
LS18XLS24	52.17	87.50	2.00	4.00	6.50	9.75	4.33	5.65	37.00	26.00	7.30	11.50	28.24	2.89	39.10	42.20	14.00	4.00	1.85	0.69
LS21XLS24	61.67	69.33	6.17	3.50	3.67	5.50	5.75	4.59	47.50	86.83	29.16	11.05	19.06	12.27	26.77	28.60	2.50	3.50	2.11	0.61
Mean	58.25	65.25	7.33	4.37	9.93	5.92	7.03	3.83	52.40	50.52	16.59	10.38	13.80	11.52	45.23	26.41	7.60	3.68	1.09	0.77
P. value	ns	*	*	ns	*	ns	ns	*	ns	*	*	ns	ns	*	*	*	ns	*	ns	*
S.E.	7.01	11.70	2.57	2.52	3.97	3.51	3.23	2.72	25.50	23.54	3.63	4.01	9.10	6.23	38.66	32.84	4.31	4.42	0.59	0.69
LSD (5%)	11.74	19.61	4.30	4.22	6.68	5.89	5.41	4.61	42.73	39.45	7.65	8.91	19.09	14.16	64.77	55.03	8.71	5.11	1.59	1.18
CV(%)	12.00	16.98	39.91	57.77	29.67	59.89	45.82	31.71	48.64	31.42	27.80	39.17	67.35	32.11	19.66	26.24	54.73	27.42	88.31	10.20

DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight,*: significant at 5% probability level, **: significant at 1% probability level , ns: non-significant.

5.3.3 Combining ability analysis of okra parents and F_1 progenies for agronomic traits

Mean squares and significant tests for GCA and SCA effects for the assessed traits across the test sites are presented in Table 5.5. The GCA effect was significant for DTF, NOL, SD, PH, FPL, NFPP and PYP, whereas the SCA effect was significant for all studied traits except SD and HSW. The GCA × site interaction effect was significant for DTF, NOL, FPL and NFPP, whereas the SCA × site interaction effect was significant for DTF, NOL, FPL and PYP.

5.3.3.1 General combining ability effect of parental genotypes for agronomic traits

The GCA effect of parental genotypes for assessed agronomic traits under NS and DS conditions for Loskop and Brits sites are presented in Table 5.6. Negative and significant ($P<0.05$) GCA effects for DTF in a desirable direction were recorded for LS01 and LS18 under the NS condition at the Loskop site. Parental genotype LS21 recorded a desirable significant ($P<0.05$) GCA effect for NOB under NS condition in Brits. Under DS condition, a positive and significant ($P<0.05$) GCA effect for NOL was recorded for parental genotypes LS15 in Loskop, whereas positive and significant ($P<0.05$) was recorded for LS10 and LS21 in Brits sites.

Parental genotypes LS09, LS17 and LS24 yielded the highest and positive GCA effect for SD under NS in the Loskop and Brits sites, whereas LS01, LS15, LS21 and LS24 were positive and significant ($P<0.05$) under DS for Loskop and Brits sites. The GCA effect for PH was positive and significant ($P<0.05$) for LS17 and LS18 under NS condition for Loskop and Brits sites, whereas LS10, LS17 and LS18 recorded the highest and positive GCA effect for PH under DS condition for Loskop and Brits sites. Regarding FPL, positive and significant ($P<0.05$) GCA effects were recorded for LS09, LS15, LS17 and LS24 under NS in Loskop and Brits sites. The GCA effect for FPL was positive and significant for LS15 and LS24 under DS conditions at the Loskop and Brits sites.

Parental genotypes LS15 and LS17 recorded positive and significant ($P<0.01$) effects for NFPP under NS conditions at Loskop and Brits sites. The GCA effect for NFPP was positive and significant ($P<0.05$) for LS09, LS15 and LS24 under DS conditions for Loskop and Brits sites. LS09, LS10, LS18 and LS21 recorded positive and significant ($P<0.05$) GCA effect for PYP under NS condition for Loskop and Brits sites, whereas positive and significant ($P<0.05$) effect were noted for LS10 and LS24 under DS condition for Loskop and Brits sites. Regarding HSW, parents LS09 and LS21 recorded positive and significant ($P<0.05$) GCA effect under the NS condition in Loskop and Brits sites, whereas LS10 and LS17 showed positive and significant ($P<0.05$) differences for HSW under the DS condition at Brits site.

Table 5.5: Mean squares and significant tests for GCA and SCA effects for agronomic traits among 8 okra parents and their 21 F_1 progenies in the Loskop and Brits sites

Source of variation	d.f.	Traits									
		DTF	NOB	NOL	SD	PH	FPL	NFPP	PYP	DPW	HSW
Sites	1	12082.08**	97.95**	39130.63^{ns}	19917^{ns}	60235^{ns}	1712.38^{ns}	126.27*	9028.63**	9239.30**	7.89**
Replication (Rep)	3	61.49**	18.91**	236.41**	16.89^{ns}	758.55**	100.24**	54.54^{ns}	72.54^{ns}	45.15^{ns}	0.54*
Genotype (G)	35	102.39*	16.91*	118.30*	50.75*	1304.29*	37.01*	103.18*	903.80*	98.5*	0.63^{ns}
GCA	7	43.88*	5.32^{ns}	61.41*	41.34*	1378.72*	144.84**	133.33**	518.67*	47.07	0.13^{ns}
SCA	28	53.02*	9.24*	58.59*	21.38	497.04*	17.03*	147.26**	435.21*	63.76*	0.36^{ns}
Gen × site	35	170.77^{ns}	14.96**	123.55**	47.54**	1302.90^{ns}	22.28*	53.96**	544.45*	62.45*	0.91**
GCA × site	7	54.98*	3.41^{ns}	84.14*	25.1^{ns}	850.68^{ns}	15.75*	70.13*	300.36^{ns}	21.78^{ns}	0.41^{ns}
SCA × site	28	92.98**	8.5	56.18*	23.44^{ns}	584.9^{ns}	12.10*	40.98^{ns}	465.19*	25.89ns	0.48^{ns}
Residual	280	68.92	7.54	62.06	22.45	542.2	28.16	35.17	771.42	69.82	0.51

Note: Gen: genotype, d.f: degree of freedom, DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight, GCA: general combining ability, SCA: specific combining ability, *: significant at the 5% probability level, **: significant at 1% probability level ns: non-significant.

Table 5.6: General combining effects for agronomic traits among 8 parental okra genotypes evaluated under non-stressed (NS) and drought-stressed (DS) conditions at Loskop and Brits sites

Genotypes	Traits																			
	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFPP		PYP (g/plant)		DPW (g/plant)		HSW (g)	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
Loskop																				
LS01	-1.89*	0.80ns	-0.06ns	-0.50*	3.89*	0.30ns	-2.87*	-1.82ns	2.65ns	1.83ns	0.69ns	-0.10ns	0.33ns	-1.68*	-3.83*	-2.96*	-4.09*	4.58*	0.13ns	-0.03ns
LS09	0.34ns	0.42ns	-0.32ns	-0.15ns	-1.18ns	-0.37ns	2.84*	-0.69ns	-10.02*	-14.29*	-0.78ns	-1.98*	-3.12**	1.77*	3.58*	2.37*	-2.29ns	0.84ns	0.27*	0.07ns
LS10	0.14ns	0.71ns	0.01ns	-0.40ns	-0.58ns	-1.87ns	-0.28ns	-1.85ns	0.71ns	-6.05ns	-0.90ns	-0.15ns	0.55ns	0.59ns	5.05*	3.18**	3.85*	-1.25ns	0.07ns	0.06
LS15	1.99*	-0.98*	0.82*	1.65**	1.33ns	6.27*	2.19*	3.63*	0.45ns	18.91	1.81*	1.59*	2.48**	1.63*	-1.93ns	-0.41ns	-2.19ns	-1.95	-0.14ns	-0.17*
LS17	0.54ns	1.20*	0.51ns	-0.56*	1.91ns	-3.28ns	-0.56ns	0.44ns	8.26*	4.01**	0.58ns	-1.78	-0.3	0.26ns	0.45ns	1.65ns	5.98**	-3.21*	0.06ns	-0.04ns
LS18	-2.53**	-1.04*	0.18ns	-0.13ns	-1.93ns	2.47ns	-0.29ns	1.07ns	-1.21ns	8.41ns	1.37ns	-1.03ns	-1.44ns	0.38ns	1.61ns	0.48ns	-0.73ns	-1.55ns	-0.03ns	-0.04ns
LS21	-0.12	1.34*	-0.37ns	-0.30ns	1.88ns	-3.88*	0.13ns	-2.38*	4.87ns	-9.22*	1.57ns	-0.95*	0.51ns	0.78ns	3.01*	-2.33*	1.03ns	-0.73	0.21*	0.11ns
LS24	1.54*	0.21ns	0.87*	0.37ns	-1.58ns	0.37ns	2.87*	1.60ns	-1.72ns	-3.59ns	3.17**	0.77ns	0.99ns	-0.20ns	0.13ns	2.75*	-1.56ns	3.20*	-0.07	0.04ns
Genotypes	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFPP		PYP (g/plant)		DPW (g/plant)		HSW (g)	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
Brits																				
LS01	0.69ns	2.85ns	0.09ns	0.19ns	0.03ns	0.60ns	-0.13ns	0.76*	-3.50ns	-1.66ns	0.75ns	*1.30*	-4.45**	-2.78*	-10.29	-10.17*	1.88*	-0.71ns	-0.80*	-0.90*
LS09	-0.31ns	-2.52ns	-0.21ns	0.80*	0.70ns	-0.68ns	-1.50*	-0.14ns	-6.63*	-4.08ns	1.04*	-0.48ns	-2.68*	-0.98ns	-13.36*	5.74ns	-1.88*	-0.86*	1.07*	-0.03ns
LS10	0.86ns	-0.18ns	0.59ns	0.43ns	-0.75ns	1.39*	-1.46*	0.32ns	4.52ns	7.86*	-1.99*	-2.09**	-2.44*	-0.89ns	15.65*	11.74*	-0.53ns	-0.31cf	-0.15ns	0.58*
LS15	-0.86ns	-0.05ns	-0.19ns	-0.23ns	-0.25ns	-0.01ns	0.22ns	0.30ns	-1.52ns	-6.41*	1.38*	1.34*	4.44**	2.70*	4.63ns	4.43ns	-0.88ns	0.94*	0.20ns	-0.01ns
LS17	1.78*	-0.65ns	-0.63ns	-0.81*	-1.37*	-1.38*	1.62*	-0.87*	1.12	-3.06	1.02*	0.41	5.09**	-0.82	-7.9	-15.77**	-0.18	-0.31	0.41	0.59*
LS18	-1.95*	5.08*	0.04ns	-0.13ns	0.53ns	-0.50ns	0.08ns	-0.41ns	9.65*	11.86**	0.58ns	-0.08ns	0.08ns	0.87ns	18.99*	-15.07ns	-0.38ns	0.89*	0.14ns	0.40ns
LS21	-3.46**	-2.35ns	-0.84*	0.75*	1.67*	1.85*	-0.43ns	0.89*	1.55ns	-5.48ns	-1.27*	0.67ns	-0.04ns	-0.69ns	-7.68ns	-2.40ns	-0.73ns	0.19ns	-1.50*	0.37ns
LS24	3.26**	-2.18	1.14*	-0.01	-0.57	-0.28ns	0.59ns	0.73*	-5.18*	0.96ns	-1.52*	1.53*	-0.05ns	2.59*	-5.75ns	11.44*	2.68**	0.19ns	-0.04ns	0.20ns

DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, DPW: dry pod weight per plant, HSW: hundred seed weight, PYP: pod yield per plant, *: significant at 5% probability level, **: significant at 1% probability level, ns: non-significant.

5.3.3.2 Specific combining ability effects for agronomic traits for crosses

The SCA effects of crosses for studied agronomic traits under NS and DS conditions for Loskop and Brits sites are presented in Tables 5.7 and 5.8, respectively. Crosses LS01 × LS24 and LS09 × LS21 displayed high and negative ($P<0.001$) SCA effects for DTF under NS condition for Loskop and Brits sites, whereas LS01 × LS18, LS10 × LS17, LS10 × LS24 and LS18 × LS24 recorded high and negative ($P<0.001$) SCA effects under DS condition in the same sites. Crosses LS01 × LS15, KS01 × LS24, LS10 × LS18, LS10 × LS24, LS15 × LS24, LS17 × LS24 and LS21 × LS24 recorded the highest positive and significant ($P<0.05$) SCA effects for NOB under NS condition for Loskop and Brits sites, whereas under DS condition crosses LS15 × LS21, LS17 × LS24 and LS18 × LS21 recorded positive and significant ($P<0.001$) SCA effects for NOB for Loskop and Brits sites. Regarding NOL, crosses LS01 × LS18, LS15 × LS24, LS17 × LS24, LS09 × LS15, LS09 × LS17, and LS15 × LS21 recorded positive and significant ($P<0.01$) SCA effects under NS condition for Loskop and Brits, whilst crosses LS09 × LS24, LS10 × LS17, LS10 × LS18, LS15 × LS21, LS18 × LS21 and LS21 × LS24 recorded positive and significant ($P<0.05$) SCA effects under DS condition under similar sites. Under DS condition, crosses LS09 × LS10 and LS15 × LS17 had desirable SCA effects for SD at Brits site.

Crosses LS01 × LS09, LS09 × LS24 and LS17 × LS24 had desirable SCA effects for PH under DS conditions in both sites. Crosses LS01× LS09, LS09 × LS17, LS10 × LS17, LS10 × LS18, LS10 × LS24, LS15 × LS17 and LS21 × LS24 displayed positive and significant ($P<0.05$) SCA effects for FPL under NS condition in Loskop and Brits sites, whereas crosses LS01 × LS17, LS01 × LS18, LS01 × LS21, LS09 × LS18, LS10 × LS15 and LS21 × LS24 had desirable effects for the same trait under DS condition in both sites. A positive and highly significant ($P<0.001$) SCA effect was exhibited by crosses LS10 × LS15, LS10 × LS24 and LS15 × LS17 for NFPP under NS condition at the Loskop and Brits, whereas LS09 × LS15, LS10 × LS17, LS10 × LS21 and LS15 × LS21 had desirable SCA effects under DS condition in the same sites.

In Loskop under NS condition, crosses LS01 × LS01, LS01 × LS21, LS09 × LS10, LS10 × LS15, LS15 × LS17, LS17 × LS18 and LS18 × LS24 recorded positive and significant ($P<0.05$) SCA effect for PYP, whereas in Brits crosses LS01 × LS09, LS01 × LS10, LS01 × LS18, LS01 × LS21 and LS10 × LS15 had desirable effects for PYP. Under DS condition, crosses LS01 × LS17, LS01 × LS18 and LS18 × LS21 recorded positive and highly significant ($P<0.001$) SCA

effects and crosses LS01 × LS10, LS01 × LS21, LS09 × LS10, LS09 × LS18, LS09 × LS24, LS15 × LS18, LS15 × LS21 and LS17 × LS21 recorded positive and significant ($P<0.05$) SCA effects for PYP at Loskop. Whereas crosses LS09 × LS10, LS09 × LS18, LS10 × LS15, LS15 × LS18, LS15 × LS21, LS15 × LS24 and LS18 × LS24 displayed positive and significant ($P<0.05$) SCA effects at Brits site under DS condition. Crosses LS09 × LS17 and LS18 × LS21 recorded desirable SCA effects under DS conditions at Loskop for DPW. Positive and significant ($P<0.05$) SCA effect for DPW was recorded for crosses LS09 × LS15, LS09 × LS18 and LS18 × LS21 under NS conditions at Loskop and Brits sites. Under DS condition, crosses LS01 × LS10, LS01 × LS17, LS01 × LS18, LS10 × LS18 and LS15 × LS24 exhibited desirable SCA effects for DPW at Brits. Crosses LS18 × LS21 and LS15 × LS21 recorded positive and significant ($P<0.001$) SCA effects for HSW under NS conditions for Loskop and Brits sites, respectively, whereas LS01 × LS17 recorded positive and significant ($P<0.001$) SCA effects for HSW under DS condition in Loskop and in Brits sites.

Table 5.7: Specific combining effects for agronomic traits among 28 F_1 okra progenies evaluated under non-stressed (NS) and drought-stressed (DS) conditions at the Loskop site in Limpopo Province

Crosses	Traits																			
	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFPP		PYP (g/plant)		DPW (g/plant)		HSW (g)	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
LS01×LS09	2.46	10.56**	-1.12	-0.72	2.39	8.86	-7.99*	1.43	21.21*	-4.21	6.99*	1.14	-3.31	1.54	8.48*	-6.23	-2.66	2.67	-0.39	0.46*
LS01×LS10	-0.62	-4.04*	-0.96	-0.64	0.95	-13.14*	4.95*	0.50	-2.15	-31.11*	-1.29	-2.97*	2.53	-3.83*	-3.97	7.74*	-4.35	9.21*	0.05	-0.11
LS01×LS15	-2.65	-0.03	5.13**	-2.19*	*2.25	2.06	5.13*	1.92	8.42	0.77	-1.17	0.72	-0.91	-2.19	1.04	5.8	-2.14	1.53	-0.46	-0.13
LS01×LS17	-3.42*	4.62*	-1.46	4.86	-3.98	6.11	5.02*	1.92	12.11	10.64	3.40	2.97*	2.33	-0.99	-1.21	15.31**	-2.65	6.72*	-0.51*	0.55**
LS01×LS18	3.28*	-2.30	-0.03	-0.57	8.77*	-13.97*	-1.11	0.39	-1.54	12.09	2.11	-1.88	2.63	5.56**	-6.41	10.53**	2.24	-8.49*	0.57*	0.15
LS01×LS21	0.69	1.81	-2.2	-1.24*	3.54	0.71	0.36	2.34	-22.75*	-1.91	0.68	3.77*	1.84	-3.34	9.06*	7.93*	9.72*	-1.78	0.02	-0.21
LS01×LS24	-3.68	-0.23	4.68**	1.26*	1.51	8.96	-0.59	7.44*	36.81**	13.14	-3.41	-1.28	0.23	-6.97**	-4.04	1.39	1.21	-9.19*	-0.55*	-0.26
LS09×LS10	13.09	4.77*	-2.53*	-0.16	4.81	1.53	-5.38*	2.81	-18.79*	-20.16	-7.14*	-2.31*	4.52	-3.24*	5.68*	7.23*	-5.30	3.42	-0.09	-0.25
LS09×LS15	-2.34	-0.02	2.64*	6.13	4.55	5.06	1.71	-0.19	7.07	-8.08	2.01	-6.56**	-2.29	1.77	-3.98	-16.68	9.88*	4.32	1.13**	0.22
LS09×LS17	0.03	3.53	-2.36	-0.66	-2.21	4.11	0.99	-0.19	-4.50	-12.66	4.63*	1.92	1.66	3.99*	2.14	4.70	6.33	2.11	0.83*	-0.32*
LS09×LS18	-0.45	-1.35	-1.3	-0.26	3.37	-1.30	4.26	0.21	8.96	0.08	2.54	4.67*	1.13	-3.19	-10.22	9.95*	-10.24*	-2.43	-0.05	-0.1
LS09×LS21	-3.61*	1.01	1.09	-1.59*	5.55	-0.28	3.38	-1.17	1.95	-5.28	3.86	-2.84	5.91*	0.91	2.69	-6.27	10.21*	-6.95*	-0.63*	-0.38*
LS09×LS24	-1.64	1.41	-0.76	-0.59	-0.76	10.30*	-9.59**	2.45	19.14*	35.75**	2.46	3.60	5.59*	1.81	-8.98	7.82*	6.11	-1.88	0.36	-0.43*
LS10×LS15	2.68	4.10*	0.81	3.88	-4.21	1.40	-1.27	0.89	-12.53	-4.8	-1.09	2.44	7.88**	2.36	9.76*	-2.47	5.79	-3.12	1.72	-0.10
LS10×LS17	-1.03	-0.28	-2.36	1.43*	1.86	12.78*	0.38	0.89	23.41*	-1.55	1.79*	1.75	-0.44	-0.61	-5.17*	3.35	-7.49*	0.08	-0.09	-0.17
LS10×LS18	-1.09	-0.30	5.23**	-0.01	-2.19	14.2*	5.81*	2.95	5.96	3.20	0.32*	-2.83*	-0.02	2.78	-7.02	-5.8	0.61	9.91*	-0.29	-0.07
LS10×LS21	-1.03	5.64*	0.48	-0.84	-3.17	-0.79	-1.28	-1.19	-13.02	16.34	3.03	-4.68	-1.31	9.04**	-9.14	-2.8	-1.3	6.06	0.61*	0.03
LS10×LS24	-3.01	-3.00	6.35**	-0.51	-5.59	6.96	-8.25**	-8.75*	0.28	17.04	5.22*	2.60	8.55**	2.23	5.50	2.17	-7.69	4.33	0.02	-0.37*
LS15×LS17	-2.94	-3.89	2.81*	-2.29**	-0.29	8.15	3.56	-0.19	7.13	23.39*	-0.65	0.00	-1.49	-2.64	7.82*	0.98	-4.44	-4.47	-0.37	-0.09
LS15×LS18	0.85	-0.11	-2.2	-2.39*	6.04	-2.77	0.12	-2.59	8.21	10.64	-1.00	0.42	-2.94	-1.26	1.92	8.11*	13.99**	0.23	0.34	-0.12
LS15×LS21	1.88	-3.68	-1.81	2.61**	4.23	0.25	8.78**	0.37	-13.9	-10.9	1.63	-0.26	-1.84	7.84**	5.37	9.52*	-5.90	0.81	-0.5	0.28
LS15×LS24	10.18**	10.00**	1.39	-3.39	10.45*	-3.17	1.21	0.26	-14.84	15.97	-1.53	1.85	-0.16	-2.64	-0.34	5.87	6.89	9.32*	-0.02	-0.07
LS17×LS18	-2.67	-2.46	-2.03	-0.84	3.94	2.61	0.58	-3.66	19.46*	-10.83	0.47	-0.27	3.34	-3.89*	9.86*	5.92	2.57	2.01	-0.38	0.42*
LS17×LS21	0.95	5.32*	0.89	-1.17	0.32	2.37	12.24*	-1.41	6.11	1.47	0.19	-5.12*	-0.59	-4.46*	0.51	8.72*	-11.48*	8.92*	-0.61*	-0.13
LS17×LS24	11.49	8.78*	-1.22	2.17**	11.35*	-16.79**	-0.32	-1.33	33.14**	1.50	-1.66	-0.26	-3.44	0.33	3.99	-16.21	-8.61*	-7.96*	-0.29	0.19
LS18×LS21	6.22**	-0.48	0.64	6.39	3.06	7.55*	7.85**	1.29	-2.76	-7.11	-7.05*	1.97	1.19	5.59*	2.92	10.01**	5.93	-10.07*	1.27**	-0.02
LS18×LS24	-3.74*	-1.61	2.89*	1.11	-3.42	3.04	-3.41	0.90	-11.37	-23.58*	-7.90**	2.92	2.91	4.53*	10.55*	4.89	-6.63	1.08	-0.03	0.12
LS21×LS24	-2.24	3.84	6.28**	0.94	-0.02	1.31	6.29*	6.59	10.09	-2.83	5.29*	490*	-0.89	-1.66	8.69*	4.99	14.08**	-0.69	-0.45	0.17

DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, , PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight *: significant at 5% level of probability, **: significant at 1% level of probability.

Table 5.8: Specific combining effects for agronomic traits among 28 F_1 okra progenies evaluated under non-stressed (NS) and drought-stressed (DS) conditions at the Brits site in North-West Province

Crosses	Traits																			
	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFPP		PYP (g/plant)		DPW (g/plant)		HSW (g)	
	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS	NS	DS
LS01×LS09	-8.33**	-0.91	-0.3	1.87	-1.4	1.71	1.79	0.21	-3.96	32.25**	-3.55*	-2.09	-1.19	-1.88	33.03*	-6.25	2.53	-0.49	-0.21	0.03
LS01×LS10	3.06	15.09*	0.23	-1.14	2.72	-2.02	1.06	-1.00	8.06	18.86*	-0.59	-3.38*	4.76	-2.88	44.12*	-13.82	-0.88	1.96	0.39	-0.2
LS01×LS15	5.89*	-0.04	0.52	-0.16	-0.45	-0.12	-0.15	-0.47	-8.41	-4.09	-2.68	2.36	-4.04	2.40	-2.13	-3.00	1.97	-2.29*	-0.17	-0.19
LS01×LS17	0.69	-0.44	0.28	0.92	-1.67	1.41	-1.39	0.24	-6.21	-10.77	2.18	-0.84	7.58*	-3.17	-33.43*	7.17	-4.73**	-0.54	-0.67	0.11
LS01×LS18	1.54	-0.44	0.62	-0.09	1.77	-0.64	0.40	0.91	28.92**	-14.36	2.16	3.08*	6.89*	3.08	43.04*	11.7	-0.53	5.26*	0.41	0.53
LS01×LS21	5.60*	10.96*	1.33	-0.86	-0.03	0.68	1.54	-1.12	-0.31	6.14	-2.24	3.35*	-0.96	-0.93	42.55*	-5.24	1.32	-1.54	0.33	-0.07
LS01×LS24	-5.56*	8.79*	-3.82*	0.62	-1.8	2.48	-2.22	-1.12	-11.24	-1.62	-6.01**	0.87	-7.02*	6.01*	-8.78	7.98	1.92	-0.04	0.33	-0.04
LS09×LS10	2.06	-1.54	-1.3	0.64	-2.12	1.09	-0.16	4.38**	*16.31*	-3.77	-0.76	-0.18	0.97	-2.78	6.52	26.14*	3.87*	-0.89	-0.33	-0.01
LS09×LS15	7.11**	12.99*	-0.68	-1.38	3.72*	-1.17	-1.35	-1.18	*15.94*	-5.67	2.73	1.37	*6.36*	8.90**	0.98	5.78	-0.78	*2.64*	0.51	0.05
LS09×LS17	1.14	5.26	1.25	-1.29	4.83*	-1.31	4.19**	1.66*	49.42**	-12.02	0.54	-0.21	-2.82	-2.65	27.01	-12.28	2.52	-0.39	0.72	-0.17
LS09×LS18	5.54*	5.26	1.42	1.69	-0.57	1.98	1.18	1.51*	31.22**	4.89	0.73	2.38	-4.28	-0.29	-12.18	27.75*	6.22**	2.41	-0.21	0.34
LS09×LS21	-3.18	12.63*	1.47	2.26*	-0.53	-0.71	1.50	-0.47	6.49	-7.27	1.92	-6.81**	-4.48	-7.00**	-3.84	6.84	1.07	-2.39*	0.13	0.41
LS09×LS24	1.44	7.13	0.82	-0.43	-2.13	-2.24	0.62	-0.47	1.39	20.96*	-0.41	-1.92	-0.02	-0.32	7.40	-6.60	-2.83	1.11	0.82*	-0.20
LS10×LS15	1.39	6.33	-0.82	-1.56	-0.5	-2.24	0.53	-1.24	-0.76	-18.94*	-4.99*	4.39*	11.78**	-6.29**	37.6*	27.81*	-3.13*	*2.69	0.42	0.27
LS10×LS17	2.64	-0.41	-0.55	0.36	0.78	-1.04	-2.72*	-0.77	-23.06*	-18.12*	-1.08	1.90	-9.39**	9.88**	-19.53	-23.82*	-3.33	-1.94	-0.83*	-0.41
LS10×LS18	5.04*	-0.41	-1.55	1.17	0.88	4.08*	2.33	-0.05	-20.26*	24.96*	3.10*	2.69	1.15	1.72	5.87	11.02	*3.13*	-0.64	0.27	0.36
LS10×LS21	-0.12	-6.51	-2.17*	0.24	-2.92	1.23	0.96	1.24	-18.66*	-3.04	0.37	0.84	4.36	-0.23	-5.85	14.94	1.22	1.06	-0.53	0.09
LS10×LS24	2.16	-15.67**	-0.32	1.72	1.15	-1.14	1.52	1.24	-7.76	*20.14*	3.65*	0.25	-2.38	-0.76	38.11*	-10.2	-0.68	2.06	0.48	-0.15
LS15×LS17	4.36	17.79**	-1.77	1.17	-2.72	2.03	-4.45*	6.46**	25.81*	-3.69	3.39*	-2.73	10.23**	-2.62	-7.41	-5.04	2.98*	-0.69	-0.13	-0.11
LS15×LS18	4.42	7.79	0.23	-0.18	0.72	-0.02	-0.79	0.19	3.11	-11.61	1.50	2.23	3.55	-0.58	10.09	26.17*	2.72	1.11	-0.57	0.13
LS15×LS21	1.60	9.76*	-0.55	0.22	3.42*	4.79*	-2.08	-0.19	9.21	26.39*	-2.52	1.43	18.65	7.24**	-32.3	39.09*	0.07	-0.19	1.00**	0.74*
LS15×LS24	2.55	-17.07*	3.47*	-0.63	-0.85	-1.24	0.68	-0.19	1.11	-6.37	-1.63	0.36	1.70	2.60	22.74	28.32*	3.67*	1.31	-0.27	-0.45
LS17×LS18	5.89*	7.59	-0.83	*2.43*	-1.17	-2.66	-1.05	-1.79	-10.36	22.71*	-2.01	0.28	5.44*	3.04	-27.67	-29.23*	1.02	1.86	-0.6	-0.36
LS17×LS21	-10.15**	-6.97	-0.45	-1.03	-3.13	-1.01	-1.62	0.21	-10.09	0.88	-0.75	1.01	-7.19*	0.22	-1.66	-11.81	1.87	2.56*	-0.32	-0.19
LS17×LS24	-3.54	0.19	3.40*	1.79	-0.07	2.46	4.08*	0.21	-8.53	39.78**	-0.15	1.53	14.91	-7.29**	-5.83	3.48	1.97	-0.94	-0.25	0.09
LS18×LS21	-2.42	8.09*	-0.62	3.46**	2.47	0.61	-0.84	-1.00*	4.21	-13.04	-0.35	-0.45	5.60*	8.74**	-22.19	-16.24	-0.43	1.36	-0.22	-0.33
LS18×LS24	3.41	-1.04	1.07	0.27	-2.3	1.41	0.45	-1.00*	-2.73	-15.47	1.89	1.40	-4.00	-1.68	11.18	27.92*	-3.33	-1.64	-0.46	0.18
LS21×LS24	6.26*	4.79	-2.88*	1.34	1.73	3.56*	-2.80*	-0.89	-22.79*	4.36	0.52	-2.80	-9.83**	-4.05*	-36.61*	5.41	2.02	2.06	0.17	0.07

DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight *: significant at 5% level, **: significant at 1% level of significance.

5.3.4 Heterosis estimates

Heterosis estimates for the 28 crosses for the studied traits under NS and DS conditions for Brits and Loskop sites are presented in Tables 5.9, 5.10, 5.11 and 5.12. Positive values for MHP and BPH were regarded as desirable. Regarding NOB, crosses LS01 × LS21 and LS10 × LS24 recorded positive (>0.70) MPH and BPH values under DS conditions at the Loskop site. Cross LS09 × LS15 recorded the highest positive MPH (0.60) and BPH (0.41) for NOL in the Loskop site. About 61% and 79% of crosses had negative MPH% and BPH%, respectively, for SD under the NS condition in the Loskop site. Crosses LS09 × LS15, LS15 × LS24 and LS17 × LS24 had high and positive MPH and BPH values (≥0.40) for PH under NS condition. Cross LS15 × LS17 recorded positive values (>0.50) for PH under DS condition in Loskop site. Under DS condition, crosses LS01 × LS21, LS01 × LS24 and LS15 × LS21 recorded desirable MPH and BPH for FPL in the Loskop environment.

For NFPP, crosses LS09 × LS10 and LS09 × LS15 had high and positive MPH and BPH values (>0.80) under NS condition, whereas under DS cross LS01 × LS15 recorded desirable MPH and BPH for NFFP in the Loskop site. Fourteen crosses recorded positive MPH values for PYP under NS conditions, whereas nine exhibited positive MPH values for PYP under NS conditions in the Loskop site. Crosses LS01 × LS17, LS01 × LS24, LS09 × LS24 and LS17 × LS18 recorded the highest and positive MPH and BPH values (>0.50) for PYP under DS condition in the Loskop site. About 61% and 39% of the crosses recorded positive values for MPH and BPH for DPW under the DS condition in the Loskop site. MPH values ranged from -0.93 for LS01 × LS10 to 2.24 for LS17 × LS24, whereas BPH values ranged from -0.93 for LS01 × LS10 to 1.26 for LS09 × LS17 for HSW under DS condition in Loskop site.

Desirable MPH and BPH for DTF were recorded for crosses LS09 × LS18, LS09 × LS21, LS15 × LS18, LS17 × LS18 and LS18 × LS21 under NS conditions at the Brits and Loskop sites. Under DS condition, crosses LS10 × LS15, LS10 × LS24, LS15 × LS24 and LS21 × LS24 had low and negative MPH and BPH values for the Brits and Loskop sites. Cross LS10 × LS21 had the highest and positive BPH for NOB under DS condition in Brits site, whereas LS01 × LS21 had high and positive MPH and BPH of 2.03 and 1.10 for NOL in the same site. Crosses LS01 × LS17, LS01 × LS21, and LS17 × LS21 had the highest MPH and BPH for SD, whereas crosses LS0 × LS17 and LS17 × LS18 had desirable heteroses for PH under DS condition in the Brits site. Regarding NFPP, LS01 × LS15, LS01 × LS21, LS10 × LS21, LS10 × LS24, LS15 × LS21, and LS15 × LS24 had MPH and BPH of > 0.50 in the positive direction under

NS condition in Brits site. Eight crosses recorded positive MPH and BPH for PYP under DS condition in the Brits site. Cross LS15 × LS24 recorded positive MPH and BPH values of 4.20 and 3.33% for DPW under DS conditions in the Brits site.

Table 5.9: Estimates of mid-parent heterosis (MPH, %) and better parent heterosis (BPH, %) for agronomic traits among 28 F_1 crosses of okra evaluated under non-stressed (NS) condition at the Loskop site in Limpopo Province

Crosses	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFFP		PYP (g/plant)		DPW (g/plant)		HSW (g)	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
LS01×LS09	-0.01	-0.12	-0.10	-0.33	-0.14	-0.40	-0.04	-0.31	-0.24	-0.45	0.20	-0.16	0.05	-0.33	-0.13	-0.38	-0.12	-0.40	-0.81	-0.90
LS01×LS10	0.13	0.12	-0.25	-0.33	-0.17	-0.17	-0.50	-0.50	-0.54	-0.60	-0.80	-0.80	0.02	-0.13	0.35	0.02	-0.22	-0.24	-0.50	-0.54
LS01×LS15	0.00	-0.09	0.54	0.11	-0.37	-0.54	-0.21	-0.32	-0.26	-0.41	0.01	-0.08	0.07	-0.30	0.21	0.09	-0.61	-0.70	-0.32	-0.40
LS01×LS17	0.04	0.02	0.70	0.50	-0.29	-0.43	-0.06	-0.25	-0.26	-0.37	-0.20	-0.24	-0.08	-0.30	-0.63	-0.70	-0.51	-0.52	-0.34	-0.50
LS01×LS18	0.00	-0.07	0.13	-0.13	-0.22	-0.42	-0.05	-0.30	-0.33	-0.43	0.34	-0.06	0.03	-0.21	0.01	-0.13	0.07	0.03	-0.60	-0.64
LS01×LS21	0.10	0.08	0.40	-0.05	-0.03	-0.06	-0.17	-0.20	0.10	0.03	-0.12	-0.21	-0.20	-0.34	0.03	-0.11	-0.20	-0.32	0.30	0.11
LS01×LS24	-0.10	-0.16	0.24	-0.11	-0.18	-0.01	-0.20	-0.30	0.02	-0.15	-0.10	-0.20	-0.40	-0.41	0.16	0.06	0.70	0.37	0.22	-0.31
LS09×LS10	0.08	-0.06	0.38	0.08	0.11	-0.03	0.17	-0.11	0.21	-0.04	0.42	0.07	1.80	0.86	-0.12	-0.21	0.42	0.40	-0.94	1.04
LS09×LS15	-0.18	-0.23	-0.20	-0.20	0.51	0.46	0.53	0.20	0.60	0.40	0.00	-0.23	1.13	0.80	-0.22	-0.41	-0.08	-0.21	-0.11	-0.22
LS09×LS17	0.00	-0.09	0.22	-0.17	0.00	0.00	-0.06	-0.18	-0.29	-0.43	-0.01	-0.25	0.00	-0.30	-0.58	-0.67	0.03	0.01	-0.33	-0.60
LS09×LS18	-0.20	-0.23	-0.33	-0.40	0.18	0.09	0.20	0.05	0.45	0.20	2.01	1.40	0.92	0.42	-0.37	-0.51	-0.73	-0.73	1.04	-0.96
LS09×LS21	-0.14	-0.26	0.30	0.22	-0.53	-0.70	-0.24	-0.43	-0.37	-0.54	0.21	-0.05	-0.08	-0.33	-0.27	-0.42	-0.02	-0.18	0.44	-0.43
LS09×LS24	-0.19	-0.24	-0.09	-0.31	-0.13	-0.33	0.57	0.25	0.09	-0.10	0.24	-0.04	0.02	-0.33	-0.18	-0.45	0.30	-0.11	3.20	1.93
LS10×LS15	0.01	-0.07	0.20	-0.08	0.12	0.02	-0.12	-0.20	0.11	0.05	-0.09	-0.12	0.17	-0.10	0.02	-0.16	-0.42	-0.50	0.91	0.88
LS10×LS17	0.09	0.06	0.17	-0.05	-0.09	-0.14	0.24	0.04	-0.18	-0.20	-0.02	-0.03	0.40	0.30	-0.01	-0.16	-0.45	-0.50	-0.50	-0.62
LS10×LS18	0.00	-0.07	-0.37	-0.50	0.28	0.22	-0.04	-0.22	0.26	0.22	0.51	0.10	0.21	0.07	0.00	-0.15	-0.25	-0.25	-0.06	-0.27
LS10×LS21	0.24	0.22	1.01	0.50	0.08	-0.15	-0.26	-0.28	-0.15	-0.23	-0.11	-0.20	-0.10	-0.20	0.33	0.13	-0.62	-0.70	-0.71	-0.80
LS10×LS24	-0.04	-0.17	0.20	0.14	-0.03	-0.18	-0.22	-0.28	0.01	-0.05	0.17	0.09	0.20	0.10	0.00	0.00	-0.27	-0.40	-0.80	-0.88
LS15×LS17	-0.05	-0.09	0.10	-0.25	-0.11	-0.23	0.00	-0.10	0.12	0.02	0.12	0.06	0.64	0.37	-0.51	-0.53	-0.41	-0.50	-0.04	-0.30
LS15×LS18	-0.08	-0.08	0.20	0.00	0.73	0.65	0.07	-0.05	-0.26	-0.31	0.28	0.11	0.50	0.28	-0.16	-0.20	-0.10	-0.17	-1.01	-1.04
LS15×LS21	0.04	-0.05	-0.53	-0.55	-0.17	-0.35	-0.26	-0.35	-0.17	-0.32	0.14	0.11	0.21	0.00	0.32	0.27	0.80	0.70	-0.70	-0.73
LS15×LS24	-0.09	-0.09	0.60	0.17	-0.23	-0.39	-0.07	-0.07	0.57	0.49	0.20	0.12	-0.16	-0.40	0.26	0.04	0.22	0.16	-0.14	-0.40
LS17×LS18	-0.08	-0.12	0.50	0.07	-0.22	-0.32	0.10	0.07	-0.16	-0.01	0.30	-0.10	-0.05	-0.10	-0.32	-0.32	0.00	-0.02	-0.65	-0.65
LS17×LS21	0.05	0.00	0.13	-0.25	0.11	-0.09	0.10	-0.11	0.22	0.20	0.01	-0.05	-0.03	-0.04	-0.40	-0.53	0.75	0.42	-0.21	-0.25
LS17×LS24	0.00	-0.04	0.40	0.18	-0.13	-0.22	-0.25	-0.33	0.52	0.47	-0.20	-0.22	0.28	0.08	0.07	-0.14	-0.60	-0.70	0.43	-0.24
LS18×LS21	-0.03	-0.11	-0.40	-0.48	-0.33	-0.50	-0.21	-0.37	-0.41	-0.50	0.30	0.01	-0.01	-0.04	0.50	0.50	0.24	0.20	-0.70	-0.70
LS18×LS24	0.00	-0.01	1.10	0.55	0.05	-0.14	0.53	0.34	0.29	0.26	0.55	0.20	0.52	0.26	0.32	0.15	-0.60	-0.70	-0.21	-0.60
LS21×LS24	0.09	0.00	0.63	0.20	-0.40	-0.45	-0.35	-0.42	-0.65	-0.70	-0.26	-0.26	-0.64	-0.70	-0.53	-0.62	0.14	0.06	1.16	0.20

NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight.

Table 5.10: Estimates of mid-parent heterosis (MPH, %) and better parent heterosis (BPH, %) for agronomic traits among 28 F_1 crosses of okra evaluated under drought-stressed (DS) condition at the Loskop site in Limpopo Province

Crosses	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFFP		PYP (g/plant)		DPW (g/plant)		HSW(g)	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
LS01×LS09	0.06	-0.16	-0.25	-0.37	-0.25	-0.40	-0.31	-0.37	-0.41	-0.54	0.14	-0.10	0.58	0.21	-0.18	-0.41	-0.70	-0.70	0.26	0.12
LS01×LS10	0.03	-0.01	0.00	-0.59	-0.22	-0.40	-0.16	-0.20	-0.28	-0.38	0.30	0.20	0.03	-0.30	0.24	0.06	0.16	-0.13	-0.93	-0.93
LS01×LS15	-0.03	-1.10	0.09	-0.07	-0.12	-0.24	-0.20	-0.26	0.32	-0.32	0.33	0.29	1.20	1.10	-0.30	-0.45	0.23	0.02	-0.53	-0.63
LS01×LS17	-0.13	-0.15	0.22	0.05	-0.40	-0.50	-0.23	-0.32	-0.24	-0.40	-0.14	-0.34	0.02	-0.10	1.80	1.50	-0.50	-0.63	0.15	0.15
LS01×LS18	0.04	0.00	0.27	0.03	0.25	0.02	0.21	0.11	0.23	-0.08	0.20	0.00	0.31	-0.02	0.68	0.64	-0.50	-0.60	-0.57	-0.63
LS01×LS21	-0.01	-0.01	1.25	1.04	0.12	0.04	0.20	0.14	0.08	0.04	0.42	0.34	0.33	0.08	0.52	0.49	0.50	-0.04	-0.74	-0.80
LS01×LS24	-0.05	-0.07	-0.13	-0.19	0.03	-0.01	-0.22	-0.23	-0.07	-0.35	0.70	0.60	-0.40	-0.60	0.72	0.70	-0.52	-0.62	1.24	0.41
LS09×LS10	0.11	0.02	0.03	-0.06	0.40	0.32	-0.07	-0.14	0.30	0.16	0.24	0.12	-0.21	-0.30	-0.19	-0.34	-0.20	-0.37	-0.39	-0.49
LS09×LS15	-0.03	-0.09	0.02	-0.23	0.60	0.41	-0.01	-0.20	0.23	0.12	0.30	0.00	-0.20	-0.35	-0.61	-0.63	-0.71	-0.75	-0.48	-0.51
LS09×LS17	-0.03	-0.09	0.45	0.40	0.52	0.38	-0.04	-0.21	-0.15	-0.20	0.02	0.00	-0.16	-0.40	0.26	-0.03	0.10	-0.23	1.77	1.26
LS09×LS18	0.02	-0.07	0.33	-0.06	-0.31	-0.33	-0.41	-0.50	0.31	0.20	-0.53	-0.60	0.10	-0.30	-0.20	-0.43	-0.34	-0.51	0.06	-0.23
LS09×LS21	-0.04	-0.10	0.09	0.00	-0.04	-0.27	-0.32	-0.40	0.04	-0.22	0.24	-0.03	-0.02	-0.10	1.20	0.60	-0.51	-0.70	0.54	0.45
LS09×LS24	-0.14	-0.17	0.00	-0.35	-0.40	-0.53	-0.44	-0.50	0.19	0.01	0.04	-0.18	-0.24	-0.40	-0.27	-0.50	-0.31	-0.45	-0.60	-0.80
LS10×LS15	-0.04	-0.07	-0.40	-0.50	-0.14	-0.24	0.00	-0.13	0.11	0.00	0.16	0.00	-0.01	-0.31	-0.21	-0.32	0.70	0.60	0.03	-0.18
LS10×LS17	0.00	-0.02	0.62	0.44	0.01	-0.11	-0.10	-0.17	-0.44	-0.48	0.10	-0.03	0.25	-0.15	-0.10	-0.16	2.01	1.60	0.25	0.21
LS10×LS18	0.06	0.04	-0.22	-0.42	0.21	0.14	-0.17	-0.30	0.21	0.01	-0.20	-0.22	-0.40	-0.44	-0.21	-0.31	0.29	0.24	-0.75	-0.80
LS10×LS21	-0.03	-0.06	0.22	0.22	-0.16	-0.39	-0.10	-0.10	-0.20	-0.28	0.22	0.03	-0.10	-0.22	0.77	0.53	-0.13	-0.31	0.08	-0.14
LS10×LS24	-0.02	-0.07	1.10	0.77	0.45	0.10	0.13	0.10	0.01	-0.05	0.43	0.22	0.12	0.02	-0.05	-0.20	0.05	-0.02	1.80	0.72
LS15×LS17	-0.05	-0.09	-0.59	-0.70	-0.40	-0.40	-0.48	-0.50	0.70	0.50	-0.43	-0.55	-0.40	-0.40	0.50	0.21	1.01	0.60	0.30	0.00
LS15×LS18	0.09	0.04	-0.21	-0.30	-0.43	-0.50	-0.07	-0.10	-0.28	-0.40	-0.33	-0.45	0.40	0.00	-0.29	-0.45	0.60	0.33	1.38	0.65
LS15×LS21	-0.10	-0.10	0.40	0.10	-0.51	-0.60	0.04	-0.05	0.13	-0.20	0.42	0.39	0.50	0.15	-0.31	-0.50	0.23	-0.11	0.24	0.24
LS15×LS24	-0.11	-0.13	-0.43	-0.50	-0.63	-0.70	-0.26	-0.33	0.01	-0.06	0.12	0.11	-0.38	-0.60	0.20	-0.10	0.10	0.03	2.00	0.71
LS17×LS18	-0.02	-0.05	0.20	-0.20	0.45	0.36	0.30	0.26	0.40	0.22	-0.23	-0.30	1.01	0.33	1.10	1.10	0.30	0.17	0.35	0.15

LS17×LS21	-0.04	-0.04	1.01	-0.22	-0.29	-0.42	0.06	0.00	-0.32	-0.46	-0.12	-0.33	-0.50	-0.60	1.30	0.20	1.31	1.04	-0.11	-0.31
LS17×LS24	-0.09	-0.11	1.01	0.30	0.05	-0.12	-0.09	-0.18	0.65	0.34	-0.30	-0.43	-0.15	-0.50	0.20	0.03	0.30	0.08	2.24	1.04
LS18×LS21	0.13	0.09	0.18	-0.11	-0.37	-0.51	-0.15	-0.21	0.44	-0.04	-0.50	-0.60	-0.35	-0.42	0.54	0.53	-0.80	-0.81	-0.06	-0.35
LS18×LS24	-0.05	-0.11	0.11	-0.04	0.00	-0.21	0.09	0.01	0.03	-0.08	-0.30	-0.40	0.01	-0.14	-0.14	-0.20	0.13	-0.05	1.85	1.06
LS21×LS24	-0.02	-0.04	0.32	0.11	-0.52	-0.53	-0.12	-0.13	-0.30	-0.50	0.02	0.01	-0.25	-0.40	0.24	0.19	1.37	0.80	1.14	0.22

NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight.

Table 5.11: Estimates of percentage mid-parent heterosis (MPH, %) and better parent heterosis (BPH, %) for agronomic traits among 28 F_1 crosses of okra evaluated under non-stressed (NS) condition at the Brits site in North-West Province

Crosses	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFFP		PYP (g/plant)		DPW (g/plant)		HSW (g)	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
LS01×LS09	0.27	-0.05	-0.26	-0.48	0.22	0.16	0.22	0.14	0.88	0.71	-0.38	-0.44	-0.16	-0.48	0.64	0.25	-0.06	-0.27	0.53	0.16
LS01×LS10	7.86	0.02	-0.06	-0.17	-0.47	-0.61	0.64	0.54	-0.25	-0.31	-0.32	-0.47	0.31	0.10	0.74	0.59	0.20	0.15	1.01	0.65
LS01×LS15	-0.06	-0.11	-0.41	-0.56	-0.02	-0.31	0.19	0.06	0.24	0.17	-0.41	-0.63	1.01	0.59	0.53	0.24	0.14	0.00	0.01	0.00
LS01×LS17	0.48	-0.03	-0.55	-0.59	0.16	-0.20	-0.01	-0.12	0.49	0.13	-0.41	-0.55	-0.12	-0.38	-0.01	-0.20	-0.04	-0.13	0.16	-0.15
LS01×LS18	-0.11	-0.15	-0.65	-0.74	-0.19	-0.54	-0.12	-0.25	0.27	0.09	-0.80	-0.83	-0.28	-0.57	-0.08	-0.31	0.04	0.00	-0.28	-0.37
LS01×LS21	5.18	0.00	-0.40	-0.56	2.03	1.10	-0.12	-0.18	0.26	0.11	0.40	0.08	2.70	2.06	-0.37	-0.50	-0.31	-0.41	-0.05	-0.07
LS01×LS24	0.90	0.02	0.35	-0.08	0.07	0.00	0.05	-0.01	-0.04	-0.12	-0.43	-0.62	0.36	0.18	-0.35	0.37	1.08	0.93	0.10	-0.16
LS09×LS10	-2.20	-0.03	0.67	0.29	-0.37	-0.60	-0.01	-0.02	0.44	0.19	-0.09	-0.24	-0.09	-0.24	0.27	-0.09	0.26	0.00	0.25	0.13
LS09×LS15	1.94	0.01	0.31	0.21	-0.25	-0.57	0.14	0.07	0.06	-0.07	0.95	0.53	0.06	-0.50	-0.20	-0.50	0.48	0.05	0.23	-0.06
LS09×LS17	-0.88	-0.03	-0.31	-0.48	0.73	0.00	0.02	-0.04	0.06	-0.07	-0.35	-0.44	-0.59	-0.67	1.36	0.23	0.03	-0.14	0.17	-0.18
LS09×LS18	-0.15	-0.23	0.14	0.05	-0.27	-0.58	-0.11	-0.12	0.13	-0.08	-0.36	-0.48	-0.46	-0.51	-0.63	-0.77	-0.52	-0.64	-0.12	-0.39
LS09×LS21	-0.12	-0.12	0.26	-0.03	-0.24	0.09	-0.04	-0.04	-0.04	-0.20	-0.51	-0.61	-0.48	-0.64	0.58	0.04	-0.13	-0.23	-0.10	-0.33
LS09×LS24	0.00	-0.07	0.04	-0.03	-0.06	-0.05	-0.12	-0.19	0.40	0.38	0.15	0.15	0.53	0.02	-0.61	-0.70	0.33	0.09	-0.64	-0.64
LS10×LS15	0.45	-0.11	0.29	0.06	-0.65	-0.67	-0.18	-0.34	0.70	0.63	1.76	1.63	-0.15	-0.31	-0.47	-0.54	1.09	0.77	0.53	0.27
LS10×LS17	-5.87	-0.07	-0.84	-0.85	-0.21	-0.24	-0.82	-0.85	-0.54	-0.60	-0.37	-0.39	-0.64	-0.65	-0.88	-0.90	-0.71	-0.73	-0.02	-0.27
LS10×LS18	-0.12	-0.14	-0.28	-0.41	-0.25	-0.31	-0.31	-0.40	0.02	-0.05	-0.45	-0.61	-0.12	-0.32	-0.43	-0.54	0.58	0.46	-0.47	-0.61
LS10×LS21	-0.52	-0.01	-0.07	-0.40	-0.45	-0.60	-0.31	-0.33	0.06	0.03	-0.32	-0.33	1.25	0.76	-0.65	-0.65	0.27	0.12	-0.61	-0.68
LS10×LS24	5.20	-0.02	-0.36	-0.53	-0.16	-0.41	0.27	0.09	-0.02	-0.17	1.01	0.61	1.37	0.80	-0.35	-0.40	-0.11	-0.14	0.13	0.02
LS15×LS17	-0.19	-0.21	-0.86	-0.90	-0.56	0.57	-0.38	-0.45	-0.13	-0.26	1.45	1.20	-0.35	-0.46	-0.70	-0.90	-0.30	-0.47	-0.62	0.70
LS15×LS18	-1.46	-0.03	-0.19	-0.20	-0.67	-0.67	-0.25	-0.29	0.00	-0.20	-0.51	-0.67	0.19	-0.20	-0.92	-0.93	1.21	0.91	-0.70	-0.74
LS15×LS21	-0.09	-0.09	0.49	0.09	0.47	0.06	0.02	-0.06	0.33	0.24	0.12	0.04	0.77	0.68	0.23	0.21	0.60	0.18	0.24	0.21
LS15×LS24	-0.15	-0.20	-0.25	-0.35	-0.18	-0.44	-0.22	-0.27	-0.07	0.82	-0.14	-0.25	0.77	0.61	0.00	-0.50	-0.13	-0.29	-0.46	-0.60
LS17×LS18	-0.01	-0.12	-0.58	-0.66	-0.50	-0.52	-0.43	-0.27	-0.30	-0.38	-0.48	-0.63	-0.41	-0.57	-0.28	-0.50	-0.46	-0.53	-0.28	-0.29
LS17×LS21	-0.09	-0.11	-0.08	-0.41	-1.15	-0.79	-0.43	-0.46	-0.03	-0.14	0.00	-0.04	0.85	0.49	-0.47	-0.61	-0.44	-0.47	-0.66	-0.70

LS17×LS24	0.10	0.05	0.69	0.23	-0.37	-0.56	-0.24	-0.36	-0.12	-0.34	1.18	0.74	0.00	-0.27	-0.55	-0.58	0.31	0.27	-0.82	-0.88
LS18×LS21	-0.08	-0.05	0.00	-0.27	-0.29	-0.50	0.11	-0.02	0.01	-0.02	-0.54	-0.67	-0.54	-0.70	-0.52	-0.56	-0.14	-0.29	-0.66	-0.70
LS18×LS24	-0.09	-0.13	-0.68	-0.72	-0.49	-0.65	-0.49	-0.54	-0.24	-0.39	-0.66	-0.79	0.73	0.11	-0.43	-0.58	1.24	1.00	0.55	0.08
LS21×LS24	-0.07	-0.03	0.43	0.16	-0.49	-0.53	-0.24	-0.24	0.03	-0.16	1.53	1.10	1.58	1.46	-0.56	-0.66	-0.68	-0.71	1.10	0.57

NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight.

Table 5.12: Estimates of percentage mid-parent heterosis (MPH, %) and better parent heterosis (BPH, %) for agronomic traits among 28 F_1 crosses of okra evaluated under drought-stressed (DS) condition at the Brits site in North-West Province

Crosses	DTF		NOB		NOL		SD (mm)		PH (cm)		FPL (cm)		NFFP		PYP (g/plant)		DPW (g/plant)		HSW (g)	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
LS01×LS09	-0.10	-0.29	0.22	-0.26	-0.21	-0.94	2.5	0.25	-0.54	-0.59	0.42	0.19	0.90	0.14	0.23	-0.30	-0.14	-0.64	0.25	-0.32
LS01×LS10	-0.35	-0.36	0.73	0.25	0.33	0.13	2.45	0.38	-0.60	-0.70	-0.2	-0.33	3.25	1.64	-0.38	-0.49	0.57	-0.15	0.41	0.29
LS01×LS15	4.45	-0.06	0.07	-0.35	-0.27	-0.37	0.82	-0.5	-0.7	-0.76	-0.14	-0.18	1.73	0.92	-0.80	-0.86	1.00	0.33	-0.85	-0.89
LS01×LS17	-0.27	-0.42	0.15	0.14	0.12	-0.13	1.5	0.68	-0.65	-0.77	0.08	0.07	3.81	2.17	0.06	-0.07	1.80	0.60	-0.57	-0.63
LS01×LS18	-0.27	-0.32	0.83	0.11	1.05	0.35	-0.13	-0.46	0.62	0.23	-0.19	-0.2	0.41	-0.23	1.05	0.06	1.46	0.33	0.10	-0.74
LS01×LS21	0.03	0.00	1.59	0.83	1.77	0.80	2.19	1.02	-0.52	-0.6	-0.15	-0.18	0.71	0.07	2.70	2.41	0.14	-0.14	1.21	0.75
LS01×LS24	-0.25	0.01	1.85	0.91	0.65	-0.33	-0.25	-0.57	-0.47	-0.5	0.26	0.02	-0.3	-0.63	-0.56	-0.71	-0.45	-0.7	-0.64	-0.79
LS09×LS10	-0.21	-0.39	0.04	-0.24	-0.5	-0.45	0.03	-0.19	0.23	-0.14	0.32	0.07	0.25	0.14	-0.44	-0.70	0.25	0.15	-0.84	-0.90
LS09×LS15	0.72	0.70	-0.21	-0.21	-0.53	0.28	-0.27	-0.27	0.54	0.12	-0.25	-0.27	1.84	1.12	-0.70	-0.72	0.14	-0.27	-0.18	-0.56
LS09×LS17	0.25	0.23	0.61	0.09	-0.19	-0.16	0.07	-0.19	-0.14	-0.46	-0.25	-0.27	0.07	-0.21	0.03	-0.40	-0.07	-0.36	-0.59	-0.78
LS09×LS18	0.11	-0.13	-0.08	-0.15	-0.39	-0.30	0.43	0.11	-0.54	-0.67	-0.35	-0.38	-0.62	-0.72	0.00	-0.40	-0.07	-0.36	-0.01	-0.63
LS09×LS21	0.27	0.04	-0.01	-0.26	-0.5	0.31	0.32	0.01	-0.5	-0.52	-0.26	-0.33	-0.59	-0.69	-0.17	-0.52	-0.3	-0.33	-0.67	-0.81
LS09×LS24	0.28	0.04	0.63	0.32	0.11	-0.12	-0.13	-0.15	-0.40	-0.44	-0.65	-0.65	-0.86	-0.91	1.21	-0.04	-0.76	-0.82	-0.08	-0.30
LS10×LS15	-0.11	-0.18	-0.44	-0.6	-0.12	-0.26	-0.4	-0.51	0.41	0.28	0.44	0.44	2.40	1.72	-0.01	-0.98	-0.81	-0.82	-0.63	-0.75
LS10×LS17	0.19	-0.11	1.21	0.94	1.21	0.70	0.53	0.32	2.21	1.70	0.71	0.40	2.47	2.03	2.70	2.70	0.75	0.08	2.95	2.75
LS10×LS18	-0.09	-0.25	-0.07	-0.28	-0.02	-0.03	0.14	0.14	-0.29	-0.32	0.43	0.21	-0.07	-0.36	1.30	1.04	-0.06	-0.38	2.05	1.90
LS10×LS21	-0.20	0.00	1.85	1.77	0.86	0.77	0.25	0.21	0.16	-0.22	0.35	0.14	-0.61	-0.72	5.07	4.31	-0.68	-0.69	3.65	2.95
LS10×LS24	-0.17	-0.22	0.24	0.09	0.23	0.05	0.15	-0.12	-0.52	-0.65	-0.28	-0.42	0.34	-0.13	0.4	0.04	0.16	-0.15	-0.43	-0.68
LS15×LS17	0.45	0.42	0.04	-0.29	-0.49	-0.65	0.01	-0.36	-0.54	-0.64	-0.22	-0.38	0.94	0.75	-0.82	-0.9	-0.65	-0.69	1.00	-0.36
LS15×LS18	0.12	-0.13	-0.18	-0.24	-0.09	-0.26	0.25	-0.04	0.06	0.03	0.24	0.05	-0.05	-0.42	0.82	0.07	-0.25	-0.25	3.95	2.27
LS15×LS21	0.72	-0.21	-0.09	-0.32	-0.09	-0.26	0.00	-0.34	0.13	-0.21	0.41	0.19	0.63	0.04	-0.81	-0.90	-0.50	-0.67	1.10	0.30
LS15×LS24	-0.16	-0.33	0.32	0.00	0.12	0.12	-0.35	-0.35	0.23	-0.07	-0.12	-0.29	-0.56	-0.62	-0.55	-0.71	4.20	3.33	-0.58	-0.71
LS17×LS18	0.12	-0.11	1.19	0.55	1.01	0.57	0.51	0.29	2.31	1.64	-0.1	0.17	0.14	-0.27	1.7	1.34	0.14	-0.2	4.29	4.29

LS17×LS21	0.07	-0.12	0.52	0.29	0.02	-0.18	1.02	0.65	-0.44	-0.70	-0.04	-0.12	-0.34	-0.56	2.92	2.52	-0.63	-0.75	16.06	15.06
LS17×LS24	0.08	-0.18	0.33	0.05	0.07	-0.39	0.41	-0.12	-0.52	-0.70	-0.42	-0.6	-0.15	-0.48	-0.30	-0.50	0.20	0.00	-0.73	-0.85
LS18×LS21	-0.13	0.11	0.13	-0.10	0.31	0.28	0.52	0.48	-0.30	-0.52	-0.42	-0.42	-0.32	-0.36	4.30	3.23	-0.71	-0.8	13.12	12.29
LS18×LS24	0.16	0.20	-0.04	-0.17	0.65	0.41	0.68	0.26	-0.53	-0.65	0.00	-0.06	-0.87	-0.88	2.17	0.93	-0.67	-0.75	-0.15	-0.52
LS21×LS24	-0.03	-0.03	0.11	0.00	0.05	-0.23	0.43	0.05	0.01	-0.1	0.00	-0.09	-0.4	-0.49	2.62	0.31	-0.13	-0.3	-0.24	-0.58

NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight.

5.3.5 Associations among traits under non-stressed and drought-stressed conditions

Pearson's correlation coefficients (*r*) indicating associations among studied agronomic traits under NS and DS conditions in the Loskop and Brits sites are presented in Tables 5.13 and 5.14, respectively. Positive and significant correlation was observed between HSW and NOB ($r = 0.49$, $P<0.05$) under NS in Loskop (Table 5.13). Positive and significant correlations were observed between SD ($r = 0.47$, $P<0.01$), PH ($r = 0.63$, $P<0.01$), NFPP ($r = 0.42$, $P<0.01$), SW ($r = 0.81$, $P<0.01$) and PYP ($r = 0.36$, $P<0.05$) with NOL under NS condition in the Loskop site (Table 5.13). Positive and significant correlations between PH ($r = 0.49$, $P<0.01$), FPL ($r = 0.40$, $P<0.05$), NFPP ($r = 0.43$, $P<0.05$) and PYP ($r = 0.39$, $P<0.05$) with SD was observed under NS in Loskop. FPL ($r = 0.47$, $P<0.01$), NFPP ($r = 0.45$, $P<0.01$) and PYP ($r = 0.40$, $P<0.05$) positively correlated with PH under NS in the Loskop site (Table 5.13). A positive and significant correlation was observed between NFPP ($r = 0.37$, $P<0.05$) and PYP ($r = 0.45$, $P<0.01$) with FPL, whereas PYP ($r = 0.41$, $P<0.05$) positively correlated with NFPP under NS condition (Table 5.13).

Under DS condition at the Loskop site, a positive and significant correlation was observed between NOL ($r = 0.38$, $P<0.05$), SD ($r = 0.33$, $P<0.05$) with NOB. SD positively and significantly correlated with NOB ($r = 0.33$, $P<0.05$) and NOL ($r = 0.59$, $P<0.01$) under DS conditions (Table 5.S1). A positive and significant correlation was displayed between PH with NOL ($r = 0.59$, $P<0.01$) and SD ($r = 0.53$, $P<0.01$) under the DS condition in the Loskop site. FPL positively and significantly correlated with NOL ($r = 0.57$, $P<0.01$) and SD ($r = 0.41$, $P<0.01$) under DS conditions. There was a positive and significant correlation between NFPP with NOB ($r = 0.43$, $P<0.01$) and SD ($r =0.46$, $P<0.01$). A positive and significant correlation was observed between PYP with NOL ($r = 0.36$, $P<0.05$), SD ($r = 0.37$, $P<0.05$), FPL ($r = 0.39$, $P<0.05$) and NFPP ($r = 0.48$, $P<0.01$) under DS condition in the Loskop site (Table 5.S1).

In Brits, positive and significant correlations were observed between NOB ($r = 0.38$, $P<0.05$) and SD ($r = 0.35$, $P<0.01$) with DTF under the NS condition (Table 5.14). DPW positively and significantly correlated with NOB ($r = 0.38$, $P<0.05$) under NS in Brits. A positive and significant correlation was displayed between FPL ($r = 0.48$, $P<0.01$), NFPP ($r = 0.57$, $P<0.01$) and PYP ($r = 0.46$, P,0.05) with NOL under NS condition. FPL ($r = 0.37$, $P<0.05$) and PYP ($r = 0.55$, $P<0.01$) positively and significantly correlated with SD under NS in Brits site. A positive and significant correlations was observed between NFPP ($r = 0.52$, $P<0.01$) and PYP

(r = 0.36, P<0.05) with FPL, whereas PYP positively correlated with SW (r = 0.51, P<0.01) under NS condition in Brits site (Table 5.14).

A positive and significant correlation was observed between NOL (r = 0.72, P<0.05) and NOB under DS conditions (Table 5.14). SD positively and significantly correlated with NOB (r = 0.35, P<0.05) and NOL (r = 0.61, P<0.01) under DS condition for Brits site. PH positively and significantly correlated with NOB (r = 0.35, P<0.05) and NOL (r = 0.36, P<0.05) under DS condition for Brits. Positive and significant correlations were observed between FPL with NOL (r = 0.50, P<0.01) and PH (r = 0.44, P<0.05), while NFPP positively correlated with FPL (r = 0.37, P<0.05) under DS condition in Brits (Table 5.14). DPW positively and significantly correlated with PH (r = 0.35, P<0.05) and FPL (r = 0.47, P<0.01) in Brits under DS condition. H SW positively and significantly correlated with NOL (r = 0.37, P<0.05) and SD (r = 0.51, P<0.01) under DS. There was a positive and significant between PYP with NOB (r = 0.50, P<0.01), SD (r = 0.62, P<0.010, FPL (r = 0.53, P<0.01) and NFPP (r = 0.62, P<0.01) under DS condition for Brits site (Table 5.14).

Table 5.13: Pearson correlation coefficients showing the association between the studied traits among okra genotypes under non-stressed (lower diagonal) and drought stress (upper diagonal) in the Loskop site in Limpopo Province.

Traits	DTF	NOB	NOL	SD	PH	FPL	NFPP	DPW	HSW	PYP
DTF		-0.16	0.04	0.08	-0.16	-0.03	-0.11	0.16	-0.01	0.16
NOB	0.28		0.38*	0.33*	0.27	-0.15	0.43**	-0.16	-0.05	-0.06
NOL	-0.22	-0.19		0.59**	0.59**	0.57**	0.24	0.12	-0.23	0.36*
SD	-0.21	0.16	0.47**		0.53**	0.41*	0.46**	0.27	-0.13	0.37*
PH	-0.35	-0.09	0.63**	0.49**		0.18	0.16	0.18	0.23	0.13
FPL	-0.36*	-0.03	0.17	0.40*	0.47**		0.15	0.34*	0.19	0.39*
NFPP	-0.13	-0.37*	0.42*	0.43*	0.45**	0.37*		-0.22	-0.16	0.48**
DPW	0.31	0.03	-0.24	-0.03	-0.11	-0.04	0.11		0.03	-0.29
SW	0.22	0.49*	0.81**	0.02	0.21	-0.15	-0.24	0.21		-0.21
PYP	-0.33	0.29	0.36*	0.39*	0.40*	0.45**	0.41*	-0.13	-0.04	

DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: fresh pod yield per plant, DPW: dry pod weight per plant, HSW: hundred seed weight,*: significant at 5% level, **: significant at 1% level, ns: non-significant.

Table 5.14: Pearson correlation coefficients showing the association between the studied traits among okra genotypes under non-stressed (lower diagonal) and drought stress (upper diagonal) in the Brits site in North-West Province.

Traits	DTF	NOB	NOL	SD	PH	FPL	NFPP	DPW	HSW	PYP
DTF		-0.04	0.08	0.01	0.01	-0.08	0.02	-0.20	-0.17	-0.11
NOB	0.38*		0.75**	0.35*	0.35*	-0.13	-0.18	-0.06	0.28	0.50**
NOL	0.13	-0.12		0.61**	0.36*	0.50**	-0.08	0.06	0.37*	0.24
SD	0.35*	0.28	0.21		0.03	-0.12	0.02	-0.12	0.51**	0.62**
PH	-0.03	0.16	0.21	0.23		0.44*	0.24	0.35*	0.14	0.1
FPL	0.24	0.27	0.48**	0.37*	0.2		0.37*	0.47**	-0.04	0.53**
NFPP	0.21	0.14	0.57**	-0.01	-0.03	0.52**		0.38*	-0.18	0.62**
DPW	0.19	0.38*	-0.21	0.21	-0.02	-0.10	0.24		0.06	0.07
SW	0.14	0.09	0.27	0.19	0.13	0.17	-0.04	-0.09		0.31
PYP	0.13	0.09	0.46*	0.55**	0.18	0.36*	-0.16	-0.01	0.51**	

DTF: days to flowering, NOB: number of branches per plant, NOL: number of leaves per plant, SD: stem diameter, PH: plant height, FPL: fresh pod length, NFPP: number of fresh pods per plant, PYP: fresh pod yield per plant, , DPW: dry pod weight per plant, HSW: hundred seed weight, *: significant at 5% level of probability, **: significant at 1% level, ns: non-significant.

5.4 Discussion

Okra is a relatively drought-resilient crop able to thrive in water-limited environments. However, recurrent, and increased drought intensities threaten the crop's sustainable production in arid and semi-arid agroecologies. Therefore, there is a need to develop drought-tolerant okra varieties. The current study estimated combining ability and heterosis for yield-related traits under non-stress and drought stress conditions to identify superior breeding parents and experimental hybrids with drought tolerance. Analysis of variance revealed a significant genotypic effect for the studied traits (Table 5.2) suggesting the existence of considerable variability among the okra genotypes and developed crosses for further selection or breeding. Significant genotypes × site interaction effects and their interaction for yield and yield-related traits suggested the existence of considerable variability among the studied okra accessions for breeding. Also, Wammanda et al. (2010) reported significant genotypes × site interaction effect for yield and yield attributing traits in okra.

There were significant GCA and SCA effects for studied traits, which suggested that both additive and non-additive effects condition the inheritance of agronomic traits in okra, agreeing with the studies of Reddy et al. (2013) and Abed et al. (2020). Early flowering is a critical

phenological trait related to yield potential, which allows drought escape in water-stressed environments (Javiya et al., 2020). Crosses LS01 × LS18, LS10 × LS17, LS10 × LS24 and LS18 × LS24 with high and negative SCA effects under drought stress for days to flowering (Tables 5.7 and 5.8) are valuable for breeding for earliness. Parental genotypes LS01, LS21 and LS24 and crosses LS01 × LS18, LS01 × LS21, LS01 × LS24, LS09 × LS15, LS09 LS17, LS10 × LS24, LS17 × LS18 and LS17 × LS24 recorded the highest number of leaves (>30) under drought stress condition at Loskop site (Table 5.3). According to Jaleel et al. (2009) the development of optimal leaves is associated with an increased dry matter yield. Additionally, crosses LS09 × LS24, LS10 × LS17, LS10 × LS18, LS18 × LS21 and LS21 × LS24 identified with high SCA effects for the number of leaves under drought-stressed conditions (Tables 5.7 and 5.8) are potential genetic resources for further selection to enhance plant biomass (Kusvuran 2012).

Plant height is essential for improving okra biomass production (Mkhabela et al. 2021). The present study found genetic variation in plant height among the newly developed progenies. For example, crosses LS01 × LS09, LS09 × LS24 and, LS17 × LS24, exhibited positive and significant SCA effects for plant height under water-limited conditions (Tables 5.7 and 5.8). In addition, crosses LS09 × LS15, LS10 × LS15 and LS17 × LS18 produced taller plants than their respective parents (Tables 5.3 and 5.4), suggesting additive gene action conditioning the inheritance of plant height.

Fresh pod length and number of pods per plant are the most important determinants of yield in okra (Eshiet and Brisibe 2015). Thus, selection based on these characters is essential in okra breeding programs. The newly developed progenies such as LS01×LS17, LS01 × LS18, LS01 × LS21, LS09 × LS18, LS10 × LS15 and LS21 × LS24 had high SCA effects for both traits under drought stress conditions (Tables 5.7 and 5.8) despite the non-significant GCA effects of their parents. The positive and significant correlation between fresh pod length and pod yield under drought stress conditions (Tables 5.13 and 5.14) suggests that selection for high fresh pod length improves yield potential. According to Wammanda et al. (2010), longer pods allow for the development of ovules to accommodate more seeds, thus enhancing yield potential.

Breeding for pod yield capacity is a key breeding goal in okra. The present study identified crosses such as LS01 × LS17, LS09 × LS21, LS09 × LS17, LS10 × LS21, LS15 × LS17 and LS17 × LS18, LS09 × LS24, LS15 × LS18, LS18 × LS21 and LS18 × LS24 with high yield under drought stress condition (Tables 5.3 and 5.4). These selected crosses require further

improvement. The parental genotypes LS01, LS09, LS10, LS17, LS21 and LS24, which had high GCA effects for pod yield under drought stress, are valuable germplasm for breeding. Additionally, LS01 had high GCA effects for SD, FPL, NFPP and SW, whereas parental genotype LS10 had high GCA effects for NOL, PH, FPL, and SW. Parental genotype LS17 had high GCA effects for NOB, NOL, SD and SW, whereas LS24 had high GCA effects for SD, FPL and NFPP (Table 5.6). The parental genotypes with high GCA effects may be used in a multiple-crossing program for selecting desirable okra accessions (Reddy et al. 2013).

Exploiting heterosis among genetically diverse okra accessions can aid in developing desirable recombinants of high yield potential and drought tolerance (Keerthana et al. 2021). There was varied heterosis for studied agronomic traits under drought-stressed conditions among crosses (Tables 5.10 and 5.12). The present study recorded positive MPH and BPH values for FPL, NFFP and FPYP, indicating that these crosses help improve okra yields under drought-stressed environments. Positive heterosis observed from the evaluated test crosses may assist in further improving and releasing promising hybrids as cultivars. Crosses LS01 × LS10, LS01 × LS17, LS01 × LS18, LS01 × LS21, LS01 × LS24, LS09 × LS21, LS10 × LS17, LS10 × LS18, LS10 × LS21, LS15 × LS17, LS15 × LS18, LS17 × LS18, LS17 × LS21, LS17 × LS24, LS18 × LS21, LS18 × LS24 and LS21 × LS24 (Tables 5.3 and 5.4) were best yielding under drought stressed condition exceeding their parental genotypes. These crosses are useful genetic resources for genetic advancement and developing improved okra varieties under water-limited conditions.

5.5 Conclusions

The current study evaluated combining ability, heterosis and drought tolerance for agronomic traits among selected okra genotypes to identify promising families for further selection and breeding. Parental genotypes LS09, LS10 and L24 showed positive GCA effects for PYP under DS conditions, priming it as a valuable germplasm for variety design. Crosses LS01 × LS17, LS01 × LS18, LS09 × LS10, LS09 × LS18, LS09 × LS24, LS15 × LS18, LS15 × LS21, LS15 × LS24 and LS17 × LS21 expressed positive SCA effects for PYP under DS condition and are recommended for further breeding and commercialization for targeted production in water-scarce environments of South Africa.

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An overview of the research findings

Introduction and objectives of the study

Okra (*Abelmoschus esculentus* L., 2n = 130) is a vegetable and seed oil crop cultivated in tropical and subtropical climates with limited rainfall conditions globally. It is an allotetraploid derived from the natural hybridization of a wild progenitor, A. *tuberculatus* (2n = 58), with another unidentified species with 2n = 72 chromosomes. Okra is a relatively drought-tolerant crop that can grow successfully under water-limited conditions with minimal supplemental irrigation. Despite being relatively drought-tolerant, the crop fails to reach its maximum yield potential, resulting in low marketable pod yields, primarily when drought stress occurs at the flowering and pod development stages. For example, 37 to 83% of yield losses attributed to drought stress occurred during the reproductive stage. The low yield performance is related with the cultivation of low-yielding and drought-sensitive varieties. Exploring the okra genetic resources available in SSA and globally is essential to identify potential sources of valuable traits associated with drought tolerance for trait introgression and breeding programmes. Therefore, the aim of this research was to determine the genetic diversity present among okra germplasm collections using phenotypic and physiological traits and SSR markers under drought-stressed and non-stressed conditions to identify and select unique and good combiner parental accessions and progenies for pre-breeding. The specific objectives of the study included:

i. To determine phenotypic response of okra (*Abelmoschus esculentus*) genotypes under drought-stressed and non-stressed conditions to select promising breeding parents.
ii. To characterize okra (*Abelmoschus esculentus*) accessions with variable drought tolerance through simple sequence repeat markers and phenotypic traits and identify genetically distinct lines for breeding.
iii. To assess drought tolerance of okra (*Abelmoschus esculentus*) genotypes based on leaf gas exchange and chlorophyll fluorescence to guide breeding for drought tolerance in okra.
iv. To determine the combining ability effects and heterosis of selected okra (*Abelmoschus esculentus*) accessions for yield and yield-related traits under drought-stressed and non-stressed conditions and select the best combiner parents and families for breeding.

Research findings in brief

Phenotypic response of okra (*Abelmoschus esculentus*) genotypes under drought-stressed and non-stressed conditions

Twenty-six okra genotypes were evaluated in greenhouse and field environments under drought-stressed and non-stressed conditions. Data were collected on the following agronomic traits: number of branches (NB) per plant, stem diameter (SD), plant height (PH), number of days to maturity (DTM), number of pods per plant (NPP), fresh pod length (FPL), mature pod length (DPL), mature pod weight (DPW) and number of seeds per plant (NSP), anthocyanin (ATY) pigmentation and yield per plant (YPP). Data were subjected to analysis of variance, Pearson's correlation analysis, principal component analysis and biplots using R version 4.0. The core findings of the study were:

- Significant ($P<0.05$) differences in yield and yield components traits were found among genotypes, water condition effect, and their interaction that allowed for the selection of okra genotypes for water-limited conditions.
- Significant and positive correlations between yield components and pod yield under both water regimes indicate that these traits are useful for improving yield potential for water-constrained environments.
- Okra genotypes such as LS01, LS09, LS15, LS17, LS18, LS21 and LS24 with higher fresh pod length, number of pods per plant and fruit yield per plant under drought stress conditions were identified, as they possess suitable phenotypic traits that promote the development of high yield potential.
- The above-selected lines are recommended for pre-breeding and breeding programs.

Characterization of okra (*Abelmoschus esculentus*) accessions with variable drought tolerance through simple sequence repeat markers and phenotypic traits

Twenty-five okra landrace accessions sourced from the Agricultural Research Council, Vegetable, Industrial and Medicinal Plants (ARC-VIMP)/South Africa and a locally adapted and grown okra variety, "Clemson Spineless", were used for this study. Genomic DNA was extracted from 20 seeds per genotype using a modified CTAB method. Bulked DNA was used for amplification and analysis. Nine polymorphic SSR markers developed for okra were selected and used. The test lines were phenotyped using the following traits: the number of

pods per plant (NPPP), fresh pod length (FPL), pod yield per plant (PYPP), dry pod length (DPL), mature dry pod weight (DPW), total above-ground biomass (AGB), root weight (RW), root to shoot ratio (RSR) and harvest index (HI). The main findings were as follows:

- High polymorphism were recorded, suggesting that the selected SSR markers are suitable for distinguishing the genetic diversity among the tested accessions.
- The okra accessions were grouped into three distinct genetic clusters, indicating the presence of genetic differences among the studied okra accessions.
- Significant ($P<0.05$) genotype, water regime and interactions effects were detected for several phenotypic traits. This allowed the identification and selection of ideal accessions suited for irrigated and drought-prone environments.
- The phenotypic and genotypic clustering of the test lines under drought-stressed conditions was relatively inconsistent compared to non-stressed conditions.
- Overall, the SSR markers were valuable in complementing phenotypic selection.

Assessment of drought tolerance in okra (*Abelmoschus esculentus*) genotypes based on leaf gas exchange and chlorophyll fluorescence

Twenty-six genetically distinct okra genotypes with varied levels of drought tolerance were used for the study. The accessions were evaluated for their morphological responses to drought stress under field and glasshouse environments. Gas exchange and chlorophyll fluorescence parameters were measured using LI-6400 XT Portable Photosynthesis system (Licor Bioscience, Inc. Lincoln, Nebraska USA). The following parameters were determined: stomatal conductance (gs), net CO2 assimilation rate (A), transpiration rate (T), intercellular CO_2 concentration (C_i) and the ratio of intercellular and ambient CO_2 (C_i/C_a) concentrations, The ratio of A and gs was used to compute intrinsic water use efficiency (WUE_i), the ratio of A and T was used to calculate instantaneous water-use efficiency (WUE_{ins}). To estimate chlorophyll fluorescence variables, the following parameters were recorded: the minimum (F_o') and maximum fluorescence (F_m') of light-adapted leaves under natural glasshouse conditions. The steady-state fluorescence (F_s) was also determined in light-adapted photosynthesis. Additional chlorophyll fluorescence parameters were estimated such as the maximum quantum efficiency of photosystem II photochemistry (Fv'/Fm'), the effective quantum efficiency of photosystem II photochemistry (ϕ_{PSII}), photochemical quenching (qP), non-photochemical quenching (qN), and electron transport rate (ETR) and the alternative

electron sink (AES). Data were subjected to analysis of variance, correlation analysis, principal component analysis, biplots using R version 4.0 and XLSTAT. The core findings of the study were:

- Significant ($P<0.05$) differences for most evaluated traits of leaf gas exchange and chlorophyll fluorescence traits were found among genotypes, drought conditions, and their interaction enabling the selection of okra genotypes for water-limited conditions.
- Seven principal components (PCs) contributing to 82% of the total variation for assessed physiological traits were identified under drought-stressed conditions.
- The study identified drought-tolerant accessions, namely LS05, LS06, LS07 and LS08 based on high A, T, *Fm'*, *Fv'/Fm'* and ETR, and LS10, LS11, LS18 and LS23 based on high *AES*, *Ci*, *Ci/Ca*,*WUEi*, *WUEins*, *ϕPSII* and AES. These selected genotypes were high yielding under drought stress conditions.
- Physiological variables complement phenotypic trait response to guide genotype selection for water-limited agroecologies.

Combining ability effects and heterosis of selected okra (*Abelmoschus esculentus*) genotypes for yield and yield related traits under drought stressed and non-stressed conditions

Eight okra genotypes were selected and crossed using an 8 × 8 half diallel mating design to generate new genetic combinations. The parents were selected based on their high yield potential and tolerance to drought stress. The eight parents and 28 crosses were field evaluated using a 12 × 3 lattice design with three replications at two different sites: Loskop in Limpopo and Brits in North-West Province in South Africa. Data were collected on days to 50% flowering (DTF), plant height (PH), number of branches per plant (NOB), number of leaves per plant (NOL), stem diameter (SD), fresh pod length (FPL), number of fresh pods per plant (NFPP), fresh pod yield per plant (PYP), dry pod weight (DPW) and hundred seed weight (HSW). The main outcomes of this study were as follows:

- Significant ($P<0.01$) effects of genotype, environment, and genotype × environment interaction was recorded for pod yield and component traits.
- General combining ability (GCA) and specific combining ability (SCA) effects were significant ($P<0.05$) for most traits, indicating the role of additive and non-additive gene action underlying the inheritance of the assessed traits.

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- Crosses LS01 × LS17, LS01 × LS18, LS09 × LS10, LS09 × LS18, LS09 × LS24, LS15 × LS18, LS15 × LS21, LS15 × LS24 and LS17 × LS21 expressed positive SCA effects for PYP under DS condition and are recommended for genetic advancement, production, and commercialization in water-scarce environments of South Africa.

Implications of the study for population improvement and breeding of okra with drought tolerance

- High levels of phenotypic variations were recorded for drought tolerance under non-stressed and drought-stressed conditions, indicating that genetic improvement is possible through directional selections.
- The currently used SSR markers provided complementary data for selecting superior okra genotypes with high yields.
- Optimal gas exchange and photoprotection enhanced drought adaptation in the assessed okra genotypes and tested water regimes, indicating that physiological traits could be beneficial for drought tolerance breeding in okra.
- Significant GCA and SCA effects for studied traits indicated the role of additive and non-additive gene action underlying the inheritance of the traits, respectively.
- The GCA effects of parents and SCA effects of crosses allowed the selection of promising breeding parents (LS09, LS10 and LS24) and new progenies (LS01 × LS17, LS01 × LS18, LS09 × LS10, LS09 × LS18, LS09 × LS24, LS15 × LS18, LS15 × LS21, LS15 × LS24 and LS17 × LS21). The selected genotypes are desirable for genetic advancement and pure line and hybrid okra cultivar development that are adapted to water-limited conditions in South Africa or similar agroecologies.