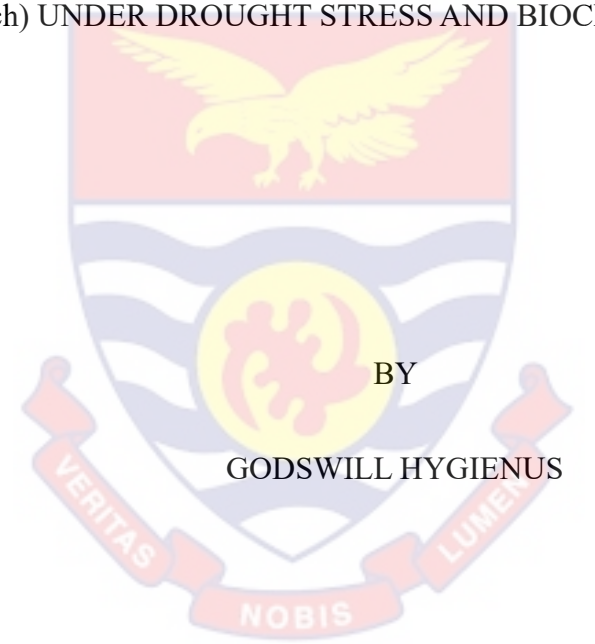
UNIVERSITY OF CAPE COAST

SELECTION FOR SUPERIOR ROOT SYSTEM ARCHITECTURE, BIOCHEMICAL AND YIELD TRAITS IN OKRA (*Abelmoschus esculentus* (L.) Moench) UNDER DROUGHT STRESS AND BIOCHAR AMENDMENT



BY

GODSWILL HYGIENUS

2025

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SELECTION FOR SUPERIOR ROOT SYSTEM ARCHITECTURE, BIOCHEMICAL AND YIELD TRAITS IN OKRA (*Abelmoschus esculentus* (L.) Moench) UNDER DROUGHT STRESS AND BIOCHAR AMENDMENT

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GODSWILL HYGIENUS

Thesis submitted to the Department of Crop Science of the School of Agriculture, College of Agriculture and Natural sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Master of Philosophy degree in Crop Science.

MARCH 2025

DECLARATION

Candidate's Declaration

I certify that this thesis is based on the results of my original research except where specific reference to other studies have been made. Neither this thesis nor any section has been previously presented for a degree at the University of Cape Coast or elsewhere.

Candidate's Signature…… Date……

Name: Godswill Hygienus

Supervisors' Declaration

I declare that the preparation and presentation of this thesis were supervised following the guidelines on supervision of the thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature……. Date……

Name: Professor Michael Osei Adu

Co-Supervisor's Signature……. Date……

Name: Professor Paul Agu Asare

ABSTRACT

A robust root system architecture (RSA) in interaction with increased antioxidant activities and osmoprotectants accumulation confer tolerance to crops when challenged by drought, resulting in improved yields. In addition to these innate plant mechanisms, various soil amendments, such as biochar, have also been proven to alleviate drought impacts on crops. Two experiments were conducted in this study. A greenhouse study was first conducted to assess genotypic variation in the RSA of 60 okra genotypes at the seedling stage. Based on the first experiment's results, ten genotypes from various clusters were selected for further screening under drought and biochar amendment in the 2nd experiment. In the 1st Experiment, genotypic variation was observed in all the RSA and biomass traits analysed. Genetic coefficient of variation (GCV) was high (>20%) for all biomass traits and the majority of RSA traits, barring lateral root angle and primary root length, which had low (<10%) GCV. High (>60%) broad-sense heritability (H^2) was recorded for all traits. Correlation analyses revealed a significant positive relationship between total root length and all other RSA traits. Population structure analysis through Ward's hierarchical clustering grouped the genotypes into two clusters, with cluster 2 membership superior in most RSA traits. In the 2nd Experiment, drought elicited hyper-antioxidant (superoxide dismutase, ascorbic acid and salicylic acid) activities, increased osmoprotectants (proline and carbohydrate) and reduced pod yield (pod length, pod diameter, number of pods per plant and total pod yield). However, there were differential genotypic responses. Some genotypes recorded higher antioxidant and osmoprotectant contents, translating into higher yields. Biochar application mitigated the drought impact at increasing rates, evidenced by reduced antioxidants and osmoprotectants content, but increased pod yield. This study, therefore, demonstrated the presence of genetic diversity in the RSA of okra and the drought-mitigating potential of oil palm empty fruit bunch biochar on the biochemical and yield traits of okra. On the whole, cluster 2 genotypes (VI060692 and GH112) with superior RSA recorded greater overall antioxidant and osmoprotectants contents, and total pod yield, suggesting that RSA can be harnessed in selecting drought-tolerant okra genotypes.

KEY WORDS

Antioxidants

Biochar

Drought stress

Okra (*Abelmoschus esculentus*)

Osmoprotectants

Root system architecture

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DEDICATION

To God Almighty; my mother: Deaconess Mrs. Roselyne Hygienus; and my siblings: Mr. Emmanuel Hygienus and Miss. Precious Hygienus.

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Maximum diameter	Mxd
Median diameter	Md
Number of branch points	Nbp
Number of first order laterals	Nfol
Number of pods per plant	Npp
Number of root tips	Nrt
Pod diameter	Pd
Pod length	Pl
Primary root length	Prl
Proline	Pro
Projected area diameter range 1	PADR1
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Root dry weight	Rdw
Root length diameter range 1	RLDR1
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Root network area	Na
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Root surface area	Sa
Root system architecture	RSA
Root-to-shoot ratio	RS
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CHAPTER ONE

INTRODUCTION

Background to the study

Okra (*Abelmoschus esculentus* (L.) Moench) is a multipurpose vegetable with a wide range of applications due to the diverse uses of its pods, leaves, buds, stems, flowers, and seeds (Yonas et al., 2014). From a nutritional perspective, the tender pods are used in various culinary preparations, including salads, soups, and stews, and can be eaten in the boiled, fried, fresh, or dried forms (Ndunguru & Rajabu, 2004). The pods contribute essential nutrients such as carbohydrates, protein, fibre, fat, and minerals such as calcium, zinc, iron, sodium, magnesium, nickel, and potassium (Khan & Rab, 2019). Okra is rich in vitamins A, B, and C, folate, antioxidants, and unsaturated fatty acids such as linoleic acid, which are vital for human nutrition (Ibitoye & Kolawole, 2022). Additionally, okra's medicinal properties are well-documented. The pods possess curative effects against dysentery, gonorrhoea, and urinary disorders (Chittora et al., 2016). Industrially, the fibre from okra stems can substitute for jute (Chanchal et al., 2018). Mucilage is applied to glazing certain papers (Markose & Peter, 1990). Additionally, baked foods and sweetened frozen foods (e.g., ice creams) utilise polysaccharides to extend the shelf life of these products (Archana et al., 2016).

In Ghana, okra is the fourth commonest vegetable, following tomatoes, capsicum pepper, and garden eggs, and it is extensively grown across various regions of the country (Oppong-Sekyere et al., 2011). Fresh okra is readily available in nearly all markets during the wet season, while in the dry season, especially in

Northern Ghana, dehydrated forms of okra are prevalent due to their significant commercial value (Oppong-Sekyere et al., 2011).

However, despite the nutritional, economic, and industrial importance of okra, the mean pod yield in Sub-Saharan Africa (SSA) is 2.5 tons per hectare (t/ha) compared to the potential yield reaching 8.8 t/ha (Mkhabela et al., 2022). Several factors contribute to this low productivity, with a significant portion attributed to drought, heat stress, and the utilization of unimproved varieties that struggle to adapt to arid and semi-arid environments (Alake, 2020). Drought is the severest abiotic stressor impeding crop production (Moussa, 2011; Rohbakhsh, 2013) as a result of the disruption of several morpho-physiological and biochemical processes which regulate the development of plants (Bahadur et al., 2013; Ewetola & Fasanmi, 2015). During drought, there is an accumulation of Reactive Oxygen Species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), and hydroxyl radicals ($OH^{\bullet-}$) (Yasar et al., 2008) which trigger oxidative stress and the obstruction of normal cells functioning in plants (Stanley & Yuan, 2019). The attack of ROS on nucleic acids, lipids, and proteins leads to lipid peroxidation, protein denaturation, DNA mutations, and damage to terpenoids and carbohydrates (Dawood et al., 2019; Guo et al., 2018). Water deficit results in a loss of turgor, restricting cell division and elongation. This, in turn, leads to reduced plant growth, decreased light interception, and reduced chlorophyll content (Lawlor & Cornic, 2002). These effects ultimately result in reduced photosynthesis, respiration, leaf size, plant biomass, root proliferation, and overall crop yields (Ayub et al., 2020; Farooq et al., 2009a).

In response to stressors, different molecular, physiological, and biochemical processes are initiated by plants to adapt to adverse conditions. These adaptation processes include the accumulation of compatible osmolytes, alterations in gene expression and the activation of antioxidant systems (Ahmed & El-Sayed, 2021). Proline, among the most crucial osmolytes, is accumulated by plants when challenged by drought, playing a vital role in helping plants to withstand stress (Lintunen et al., 2020). To further achieve osmotic adjustment, plants increase the sugar content in their roots and leaves (Seleiman et al., 2021). The enzymatic antioxidant machineries of the defense system established in response to stress include Catalase (CAT), Superoxide Dismutase (SOD), Glutathione Reductase (GR), and Ascorbate Peroxidase (APX), serving as ROS scavengers (Das et al., 2020). Tolerant crops activate their defense mechanisms when faced with water scarcity (Chaves & Oliveira, 2004). The expected rise in the occurrence of extreme weather events, such as erratic rainfall patterns due to climate change, puts more land at risk of drought globally. Therefore, screening and selecting drought-tolerant okra genotypes with heritable traits for breeding drought-resistant varieties is the most viable approach to ensure a continuous and sufficient food production for the growing world population.

Furthermore, the root system is pivotal in plants' developmental process. Root system architecture (RSA), which is the spatial distribution of roots in the soil environment (Lynch, 1995; Rich & Watt, 2013), exhibits plasticity and dynamism (Zhu et al., 2011; Sun et al., 2021), enabling plants' response to their environment for enhanced water and nutrient acquisition (Sun et al., 2021; Zhu et al., 2011).

Substantial evidence supports the significance of diverse RSA traits in bestowing resistance upon crops against drought events. For instance, the root growth angle (RGA) emerges as a critical determinant of whether a plant develops shallow or deep roots, given its role in directing the elongation of roots (Kitomi et al., 2015; Uga et al., 2015). Studies showed that a higher or nearly vertical RGA positively correlates with deep roots formation (Kato et al., 2006; Oyanagi et al., 1993). DEEPER ROOTING 1 (DRO1), a quantitative trait locus (QTL) associated with RGA, was characterized and cloned in rice (Uga et al., 2012, 2013). Upon introgression into a shallow-rooted cultivar, the resultant DRO1 near-isogenic line (NIL) demonstrated a significantly elevated yield when cultivated under water deficit (Uga et al., 2013). Similarly, in a separate study, a drought-adapted wheat genotype was observed to possess a compact root system, allocating a greater proportion of its roots at depth and with significantly greater root length. This adaptation yielded an average yield advantage of 14.5% under water deficit (Manschadi et al., 2010). These, in addition to a myriad of other RSA traits (such as surface area, number of branch points, diameter, etc.), are key characteristics that can be strategically leveraged within breeding programmes to develop drought-resistant crop varieties.

Besides the molecular, biochemical and physiological mechanisms that plants employ in adapting to drought stress, certain soil amendments have shown promise in enhancing a plant's ability to withstand drought and improve water-use efficiency, one of which is biochar (Batool et al., 2015). Biochar is a pyrolysed biomaterial which is rich in carbon (McGlashan et al., 2012). The positive effect of

biochar application on crop production is attributable to its ability to improve the physicochemical and biological properties of soils (Jabborova et al., 2021). This includes increasing organic matter content, reducing bulk density, improving aeration and cation exchange capacity, enhancing water-holding capacity, and promoting microbial activities due to the porous nature of biochar (Singh et al., 2019). As a result, Abewoy (2018) opined that investing in such soil amendments, particularly for high-value vegetable crops, is advantageous, especially for small-scale farmers, as these crops can generate significantly higher income per hectare than staple crops.

Biochar can be prepared from diverse organic materials, including the oil palm empty fruit bunch (EFB). Many studies have assessed EFB application to the soil as biochar, organic mulch, or through composting (Anyaoha et al., 2018). For example, EFB application in biochar or compost has been found to augment water and nutrient content of soil (Ahmad-Dani, 2018). With Ghana generating about 390 tons of EFB per day (Adu et al., 2022a), biochar production and use opens the avenue to turn these wastes into useful materials that can be incorporated into the mix of strategies for mitigating drought impacts on crops.

Problem statement

Agriculture heavily relies on both the timing and distribution of rainfall and water resources (Rosegrant et al., 2009). Globally, an estimated 3,830 cubic kilometres (km^3) of water are withdrawn annually, with 70% of this, approximately 2,664 km^3, allocated for agricultural purposes (Molden et al., 2011), making agriculture the largest consumer of water. Projections indicate that crops' annual

water consumption, including precipitation and irrigation, will increase by 0.7% per year, reaching 8,600 km³ by 2025 and 9,060 km³ by 2050 (Rosegrant et al., 2009). Non-irrigation water use is also expected to double by 2050, surpassing 700 km³ per year. Many regions are already experiencing water scarcity globally, which is anticipated to worsen due to climate change impacts, population growth, and economic and land-use changes (Mancosu et al., 2015). These factors cast a dim shadow over future availability of sufficient water for plant use.

As a stress factor, water scarcity exerts detrimental effects on plant growth and development, especially in the arid and warm regions of the world. Drought conditions limit crops from reaching their maximum genetic potential in yields (Begna, 2020). Severe droughts carry a significant risk of substantial yield losses and the potential for total crop failure, particularly in regions where crop production is chiefly rainfed (Begna, 2020). The escalating intensity, frequency, and duration of drought events on a global scale continuously threaten food security (Ngcamu & Chari, 2020). Over 800 million people face chronic hunger, with millions more at risk. As the world's population is estimated to reach 9.2 billion by the year 2050 (Rosegrant et al., 2009), agriculture, particularly vegetable production, is faced with the challenge of meeting this ever-expanding population's food and nutritional requirements (Singh et al., 2019).

Vegetables form key component of the world's daily cuisine, providing essential nutrients like vitamins and minerals for body growth and repair (Anyaoha et al., 2015). They are considered protective foods due to their significant contribution to maintaining good health and preventing diseases (Ngbede et al.,

2014). Okra, one of the most widely cultivated vegetables in tropical regions, has a nutritional profile with essential and non-essential amino acids comparable to soybeans (Ngbede et al., 2014). While okra is generally noted for its relative tolerance to drought (Singh et al., 2014), drought can potentially reduce yields, depending on its severity and the specific phenological stage of the crop (Romaisa et al., 2015). Bahadur et al. (2013) highlighted that drought, particularly during anthesis and pod-filling stages, could cause yield reductions exceeding 70% in okra. Given the vital role that okra plays as a key food security crop in many countries, Anyaoha et al. (2015) underscored the necessity of developing varieties that are resilient and high-yielding to help mitigate the adverse economic effects of climate change induced challenges like drought, flooding, high salinity, and low nutrient availability. Hence, breeders face the crucial task of screening and identifying tolerant genotypes to serve as parents for further breeding works.

Justification

The first organ to detect changes in soil moisture is the plant's root (Eltigani et al., 2021) and serves as the initial line of defence during drought (Fenta et al., 2014; Manavalan et al., 2010). Water deficit prompts plants to redirect assimilates towards root growth rather than shoot growth, enabling deeper soil penetration (Rich & Watt, 2013). This extensive root system promotes nutrient and water absorption, contributing to a plant's drought tolerance (Gewin, 2010). Similarly, steeper-angle roots are better suited for capturing mobile resources like water and nitrogen, which rapidly traverse the soil profile and accumulate at greater depths (Lynch & Wojciechowski, 2015; Trachsel et al., 2011). However, significant

differences in the morphology of roots, including diameter, area, length, and volume, are observed in plant species and within different genotypes of the same species (Eltigani et al., 2021). Therefore, assessing the genetic diversity in RSA is essential for developing resilient crops capable of withstanding abiotic stress, effective in soil exploration, and efficient in acquiring and utilizing soil resources to achieve higher yields.

Additionally, oil palm EFB, the residual biomass left after the extraction of fresh oil palm fruits, constitutes one-third of the dry matter generated during the production of crude palm oil (Adu et al., 2022a). Globally, large quantities of oil palm EFB are produced from oil palm plantations, with an annual production of nearly 99 billion metric tons worldwide (Geng et al., 2015), with Ghana contributing 390 tons per day (Adu et al., 2022a). Traditionally, EFB is often disposed of by allowing it to decompose naturally or by burning it, which, in turn, leads to increased greenhouse gases emissions, including carbon dioxide and methane (Adu et al., 2022a). However, there is a well-documented practice of applying EFB to soils in various forms, such as organic mulch (in its raw form), biochar, or compost (Anyaoha et al., 2018), and they serve to enhance the soil's water and nutrient retention capacity (Batool et al., 2015). The use of EFB biochar in soils has been shown to enhance crop growth and yield by approximately 78.4% compared to unamended soils (Adu et al., 2022a). Because EFB is a readily available resource in Ghana, this offers a huge potential in mitigating drought impacts and heralds a step in the right path towards attaining food security amid a changing climate. More so, incorporating the EFB into soils mitigates the emission

of greenhouse gases accompanying its incineration and decomposition (Adu et al., 2022a) thereby helping reduce climate change events.

Objectives of the study

General objective

This study seeks to quantify the variations in the RSA of sixty okra genotypes and assess the drought-mitigating potential of oil palm EFB biochar on the pod yield and biochemical aspects of the leaves.

Specific objectives

This study specifically seeks to:

1. Assess genotypic variation in the RSA of sixty okra genotypes.

2. Evaluate the effects of drought and the drought-mitigating potential of oil palm EFB biochar on the biochemical traits of selected okra genotypes.

3. Evaluate the effects of drought and the drought-mitigating potential of oil palm EFB biochar on the yield traits of selected okra genotypes.

4. Select drought-tolerant okra genotype(s) for further breeding works.

Research hypothesis

The following hypothesis will be tested in this study:

1. Genotypic variation does not exist in the RSA of okra.

2. Drought stress does not affect the biochemical traits of okra.

3. Drought stress does not affect the yield traits of okra.

4. Oil palm EFB biochar does not have modulating effect on okra's biochemical traits under drought stress.

5. Oil palm EFB biochar does not have modulating effect on okra's yield traits under drought stress.

Expected outcomes

The following outputs will be garnered at the end of this study:

1. The RSA of okra will be described, and the existing genotypic variations will be quantified.

2. The drought-mitigation potential of oil palm EFB biochar will be quantified and either recommended or rejected as a suitable soil amendment against drought.

3. Drought-tolerant okra genotype(s) will be selected for further breeding.

CHAPTER TWO

LITERATURE REVIEW

Botany, origin and distribution of okra

Okra is one of Malvaceae family's most commonly utilized species (Naveed et al., 2009). It is an annual herbaceous dicotyledonous crop with an indeterminate growth habit (National Research Council, 2006). The presently accepted binomial nomenclature for this particular species is *Abelmoschus esculentus* (L.) Moench (Siemonsma, 1982). However, *A. esculentus* goes by various local names across different regions of the world. For instance, it is referred to as "fetri" in Ewe, "nkruma" in Twi, "quillobo" in Congo, "okwuru" among the Igbos of Nigeria, "lady's finger" in English, "ocra" in Italian, "gombo" in French, "ocker" in German, and "gumbro" in Portuguese, among other names (National Research Council, 2006).

There are two primary theories explaining the origin of *A. esculentus* (Tripathi et al., 2011). The first theory revolves around *A. tuberculatus* (the wild species), which is a close relative of *A. esculentus*. *A. tuberculatus* is native to the medium altitude hilly regions in Uttar Pradesh near Saharanpur in the areas of Ajmer and Indore in India (Charrier, 1984). This posits that the origin of *A. esculentus* can be traced to these regions (Tripathi et al., 2011). An alternative perspective proposes that the domestication of *A. esculentus* occurred in Ethiopia. This theory is based on another putative ancestor, *A. ficulneus*, and the evidence of ancient cultivation in East Africa (Tripathi et al., 2011). It is believed that from this point of origin, the cultivation of *A. esculentus* permeated the Middle East and

North African regions (Lamont, 1999). However, despite the differing theories on its origin, *A. esculentus* is cultivated worldwide, from the Mediterranean to equatorial regions.

Importance of okra

Every component of the okra plant possesses one or more valuable applications, whether nutritionally, medicinally, economically or industrially. The premature green pods are employed as a dietary vegetable, and the extract from these pods are used in thickening numerous recipes for soups and sauces, enhancing their consistency (Kumar et al., 2013). A 100 g consumable portion provides dry matter (10.4 g), energy (3100 calories), protein (1.8 g), calcium (90 mg), iron (1.0 mg), carotene (0.1 mg), thiamin (0.07 mg), riboflavin (0.08 mg), and niacin, along with 18 mg of vitamin C (Grubben et al., 1977). Also, the pods contain 103 mg of potassium, 43 mg of magnesium, and 56 mg of phosphorus (Smartt & Simmonds, 1995). Notably, no variation in the protein efficiency ratio was observed between heated (130°C) and non-heated flour, indicating the absence of anti-nutritional factors (Karakoltsidis & Constantinides, 1975).

In addition to the tender pods, the okra plant leaves are also used as a vegetable. Each 100 g of edible pods exhibit a nutritional composition consisting of water content at 81.50 grams, providing 56.00 kcal of energy, along with 4.40 grams of protein, 0.60 grams of fat, 11.30 grams of carbohydrates, 2.10 grams of fiber, 532.00 mg of calcium, 70.00 mg of phosphorus, 0.70 mg of iron, 59.00 mg of ascorbic acid, 385.00 µg of β-carotene, 0.25 mg of thiamin, 2.80 mg of riboflavin, and 0.20 mg of niacin (Gopalan et al., 1971; VarmuDy, 2011). In regions

where a diverse range of leaves is consumed, notably in West Africa and Southeast Asia, tender okra leaves are commonly prepared like spinach or added to soups and stews (National Research Council, 2006). Like pods, okra leaves are often sun-dried and made into powdery form for future use (National Research Council, 2006).

A coffee substitute devoid of caffeine can be made through roasting and grinding of okra seeds (Çalışır et al., 2005). Moreover, okra possesses promise as a crop for essential oilseed production thanks to the seeds' high oil content, ranging from 20% to 40% (Benchasri, 2012). The oil yield from these seeds can be compared to most other oilseed crops, excluding soybean and oil palm (Kumar et al., 2010). A pleasing taste and aroma characterize the greenish-yellow edible oil derived from these seeds, and it is a rich source of unsaturated fats, particularly oleic and linoleic acid (Tripathi et al., 2011). Okra seed meal is notable for containing over 50% high-quality protein on a fat-free, dry-weight basis, of which the amino acid profile equals or exceeds those found in eggs, casein, and the United Nations Food and Agriculture Organization's reference protein from okra sources (Akingbala et al., 2003). Unlike the proteins found in cereals and pulses, okra seeds provide a balanced combination of amino acids (e.gs, lysine and tryptophan) (Holser & Bost, 2004; Kumar et al., 2010). Furthermore, the buds and flowers of the plant are also edible. As a result, okra serves as a valuable reservoir of essential minerals that are often lacking in developing countries' diets.

Okra mucilage is applied medically as a plasma replacement or blood volume expander. This mucilage is also utilized as a tablet binder and suspending

agent in various formulations (Kumar et al., 2009). Furthermore, okra exhibits several potential health benefits in addressing human ailments, particularly type 2 diabetes, cardiovascular diseases, cancer, and digestive disorders (Dubey & Mishra, 2017). The soluble fibre in okra lowers serum cholesterol levels, reducing cardiovascular disease risk (Gemede et al., 2015). Okra's ability to slow the absorption of sugar makes it a suitable choice as an anti-diabetic food (Nawaz et al., 2020). Research has indicated that alcohol extract from the leaves of okra has the potential to eradicate free oxygen radicals, relieve renal tubular-interstitial disorders, reduce proteinuria, and enhance renal function (Kumar et al., 2009; Liu et al., 2005). Historically, infusions and decoctions of *A. esculentus* pods have been employed in traditional medicine to treat diarrhea, acute inflammation, stomach and bowel irritation, catarrhal infections, gonorrhea, dysuria, dental ailments, bronchitis, and pneumonia (Habtemariam, 2019). Additionally, the pods are recognized for their potent aphrodisiac properties (Elkhalifa et al., 2021; Obeten et al., 2022).

From an industrial perspective, okra mucilage finds application in glazing certain types of papers (Markose & Peter, 1990). Sweetened frozen foods (e.g., ice creams) and baked foods employ the polysaccharides found in okra, not only for their health benefits, but also for extending the products' shelf life (Archana et al., 2016). The stems and roots of okra are employed in the clarification of sugarcane juice, a process used to produce gur or brown sugar. Okra stems contain longer fibre within their woody cores than most dicotyledonous plants. This unique attribute makes mature fruits and stems, with their crude fibre content, suitable as substitutes

for jute and in manufacturing paper and textiles (Chanchal et al., 2018; Tripathi et al., 2011).

Besides its numerous applications, okra cultivation holds substantial economic significance, serving as a primary source of income for many rural farmers in developing countries. Notably, in Ghana, particularly in the Ashanti Region, a considerable portion of farmers rely on okra production as their primary means of livelihood (Cobbinah & Kwoseh, 2021). The National Agriculture Research Project reported that with good management practices, a yield of 10–15 t/ha is achievable for okra (NARP, 1993). Building on this, Cobbinah and Kwoseh (2021) opined that this could position the crop as a major contributor to Ghana's foreign exchange earnings.

Okra possesses substantial importance in many regions of the world. As a highly versatile crop, each part serves multiple purposes, from nutrition and medicine to industry and economics. It can be rightly asserted that there is minimal to no waste in the production of okra, as each part has multiple useful applications.

Environmental requirements of okra

As a warm-season crop, okra thrives best within an average minimum and maximum temperature ranges of 18 °C and 35°C, respectively (Ezeakunne, 1984; Grubben et al., 1977). Under higher temperatures beyond 40°C to 42°C, the plant often experiences flower desiccation and abortion, resulting in reduced yields. Due to its frost-sensitive nature (Teets & Hummel, 1988), okra is typically grown in the summer in the temperate and subtropical areas (Diizyaman, 2010). Although okra has the potential to flourish in diverse soils, it achieves optimal growth in well-

drained, fertile sandy-loam soil, with an optimal pH range of 6.0 to 7.0 (Lamont, 1999). Under optimal conditions of soil moisture and a temperature of 35°C, seedling emergence occurs in approximately 7 days (Iremiren & Okiy, 1986). However, at lower temperatures of 18/15°C (day/night), it takes about 14 days for seedlings to emerge (Lotito & Quagliotti, 1991). Soaking of the seeds in water for 24 hours before sowing doubles both the percentage and rate of germination, as this softens the hard seed coat. For the production of young edible fruits over an extended period, okra requires a moderate and well-distributed rainfall of around 80 to 100 cm (Benchasri, 2012). With good environmental conditions, the harvest of tender okra pods typically commences approximately 8 to 12 weeks after sowing, and the process of flowering and fruiting can continue indefinitely.

Drought stress in crops

Agricultural drought pertains to the insufficiency of the necessary moisture needed for the optimal growth, development and completion of the plant life cycle. This condition arises from a continuous shortfall in precipitation, commonly called meteorological drought. While inadequate rainfall is often the primary factor contributing to drought, the soil's water loss due to evapotranspiration, triggered by factors such as high temperatures, sunlight and winds, can further exacerbate prevailing drought stress (Cohen et al., 2021). Among all abiotic stress factors that hamper crop yields, water deficit is considered the most destructive and recalcitrant to the efforts of plant breeders (Tuberosa & Salvi, 2006) due to its disruption of numerous morphophysiological and biochemical processes responsible for plant development (Bahadur et al., 2013; Ewetola & Fasanmi, 2015).

Effects of drought stress on seed germination, growth and yield of crops

Apart from the decrease in overall germination, insufficient soil moisture availability leads to delayed emergence, a critical factor influencing numerous crops' vitality and subsequent yield potential (Gul & Allan, 1976). In arid and semiarid regions with mainly rainfed conditions, limited moisture is a significant constraint during germination (Rauf et al., 2007), and plants exhibit heightened vulnerability to drought impact during these early stages of growth. Water is the primary regulator of germination, as germination commences with water imbibition by seeds (Sghaier et al., 2022). Water absorption serves crucial functions by activating enzymatic reactions and facilitating the mobilization of stored seed reserves, encompassing lipids, carbohydrates, and proteins (Szczerba et al., 2021). The imbibed water softens the hard seedcoats, promoting radicle and plumule emergence. Consequently, early-season drought has a severe adverse impact on germination, and the establishment of plant stands, primarily due to lowered water absorption at the imbibition stage of seed germination, decreased supply of energy, and impaired activities of various enzymes (Okçu et al., 2005).

Studies have explored drought impacts on seed germination seeds in various crop species, including crops like rapeseed (Haj Sghaier et al., 2022). Drought stress was found to have adverse effects on germination rate, seedling vigour, coleoptile length, shoot and root length in bread wheat (Kızılgecı et al., 2017). Similarly, in okra, drought has been observed to significantly hinder seed germination (Amin & Mahmood, 2011; Devi et al., 2017) and seedling growth (Amin & Mahmood, 2011).

Photosynthesis is fundamental to plant growth and productivity (Singh & Thakur, 2018), proceeding through multiple stages that face significant impairment when plants encounter water stress. Drought impact on carbon fixation may generally be categorised into two types of limitations: stomatal limitations and non-stomatal limitations (Farooq et al., 2012). When plants encounter drought, their immediate response is to shut their stomata (Basu et al., 2016), a measure taken to restrict further transpirational-water-loss (Flexas et al., 2004). This reduced stomatal aperture is attributable to lowered water potential and turgor loss (Farooq et al., 2012). While the closure of stomata mitigates transpirational-water-loss, it lowers carbon dioxide (CO_2) and nutrients intake, causing alterations in metabolic pathways, including those associated with photosynthesis (Xiong & Zhu, 2002).

On the other hand, the non-stomatal limitations affecting the photosynthetic activities of crops during drought are evident in the impaired functions of vital photosynthetic enzymes like ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisco), NADP-malate dehydrogenase, phosphoenolpyruvate carboxylase, pyruvate phosphate dikinase, and NADP-malic enzyme (Reddy et al., 2004). A notable effect of decreased CO_2 levels within chloroplastic cells is the deactivation of Rubisco and impaired functions of sucrose phosphate synthase and nitrate reductase, along with a diminished capacity for the regeneration of ribulose bisphosphate (RuBP) (Reddy et al., 2004). Since the biochemical efficiency of photosynthesis under drought conditions primarily hinges on the regeneration of RuBP and the activity of RuBisCO, they become the predominant limiting factors

under severe drought, impeding the assimilation of CO_2 in the photosynthetic process (Lawlor & Cornic, 2002; Medrano et al., 1997).

ROS are highly reactive molecules that, without effective mechanisms of protection, can engender oxidative injury to lipids, proteins, and other macromolecules, disrupting normal plant metabolism (Rout & Shaw, 2001). ROS can significantly diminish the rate of photosynthesis during water stress, primarily by interfering with the photosynthetic apparatus. This interference encompasses elements like the D1 and D2 proteins found in the PSII complex, thylakoid, and chlorophyll pigments, and additionally, it has the potential to impede the synthesis of new D1, D2, and other proteins within the cell (Zlatev, 2009).

The growth process involves an irreversible increase in volume, size, or weight, and encompasses stages of cell division, elongation, and differentiation. Drought stress negatively affects both cell division and enlargement as a result of factors such as reduced enzymatic activities, loss of turgor pressure, and reduced energy supply (Kiani et al., 2007). Higher plant's foliar photosynthetic rate decreases as relative water content (RWC) and leaf water potential decrease (Lawlor & Cornic, 2002). In response to water-deficit, the stomata gradually close, leading to a subsequent reduction in net photosynthetic rates (Reddy et al., 2004). As a result, the decrease in crop productivity during drought can be attributed to diminished leaf growth and decreased photosynthetic output (Kannan & Kulandaivelu, 2011).

Moreover, limited water availability triggers signals that prompt a premature transition in plant growth and development from the vegetative stage to

the reproductive stage (Desclaux & Roumet, 1996). This shift shortens the crop's growth cycle and produces a substantial yield penalty (Farooq et al., 2012). The devastating impact of drought on the growth of various crop species, such as chickpeas (Pushpavalli et al., 2014), wheat and corn (Ray et al., 2018), among others, is well documented.

While okra is commonly perceived as a hardy vegetable under drought stress, several studies have documented significant growth impediments and yield reductions during drought occurrences. Adejumo et al. (2019) observed that drought reduced relative water content, leaf chlorophyll levels, and biomass yield relative to their well-watered (100% FC) counterparts. In a study, drought stress significantly decreased yield per plant from 7.20 g/plant under well-watered condition to 4.31 g/plant under drought-stress (Mkhabela et al., 2022). Furthermore, drought occurrence during anthesis and pod-filling stages resulted in yield loses exceeding 70% in okra (Bahadur et al., 2013). Ahmed and El-Sayed (2021) reported a reduction in the transpiration rate and the maximum quantum yield of PSII (Fv/Fm) in okra cultivars and their hybrids as the soil moisture content decreased. Reduced plant height, leaf area, number of leaves, delayed flowering, and instances of total plant mortality have also been observed in drought stressed crops relative to control conditions (Anyaoha et al., 2015). Additionally, it was found that water deficit at 25% FC significantly reduced the carotenoid and total protein contents of okra (Ayub et al., 2021).

These findings collectively emphasise the detrimental effects of drought on the physiological and biochemical processes of okra growth and development. It

further buttresses the fact that although okra is relatively drought tolerant, there could be significant growth and yield penalties during drought events. Unfortunately, okra is one of the most neglected crops with regard to improvement efforts. Hence, considering its role as a key food security crop in many regions of the world, there is a pressing demand to develop drought-resilient and high-yielding varieties to counteract climate change-induced drought effects.

Effects of drought on nutrient availability and uptake

Higher plants primarily acquire mineral nutrients through their root systems, and the uptake of these nutrients is shaped by both the demand for them and their availability at the root surface (Bederedse et al., 2007). Drought may result in reduced nutrient uptake for various reasons, such as a reduction in nutrient supply due to decreased mineralization (Sanaullah et al., 2012) and a reduced diffusion and mass movement of nutrients in the soil. Mineral nutrients, crucial for growth and reproduction in plants, are predominantly obtained by plants from the soil as inorganic ions (Barker & Pilbeam, 2015; Taiz & Zeiger, 2006). For example, nitrogen (N) is highly mobile. It is accessible to plants as either NO_3^- (nitrate) or NH_4^+ (ammonium) ions, phosphorus (P) in the form of $H_2PO_4^-$ and HPO_4^{2-}, and sulfur is primarily absorbed in the form of inorganic sulfate. So, if drought negatively affects the breakdown of organic matter by microbes, which in turn affects the availability of nutrients for plants, it could impede plants' ability to obtain essential minerals. Insufficient soil moisture availability constrains the activities of microbes in the soil, and depending on the duration and severity of the drought, microbial metabolism may be halted (Borken & Matzner, 2009). As a

result, drought initially elicits impaired bacterial activities, which can lead to desiccation and dieback during prolonged drought.

Furthermore, the transfer of small solutes like ions, organic acids, and sugars mirrors the movement of water up to the walls of root cells, which occurs passively through both mass flow and diffusion. From the soil-root environment, water and minerals are taken up and transported to higher parts of the plant through the xylem. Transpiration is crucial here, affecting the flow of water within root and xylem vessels through root pressure and the rate of transpiration. Higher transpiration rates promote the absorption and movement of mineral elements within the xylem. When soil moisture is reduced, stomatal closure may occur which in turn lowers transpiration (Pinkerton & Simpson, 1986). This reduction in transpiration diminishes nutrient diffusion and mass flow in the soil toward the root absorption surface (Pinkerton & Simpson, 1986), subsequently affecting the translocation of nutrients to the leaves.

Effects of drought on water relations

Plant water relations encompass the processes involved in the uptake and movement of water within plants, as well as the exchanges between roots and their rhizosphere (Taiz & Zeiger, 2010). Water potential gradient between the soil and the plant is a critical element influencing plant water relations, and it is influenced by factors such as the water requirement of plants, soil's hydraulic conductivity, soil type, and soil moisture levels (Chavarria & Santos, 2012), and the atmospheric demand (da Silva et al., 2013). Water taken up by the roots is subsequently lost through transpiration via the stomata, establishing a water potential gradient

between the rhizosphere and plant root surface. Water movement is naturally from regions of higher (more positive) water potential to those of lower (more negative) water potential. As a result, the regular water loss through transpiration promotes a continual influx of water from the soil to the root surface for absorption and subsequent transportation throughout the plant. In situations where the availability of soil water becomes limited, plants' primary response is to cut down water loss from transpiration through stomata closure. Transpiration impedance leads to a reduced water supply to the root surface. Consequently, a water shortage generally results in decreased stomatal and root hydraulic conductivity, which compromises the overall plant water status (Siemens & Zwiazek, 2004).

Plant water relations are characterized by various key attributes, including pressure potential, relative water content (RWC), transpiration rate, leaf water potential, and osmotic potential. These attributes are largely affected during water deficit as a result of the lowered water potential in the plant's environment (Mahdieh et al., 2008). Leaf water potential, which measures the leaf's water content, is widely acknowledged as a reliable indicator for assessing the response of a plant to water drought stress (Chowdhury et al., 2017). Leaf water potential has been suggested as a prominent selection trait in enhancing crop drought tolerance (Nayyar et al., 2005). Many studies have reported reduced Relative Water Content (RWC) in a range of plants, such as tomato and caper bush, under drought (Ozkur et al., 2009; Subramanian et al., 2006). Water potential decreased markedly in the leaves, roots, and pods of soybeans under drought stress, with a more rapid decline in the water potential of roots than that of the pods and leaves (Liu et al., 2004).

Additionally, both the RWC and osmotic potential of sunflower were negatively affected during drought condition (Tezara et al., 2002). Reducing RWC leads to decreased turgor and a diminished hydrostatic pressure gradient, ultimately limiting water availability for cell extension mechanisms. Distinct plant genotypes exhibit varying responses, where those that are tolerant to drought maintain greater leaf water potential for longer periods and showing delayed wilting compared to sensitive genotypes when they encounter drought conditions (Ouvrard et al., 1996).

Drought resistance mechanisms in plants

Plants have developed various morphological, physiological, and biochemical coping mechanisms when challenged by drought stress (Bohnert et al., 1995). Drought resistance refers to the general term describing plant species having some adaptive traits that enable them to escape, avoid or tolerate drought (Levitt, 1980). Nevertheless, it is worth acknowledging that these strategies are not mutually exclusive. In practice, plants often employ a blend of these mechanisms.

Drought escape mechanisms

Drought escape is the ability of a plant to complete its entire life cycle before significant soil and plant water deficits occur. This adaptation involves a shortened plant life cycle, which allow plants' reproduction prior to the onset of drought (Farooq et al., 2009a). Successful escape strategies rely on efficient allocation of resources to developing fruits and seeds before severe stress sets in (Bacelar et al., 2007). This capability is linked to the ability of plants to store reserves in specific organs, like stems and roots, utilising them in the production of fruits, as observed in crops such as cereals (Bruce et al., 2002) and some legumes

(Chaves et al., 2002). In arid regions, indigenous annuals may exhibit shortened life cycles, rapid growth, and high gas exchange, optimising resource utilisation while soil moisture remains available (Maroco et al., 2000; Mooney et al., 1987).

The concept of drought escape becomes particularly advantageous in regions with more frequent terminal drought, as early-varieties tend to evade terminal drought more effectively than late-maturing varieties (Meyre et al., 2001). The development of early-maturing varieties has proven to be a potent strategy against the yield losses caused by terminal drought, because their early maturation allows them to circumvent the stressful period (Kumar & Abbo, 2001). However, it is important to note that there exists a general correlation between crop yield and crop life cycle when environmental conditions are favourable, and yield will be taxed when there is a reduction in the duration of crop growth below the optimum (Turner et al., 2001).

Drought avoidance mechanisms

Drought avoidance is the ability of a plant to maintain a high-water status within its tissues or cellular hydration when subjected to drought conditions (Blum, 2005). Plants that lean toward drought avoidance often possess tissues highly susceptible to desiccation, and consequently, they must employ strategies to circumvent water deficits whenever water scarcity is encountered (Ludlow, 1989). Dehydration avoidance is a characteristic shared by both annuals and perennials and is linked to various adaptive traits (Bacelar et al., 2012). These traits encompass two primary approaches: "water savers", that minimize water loss, and "water spenders", that maximize water absorption (Basu et al., 2016).

To minimise water loss, plants employ various strategies such as closing the stomata, decreasing light absorption by rolling of leaves (Ehleringer & Cooper, 1992), the adoption of steep leaf angles, and the development of a dense layer of trichomes that promote light reflectance (Larcher, 2000). Leaves with hair-like structures exhibit lower temperatures and transpiration rates (Sandquist & Ehleringer, 2003). This presence of leaf hairs increases light reflectance, particularly in high-temperature and high-radiation environments. It mitigates water loss by enhancing the resistance of the boundary layer to the movement of water vapor away from the surface of the leaf (Farooq et al., 2009a). Additionally, reducing leaf area within the canopy through limited growth and shedding of older leaves further minimises water loss. However, it is imperative to acknowledge that, while reduced leaf area and stature are useful in conserving water resources, they equally bring about decreased crop productivity due to the obvious reduction in photosynthetic rates.

Sustaining water absorption relies on an extensive and highly productive root system (Kavar et al., 2008; Turner et al., 2001) with the capability to extract water from a substantial soil volume. In line with this, characteristics such as increased root depth, root proliferation, and greater root length density are associated with enhanced acquisition of water. They are recognized as drought avoidance traits (Matsui & Singh, 2003). Kavar et al. (2008) acknowledged the advantages of a thick and deep-rooted system for extracting water from significant depths. Additionally, roots featuring low hydraulic conductance or possessing a few but elongated root structures can facilitate a gradual yet sustainable water supply to

the plant (Passioura, 1983). In a study on wheat genotypes, root growth was inhibited by drought stress, affecting both tolerant and sensitive wheat genotypes. However, the impact was more pronounced on sensitive wheat genotypes, primarily as a result of impaired synthesis of major cell wall polysaccharides such as cellulose, hemicellulose and pectins (Piro et al., 2003). Similarly, peanut (*Arachis hypogaea* L.) varieties with higher root dry biomass and greater root length density at deeper soil layers showed increased pod yield compared to those with lower root dry biomass and length when drought occurred prior to flowering (Jongrungklang et al., 2011). Naturally, genotypes exhibiting more robust root growth in drought-prone conditions are favored, which underscore the pivotal role of RSA traits in developing drought-resistant varieties.

Drought tolerance mechanisms

Drought tolerance is the ability of plants to withstand low tissue water levels through a myriad of adaptive traits (Basu et al., 2016), with or without a reduction in performance (Bacelar et al., 2012). Drought tolerance stands as the ultimate strategy to counter the effects of drought (Connor, 2005). Key adaptations associated with drought tolerance include osmotic adjustment, the antioxidant defense system, and alterations in the dynamics of phytohormones.

Osmotic adjustment, also known as osmoregulation, involves plants accumulating both organic and inorganic solutes when they encounter drought or salinity stress, effectively lowering water potential without reducing the actual water content in plants. Upon solute accumulation during water deficit, the cell's osmotic potential decreases, causing water to move into the cell, thereby

maintaining turgor pressure (Farooq et al., 2009a). These compatible solutes serve a dual purpose: not only do they sustain turgor pressure, they also safeguard enzymes and macromolecules within cells from the damaging effects of ROS (Farooq et al., 2010, 2009b). Through osmotic adjustment, normal organelle and cytoplasmic functions continue, enabling plants to exhibit improved growth, photosynthesis, and efficiently allocating assimilates to grain filling (Ludlow & Muchow, 1990; Subbarao et al., 2000). Even at higher concentrations, these solutes do not adversely affect cell membranes, enzymes, or other large molecules and are referred to as "compatible solutes" (Cechin et al., 2006; Kiani et al., 2007). Compatible solutes encompass a range of substances such as soluble sugars, proline, calcium, sugars alcohols, organic acids, sugar alcohols, potassium, and glycine betaine (Farooq et al., 2009a).

Similarly, plants' enhanced tolerance to various environmental stressors can be due to the presence of an antioxidant defense system (P. Ahmad, 2010; Jaleel et al., 2007). This defense system within plant cells consists of both enzymatic and non-enzymatic components. During drought stress, higher plants accumulate a range of enzymatic antioxidants like APX, POX, CAT, GR, and SOD, as well as non-enzymatic antioxidants such as reduced glutathione, ascorbic acid, β-carotene, α-tocopherol, zeaxanthin, salicylates, and compatible solutes to prevent oxidative injury (Ozkur et al., 2009; Scandalios, 2005). This antioxidant defense mechanism neutralises harmful radicals by functioning as scavengers of singlet and triplet oxygen, synergists, inhibitors of damaging enzymes, and peroxide decomposers (Manach et al., 1998). For instance, SOD is key in catalysing the dismutation of

$O_2^{\bullet-}$ to H_2O_2, the initial step in ROS scavenging systems, before the reduction of H_2O_2 to water by APX in conjunction with ascorbate as an electron donor. H_2O_2 is relatively stable and is effectively removed by catalase CAT (Apel & Hirt, 2004). The collective activities of various antioxidants work in tandem to maintain cellular ROS levels at a minimum to prevent damage. The presence of antioxidative compounds within most cellular compartments buttresses the critical importance of detoxifying ROS for the continuous existence of cells (Gill et al., 2011; Khan & Khan, 2014). It is important to highlight that the degree to which antioxidant enzyme activities elevate in response to drought differs markedly among plant species and may even vary among cultivars of similar species (Bacelar et al., 2007).

Plant growth regulators, when externally applied, and phytohormones, when internally produced, exert influence over the physiological processes of plants at very low concentrations. Phytohormones like ethylene, abscisic acid (ABA), gibberellic acid (GA), cytokinin (CK), and auxin are key players in regulating various processes that facilitate the adaptation of plants to water deficit (Wilkinson et al., 2012). While some phytohormones like GA3 and CKs function as growth promoters, others, such as ethylene and ABA, act as growth retardants (Taiz & Zeiger, 2010). In stressful conditions, the endogenous concentrations of growth retardants typically increase more than growth promoters to manage the water budget effectively (Farooq et al., 2009b). Typically, in conditions of limited water availability, plant roots release ABA, which acts as a root-shoot signal and triggers stomatal closure (Cornic & Fresneau, 2002). The closing of stomata reduces stomatal conductivity and transpiration rate (Kamanga et al., 2018),

ultimately slows down plant growth. Conversely, an increase in the intrinsic levels of CK via isopentenyltransferase (IPT) activation, a gene involved in CK biosynthesis, aids in stress adaptation by postponing the initiation of senescence induced by drought, thereby enhancing overall plant yield (Peleg et al., 2011).

Proline mediates drought stress tolerance.

A substantial body of evidence points to a positive association between proline (Pro) accumulation and plant stress (Ueda et al., 2001). Pro has been reported to increase in plants under diverse stressful conditions, encompassing drought, exposure to heavy metals, extreme temperatures (both low and high), salinity, UV irradiation, nutrient deficiency, anaerobic conditions, atmospheric pollution, and post pathogen infections (Hare & Cress, 1997; Siripornadulsil et al., 2002). Drought-induced hyperaccumulation of Pro is attributed to either the elevated expression of a major gene in the pro biosynthetic pathway, pyrroline-5-carboxylate synthetase (Ueda et al., 2001) or the inhibition of Pro dehydrogenase, a key enzyme responsible for Pro degradation, under drought conditions (Kamanga et al., 2018). Pro acts as a lipid peroxidation inhibitor, an efficient scavenger of $OH^{\bullet}$ and $^{1}O_2$ (Khan & Khan, 2017), an alleviator of cytoplasmic acidosis, and a stabilizer of proteins, including antioxidant enzymes (Szabados & Savouré, 2010; Zhang & Becker, 2015). Proline, classified as one of the standard amino acids, is recognized as an osmoprotectant. It serves as a vital component in osmotic adjustment during periods of stress by reducing the water potential of cells and ensuring water uptake from soils during drought events. Consequently, plants that naturally accumulate

pro are strongly associated with greater stress tolerance ability (Evers et al., 2010; Hassine et al., 2008).

Proline levels under stress conditions can surge to 100 times greater than those observed in control conditions; however, the capacity for pro accumulation is species-specific (Verbruggen & Hermans, 2008). This suggests that the accumulation of Pro can suffice for a selection criterion when evaluating most plant species under stressful conditions for stress tolerance (Ashraf & Foolad, 2007; Parida & Das, 2005). Numerous studies have affirmed the significance of Pro in conferring drought stress tolerance to various crop plants. For example, in chickpeas, Pro accumulation was notably higher in drought-tolerant cultivars when compared to their sensitive counterparts, irrespective of whether they were grown under normal or drought-stressed conditions (Mafakheri et al., 2010). The highest Pro content, amounting to 21.36 µg/g, was observed in okra subjected to drought conditions at 25% soil FC; in contrast, the lowest pro of 18.47 µg/g was recorded in the control plants at 100% FC (Ayub et al., 2021).

Carbohydrates mediate drought stress tolerance.

Adapting plants to drought stress necessitates adjustments in various metabolic processes, encompassing photosynthesis, respiration, and the accumulation of carbohydrates. Drought stress disrupts the accumulation of water-soluble carbohydrates (e.g., glucose, sucrose and fructose) and storage carbohydrates like fructan and starch (Kaur et al., 2007; Spollen & Nelson, 1994). Nevertheless, there is variation in the responses of carbohydrate metabolism to drought stress based on the type of carbohydrates, plant species involved, stress

severity and duration (Yang et al., 2013). It is commonly acknowledged that the synthesis and accumulation of soluble sugars play a direct role in the scavenging of radicals, storing carbon, and stabilizing essential protein structures, including RuBisCo (Dubey & Singh, 1999). As a result, sugar levels tend to increase when plants are exposed to various forms of stress (Strand et al., 1999). Moreover, soluble sugars play a crucial role as osmotic compounds that regulate water transport within plants, thus enhancing their resistance to drought. Sugars also serve as substrates for growth during abiotic stress conditions (Koch, 1996).

As observed in green gram, available sugars are accumulated to ensure optimal functioning of the metabolic processes (Kumutha et al., 2008). Likewise, in cowpea (*Vigna sinensis*) subjected to drought stress, there is an observed rise in trehalose levels (Khater et al., 2018). Busso et al. (1990) reported that the accumulation of total non-structural carbohydrates in perennial grasses subjected to prolonged periods of drought contributed to plant regrowth upon rehydration. Consequently, carbohydrate accumulation is one of the key traits for enhancing drought resistance in various plant species.

Salicylic acid mediates drought stress tolerance.

Phytohormones serve as crucial regulators in the plant's response to dehydration-related stresses, and there is a growing body of evidence indicating the involvement of salicylic acid (SA) in these processes (Hayat et al., 2010). Salicylic acid is a phenol-based (Klessig et al., 2018) endogenously synthesized phytohormone (Chavoushi et al., 2019). It has enormous significance in the regulation of plants' physiological processes, particularly those related to water

absorption, transportation of ions, transpiration, and photosynthesis (Klessig et al., 2018). Studies have shown that treating plants with SA generally resulted in improved drought stress resistance (Hayat et al., 2010). The mechanisms that underlie the enhanced abiotic stress tolerance mediated by SA is attributable to numerous factors, encompassing its interactions with major osmolytes, crosstalk with other hormones, ROS signalling, and its modulation of antioxidants. For example, both SA and its aspirin analogue can trigger the build-up of GB in amounts ranging from 0.5 to 2.5 mM in plants under conditions of extreme drought, salt and cold stresses (Jagendorf & Takabe, 2001) and serves as a systemic acquired resistance. Salicylic acid also engages in crosstalk with different hormones and growth regulators to modulate various plant responses under both stressed and normal conditions (Raza et al., 2019). Salicylic acid modulates the activities of H_2O_2-metabolizing enzymes (e.g., APX, CAT, and POD) as well as superoxide-dismutating enzymes (e.g., SOD) in plants under water deficit (Saruhan et al., 2012), all of which are integral components of the enzymatic antioxidant defense mechanism against drought stress (Alam et al., 2013).

The involvement of SA in mitigating the damage inflicted upon plants by water stress has been substantiated. Mutants of *Arabidopsis thaliana* that accumulate SA (cpr5 and acd6) displayed enhanced drought tolerance due to the closure of stomata, facilitated by SA-induced expression of PR genes such as PR1, PR2, and PR5 (Okuma et al., 2014). Under water-deficient conditions, the endogenous SA increased by five-fold in *Phillyrea angustifolia* (Munné-Bosch & Peñuelas, 2003). In rice, SA was observed to enhance carbon metabolism, fortify

the antioxidant system, maintain membrane stability, provide osmoprotection, and preserve photosynthetic pigments (Farooq et al., 2010). Salicylic acid treatment in water-stressed barley increased membrane stability and the levels of Pro and ABA, thereby imparting stress tolerance to the plants (Bandurska & Stroiski, 2005). Thus, SA is a versatile phytohormone with diverse functionalities ranging from ROS scavenging to improving osmotic adjustment, all aimed at mitigating the adverse effects of water deficit on crops.

Ascorbic acid mediates drought stress tolerance.

Ascorbic acid (AsA) is among the most potent antioxidants within plant cells, organelles, and the apoplast. Ascorbate, the active biological form of ascorbic acid, is generated through the deprotonation of the hydroxy group at C_3 (Akram et al., 2017). This compound exhibits a broad presence in various plant tissues, with typically higher concentrations in meristematic and photosynthetic cells, assuming critical roles in numerous physiological processes, including the growth, differentiation, and metabolism of plants. When plants encounter stress, there is an increased level of AsA, which are pivotal in the regulation of photosynthetic mechanisms and serves as effective protective mechanism against oxidative injury (Dolatabadian et al., 2010; Yazdanpanah et al., 2011).

Ascorbic acid is highly regarded as an effective scavenger of ROS due to its ability to provide electrons through enzymatic and non-enzymatic reactions (Mehla et al., 2017). Ascorbic acid serves to protect cell membranes against oxidative injury by scavenging H_2O_2, $OH^{\bullet}$ and $O_2^{\bullet-}$ as well as regenerating α-tocopherol from tocopheroxyl radical (Shao et al., 2005). In okra, AsA application

increased plant growth and Pro content along with lowered ion leakage and lipid peroxidation when exposed to drought (Baghizadeh et al., 2009). Also, it was observed that drought-resistant rice of the Taichung Native-l variety exhibited higher levels of AsA compared to its susceptible counterpart, I.R. 8 when both were subjected to wilting treatments during the tillering and shooting stages (Garg & Singh, 1971). These observations underscore the importance of AsA in enhancing drought resistance in plants, mainly through their actions as antioxidants.

Superoxide dismutase mediates drought stress tolerance.

Usually, ROS is generated as by-products in low amounts during normal processes of metabolism due to the partial reduction or excitation of molecular oxygen (O_2) within the cell (Halliwell, 2006). During optimal physiological conditions, the production and breakdown of ROS are balanced through various cellular detoxification mechanisms (Alscher et al., 1997). However, this equilibrium can be disrupted due to various biotic and abiotic factors, leading to an elevated intracellular concentration of ROS, where the rate of production exceed degradation (Mittler, 2002). The SOD antioxidant enzyme is pivotal in the first line of defense against oxidative stress as it catalyses the dismutation of $O_2^{\bullet-}$, H_2O_2 and O_2, and plays an essential role in ensuring the survival of plants when exposed to environmental stresses (Aydin et al., 2013; Gill & Tuteja, 2010).

Superoxide dismutase groups are categorised based on their metal cofactors, with various isoforms found in different cellular compartments including the peroxisomes (MnSOD and CuZnSOD), chloroplasts (FeSOD, MnSOD, CuZnSOD), cytosol, mitochondria (MnSOD), and potentially even in the

extracellular space (Alscher et al., 2002; Jaleel et al., 2009). Before their role in plants was recognized (McCord & Fridovich, 1969), SOD enzymes were initially identified as a group of metalloproteins with no known function (Berwal & Ram, 2019). Subsequent research has, however, revealed that a higher SOD activity or an increased number of isoforms is associated with a greater potential to eliminate ROS (Berwal & Ram, 2019). Consequently, elevated SOD activity is often associated with increased tolerance of plants to environmental stress factors (Shukla & Varma, 2019). A significant rise in SOD activity has been reported under drought stress across numerous plant species, including common bean (Zlatev et al., 2006), cowpea (Brou et al., 2007), and sweet potato (Shukla & Varma, 2019). Consequently, SOD has been proposed as an indirect indicator for selecting plants with drought resistance (Shukla & Varma, 2019).

Root system architecture and its contribution to drought resistance

The growth of roots involves the continual elongation and branching of root organs (Malamy, 2005), forming an intricate network of interconnected components across both time and space known as the RSA (Malamy, 2005). Thus, RSA encompasses the spatial arrangement of the root system or explicit deployment of root axes (Lynch, 1995). The root system architecture comprises three essential components: root system topology, distribution, and morphology (Fitter et al., 1991; Lynch, 1995). Topology describes the branching of individual roots (Fitter et al., 1991), taking into account characteristics such as the length, diameter, the number of roots emerging from a node, root insertion angles, the number of root tips (magnitude), and the number of branching points from the base to the farthest

root tip (altitude) (Glimskär, 2000). Root distribution is quantified by assessing traits like biomass and length. It is expressed as a function of soil depth or position in the rhizosphere (Adu, 2014) to estimate the fraction of soil resources accessible to the roots (Bengough et al., 2000). On the other hand, root morphology is the external characteristics of a root axis considered an organ, encompassing root hairs, root diameter, and the pattern of secondary root emergence (Adu, 2014).

Roots exhibit a remarkable capacity for developmental plasticity, allowing them to adapt to their surrounding environment (Karlova et al., 2021). This adaptability is a manifestation of phenotypic plasticity, which is the ability of a single genotype to display varied characteristics in different environments. Various architectural and anatomical features exhibit phenotypic plasticity in the context of root systems. The plasticity in RSA is a result of how individual root meristems respond to a range of factors, including soil water status (Eapen et al., 2005), temperature fluctuations (Walter & Schurr, 2005), and nutrient availability and concentration (Bai et al., 2013).

Drought generally induces a parsimonious RSA (Lynch, 2013) by reducing lateral root development and favouring a deeper rooting structure (Zhan et al., 2015). This means that roots tend to grow in the direction of higher water availability, often moving away from the dry topsoil layers (Gandullo et al., 2021). This directional growth towards areas with higher water content is achieved by investing in root elongation while enhancing the gravitropic response, which involves adjusting the root angles downward (Uga et al., 2013). From a simple geometric perspective, steeper root growth angles lead to the more rapid

development of deeper roots, enabling more efficient utilization of deep soil resources, particularly water and N (Lynch, 2022). The importance of a robust and deeper root system for achieving higher yields has been documented in various plant species, including *Glycine max* (Hund et al., 2011), chickpea (Dilley, 2005), wheat (Eissenstat, 1992) and maize (Prudhomme et al., 2014).

Desiccation often leads to an increase in the compactness or strength of many agricultural soils. However, several root phenes play a role in regulating the penetration of hard soil (Bengough et al., 2011). A greater number of thick roots, characterized by a larger diameter, is associated with a higher ability to penetrate hard soil (Materechera et al., 1992). Similar to axial roots, having a reduced number of lateral roots can be advantageous for root depth and, consequently, for extracting deep soil resources (Lynch, 2013). For example, maize genotypes with fewer, longer lateral roots exhibited deeper rooting, improving water absorption, plant growth, and yield under drought conditions (Zhan et al., 2015).

The RSA is of significant importance for agricultural productivity because many soils exhibit uneven distribution of resources and localized depletions, making the spatial arrangement of the root system a critical factor in a plant's ability to access and utilise these resources (Lynch, 1995). Owing to the ability of plants to adapt their RSA in response to the availability of water and nutrients in the soil environment, the study of how RSA responds to the presence of water and nutrients within the rhizosphere is essential for the development of resilient crops that can effectively explore the soil, acquire and utilize resources efficiently, and provide good yields, especially under challenging abiotic stress conditions (Ghanem et al.,

2011). Substantial progress has been made in studying RSA in crops, particularly cereals. There is now well-documented evidence supporting the genetic control of RSA and its correlation with increased productivity, particularly under conditions of stress (Khan et al., 2016). Thus, RSA traits, including but not limited to diameter, length, density, volume, and angle, could become a life-saver when properly integrated into breeding programmes for improved productivity in challenging climates.

Selection as a crop improvement method

One of the primary prerequisites for initiating a breeding program is the presence of the necessary genetic diversity required to identify potential genotypes to serve as parent plants (Carrodeguas-Gonzalez & Zuñiga-Orozco, 2023). Once this genetic diversity has been established, the subsequent step involves discerning among the diverse genotypes to identify individuals with desirable traits for the development of new potential cultivars, a process known as artificial selection (Carrodeguas-Gonzalez & Zuñiga-Orozco, 2023). First, numerous genotypes are assessed, often with a limited number of replicates, and at a few designated locations (referred to as screening) (Bänziger et al., 2000). Subsequently, the more promising genotypes or their progeny undergo a more extensive evaluation with increased replicates and at multiple locations (referred to as testing) (Bänziger et al., 2000). With each selection round, the breeder systematically reduces the number of genotypes and the variation among genotypes by eliminating the underperforming ones (Bänziger et al., 2000). Modern breeding programs

encompass a wide spectrum, ranging from straightforward mass selection methods to advanced indirect trait selection based on molecular markers.

In the mass selection method, many plants are chosen, and their seeds are aggregated and sown together, typically with much dependence on subsequent reduction in the number of plants at the level of individual rows derived from individual plants or inflorescences (Walker, 1969). Mass selection is commonly employed to quickly ameliorate land races and purify seed stocks (Walker, 1969). In contrast, pure-line breeding also begins with many single plant selections, but it differs from mass selection in that far fewer lines are retained (Walker, 1969). Hull (1945) introduced the concept of recurrent selection, which involves the repeated selection of desirable traits to increase their frequency solely through crosses between high-performing individuals. This results in an improved population with better mean performance in the trait of interest than the initial population while maintaining substantial genetic diversity (Begna, 2022).

The discovery of quantitative trait loci (QTLs), which contain genes responsible for quantitative traits like drought stress tolerance, has revolutionised the selection process into what is known as marker-assisted and genomic selection, often referred to as genomics-assisted breeding (Khan et al., 2016). Drought tolerance is a complex quantitative trait influenced by many genes, making it one of the most challenging traits to study and characterize (Maazou et al., 2016). Compared to conventional methods, genomics provides remarkable opportunities for dissecting quantitative traits and identifying their genetic determinants (QTLs), which sets the stage for marker-assisted selection (MAS) and, ultimately, the

cloning of QTLs and their direct manipulation through genetic engineering (Tuberosa & Salvi, 2006). Genomics has opened up new avenues for understanding and enhancing selection works for complex traits like drought tolerance in crops.

Implementing MAS necessitates the initial identification of molecular markers and genes/QTLs that account for the phenotypic variability associated with drought tolerance (Rosero et al., 2020). Since various changes in gene expression are triggered in response to drought stress in plants (Rosero et al., 2020), a significant strategy involves identifying candidate genes that are expressed under drought-stress conditions, and genomic technologies such as microarray and transcriptomic analyses have proven to be valuable tools for identifying these genes. Various molecular markers have been employed, with sequence-based DNA markers, particularly single nucleotide polymorphisms (SNPs), gaining popularity. The application of marker technologies eliminates the environment's confounding effects during selection, especially when dealing with polygenic traits like drought tolerance, allowing for indirect selection of traits independently of the plant's developmental stage. A typical success story in using MAS is the release of a novel upland rice variety, BirsaVikasDhan 111 (PY 84), in the Indian state of Jharkhand. This variety was bred using marker-assisted backcrossing with selection for multiple QTLs that enhance root growth for improved performance under drought conditions (Shashidhar et al., 2013). Marker-assisted recurrent selection has also been effectively employed in developing superior varieties of sweet corn, sunflower, and soybean (Eathington et al., 2007).

Drought-tolerant crop breeding relies on the use of various selection indices based on anatomical, physiological, and biochemical criteria, including seed yield, harvest index, shoot fresh and dry weight, leaf water potential, osmotic adjustment, the accumulation of compatible solutes, water use efficiency, stomatal conductance, and chlorophyll fluorescence (Ashraf et al., 2007). Traditionally, breeders have focused on selecting high-yielding genotypes under drought-stress conditions. However, response to selection is influenced by the magnitude of additive variance or narrow-sense heritability and selection intensity (Rauf et al., 2016). It is important to note that yield is a trait with relatively low heritability, and selecting based on this complex characteristic can be challenging, resulting in slow progress (Begna, 2022). In other words, yield per se is a complex trait to which several traits contribute individually and in combinations. Therefore, a multifaceted approach considering various contributing factors is often required to breed drought-tolerant varieties effectively. Among horticultural traits, the number of pods per plant has demonstrated good narrow-sense heritability and genetic advancement under drought conditions (Ben-Ahmed et al., 2006). To this end, while breeding methods are becoming ever more sophisticated, the selection remains the fundamental and most powerful step in the process, the primary weapon in the armoury of the plant breeder.

Biochar amendment ameliorates drought effects on crops.

Biochar is a carbon-rich substance created through pyrolysis, where biomass is heated in a closed container with limited or no oxygen (Lehmann & Joseph, 2009). Its unique properties, including a high surface area, cation exchange

capacity (CEC), low bulk density, neutral to alkaline pH, high carbon content, and nutrient content, make it an excellent soil conditioner for the tropical clay and sandy soils in SSA (Gwenzi et al., 2015). Unlike organic matter, biochar has a longer-term impact on soil because it is less susceptible to decay due to its recalcitrant carbon (Downie et al., 2012; Thies & Rillig, 2012), with an estimated mean residence time ranging from 90 to 1600 years (Singh et al., 2012).

Biochar, as a soil conditioner, enhances various soil biophysical properties, such as nutrient retention and water-holding capacity (Harvey et al., 2012), water permeability saturated hydraulic conductivity (Asai et al., 2009), and reduced soil strength (Busscher et al., 2010). It also promotes plant growth due to its porous structure (Tayyab et al., 2018). Moreover, the porous nature of biochar provides an excellent habitat for soil microbes to colonise, grow, and reproduce, which, in turn, enhances soil health and plant performance.

Studies have demonstrated the positive impact of biochar application on the development, biomass production, and yield of various vegetables during drought events (Singh et al., 2019). For example, biochar enrichment significantly enhanced stomatal conductance, water use efficiency, and photosynthesis in tomato plants under water deficit (Akhtar et al., 2014). Applying biochar improved soil chemical properties and okra's growth, yield, and water productivity (Farias et al., 2020). Similarly, there was an increase in photosynthetic rate, transpiration rate, relative water content, leaf water potential, and leaf turgor potential in maize when biochar was applied under drought conditions (Haider et al., 2015). Moreover, it was observed that adding biochar at a rate of 5% led to the lowest Pro content in the

leaves of tomatoes compared to untreated plants (Obadi et al., 2023). This observation can be linked to increased water availability in the soil, resulting in enhanced water uptake and reduced oxidative and osmotic stress (Kul et al., 2021). In the same manner, in maize, the activities of SOD, POD, and CAT enzymes were significantly reduced after the application of biochar, possibly because of the reduced demand for an adaptive response to ROS mediated by the presence of biochar (Cong et al., 2023).

In contrast, a study found that biochar reduced the accumulation of malondialdehyde in *Brassica oleracea* by increasing the activity of antioxidant enzymes (Yildirim et al., 2021). The contrasting effect of increasing anti-oxidant activities in one crop and decreasing in another could testify to the specificity of biochar effects on different crop species. In Ghana, research has demonstrated that biochar application improved soil organic carbon storage, root volume, nutrient uptake, and grain and straw yield in irrigated rice cropping systems (MacCarthy et al., 2020).

Biochar is rich in carbon and contains essential plant nutrients such as N and P and basic cations like Ca, Mg, and K, which are crucial for plant growth (Major et al., 2010). These nutrients play a significant role in enhancing nutrient availability for plants (Jabborova et al., 2021). A study found that biochar can enhance the absorption of N, P, and K by tomato plants (He et al., 2021). In addition, biochar treatment led to significantly higher levels of Ca and Mg in maize leaf samples than untreated samples (Major et al., 2010). There is enough evidence to say that biochar improves the nutrient status of soils. When plants have ample

nutrients for absorption, they tend to exhibit more robust growth and are better equipped to withstand various biotic and abiotic stresses.

Notwithstanding, it is important to note that the effects of biochar application on plant growth can vary, and neutral or even negative responses have been observed in some cases (Gwenzi et al., 2015). For instance, when biochar was applied at 5 and 15 t/ha, soybean yields decreased by 37% and 71%, respectively (Kishimoto, 1985). Other studies have shown that fresh biochar amendments may not consistently improve soil conditions and can even lead to phytotoxic effects (Bernardo et al., 2010). In Pennsylvania, a 40% reduction in tree density and basal area in 100-year-old charcoal hearth areas was observed compared to non-hearth areas (Mikan & Abrams, 1995) and was attributed to microbial immobilization of nutrients, which was associated with the high C: N ratio, especially during the initial phases of biochar amendment (Gwenzi et al., 2015).

Biochar can theoretically be produced from a wide range of organic materials. However, its properties vary significantly depending on the feedstock (the organic material used) and the processing conditions (Agegnehu et al., 2017; Brewer et al., 2017). For example, it was found that biochar derived from rice materials has unique chemical properties due to the incorporation of silica elements into its chemical structure. In contrast, biochar produced from wood materials often has a high carbon content and strong absorption characteristics (Jindo et al., 2014). Concerning the temperature effect, biochar was prepared at 400, 500, 600, 700, and 800°C, and it was observed that the biochar obtained at 600°C has high recalcitrant characteristics compared to the biochar obtained at other temperatures (Jindo et al.,

2014). This variability in the properties of biochar, attributed to feedstock, production temperature, and other factors, may explain the differences and sometimes contradictory effects reported in the literature. Thus, although most studies underscore the potential benefits of biochar application in enhancing crop performance, counter-effect is a possibility, which goes a long way in highlighting the complexity of biochar's effects on plant growth.

Many studies have explored using oil palm EFBs as organic mulch, after pyrolysis, or through composting before application to soils (Anyaoha et al., 2018). For example, applying EFB as biochar or compost has been found to enhance soil water and nutrient content (Ahmad-Dani, 2018). Compared to unamended soils, crops grown on soils amended with pyrolysed EFB experienced a substantial increase in growth and yield, approximately 78.4% higher (Adu et al., 2022a). As a result, biochar is gaining recognition as a solution to improve crop growth, enhance water and nutrient retention, and increase soil carbon sequestration (Ahmed et al., 2016). Using locally available bio-wastes like oil palm EFB to enhance soil water and nutrient retention is considered one of the most sustainable options for soil conservation and soil fertility improvement, especially in resource-poor regions (Moradi et al., 2015; Sung et al., 2010). This approach helps make the best use of available resources and contributes to sustainable agricultural practices and environmental health.

CHAPTER THREE

MATERIALS AND METHODS

Study area

The research was conducted at the A. G. Carson Technology Centre of the School of Agriculture, the University of Cape Coast, Cape Coast (2.07 °N, 1.14 °W) (Parker et al., 2010). This Centre is within the Coastal Savannah zone and features an Acrisol soil type (Asare-Bediako et al., 2014). The region experiences a bimodal rainfall distribution, with the primary rainy season occurring from April to July and a minor rainy season from September to November (Parker et al., 2010). The annual rainfall in this area typically ranges from 900 to 1000 mm, the dry period spanning from December to May (Parker et al., 2010). The temperature ranges from 23.2 to 33.2 ºC, with an annual average of 27.6 ºC (Owusu-Sekyere et al., 2011) and a relative humidity of 60%-80% (Adu et al., 2017a). However, the mean greenhouse temperature throughout the study ranged from 38^0 C to 47 ^{0}C with relative humidity of 28 % to 50 %.

Genetic material

This study used sixty (60) distinct okra genotypes, encompassing both landraces and improved cultivars typically cultivated in Ghana. These genotypes were procured from two reputable sources: the Ghana Plant Genetic Resources Research Institute and the World Vegetable Centre Gene bank, comprising genotypes originating from various neighbouring African countries, such as Togo, Benin, Sudan, Mali, Malawi, Cameroon, Nigeria, Niger, and Senegal. Each okra genotype and its originating country are presented in Appendix 1.

Physicochemical properties of soil and EFB biochar

The study utilized soil excavated from 0 to 20 cm from a field near the experimental site. This soil was previously classified as falling within the Sandy clay loam textural class and belonging to the Edina-Bronyibima/Benya-Udu series Acrisol (Schad, 2016). The soil's field capacity (FC) was determined through the gravimetric method as outlined by (Cassel & Nielsen, 2018). The pipette method was used to assess soil texture (Anderson & Ingram, 1990). The pH of the soil samples was determined by creating a soil-to-water ratio suspension of 1:2.5 (w/w basis), followed by agitation for 5 minutes and an additional 2-minute standing period. The pH was measured using a pH meter equipped with a cross-bridge electrode (Hanna Instrument Inc., USA). Organic carbon content was assessed using the dichromate method (Walkley & Black, 1934). Determining total nitrogen in the soils involved the micro Kjeldahl method, with the soils being digested in sulphuric acid with selenium powder as a catalyst (AOAC, 1990). The modified Molybdenum Blue method determined available phosphorus in the soils (Murphy & Riley, 1962). The extraction of exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}) was performed using buffered ammonium acetate extractant. The measurement K was done using flame photometry (Jenway PFP 7 model, Fischer Scientific, Goteborg, Sweden), while Ca and Mg were measured utilizing AAS (Buck Scientific model 210 VGP, Norwalk, USA). The results regarding the measured physicochemical properties of the soil are presented in Table 1.

The biochar used in the study was procured in a ready-made form from experts and was prepared using oil palm EFB feedstock. Various properties of the

biochar samples were also analysed, and the outcomes of these analyses are detailed in Table 2.

Table 1: Physical and chemical properties of experimental soil. OC: Organic carbon; N: nitrogen; BD: bulk density; P: phosphorous; K: potassium; Ca: calcium; Mg: magnesium.

Clay (%)	Silt (%)	Sand (%)	OC (%)	N (%)	BD (gcm^{-3})	pH	P ($mgkg^{-1}$)	K ($mgkg^{-1}$)	Ca ($mgkg^{-1}$)	Mg ($mg\ kg^{-1}$)
33	5	63	1.40	0.06	1.35	5.71	2.25	1.46	4.68	2.46

Table 2: Physical and chemical properties of the oil palm empty fruit bunch biochar used.

Property	Value	Property	Value
Ash (%)	43.7	Potassium as K_2O (%)	3.54
pH (water 1: 5 w/v)	10.1	Calcium as CaO (%)	2.49
pH ($CaCl_2$)	9.9	Magnesium as MgO (%)	1.59
Electrical conductivity ($dS\ cm^{-1}$)	3.8	Sodium as Na_2O (%)	0.15
Organic carbon (%)	7.1	Iron (%)	1.02
Total carbon (%)	47.0	Zinc (%)	0.017
Total inorganic carbon TIC (%)	0.8	Manganese (%)	0.027
Carbonate as CO_2 (%)	3.1	Copper (%)	0.005
Nitrogen (%)	0.85	Boron (%)	0.004
Phosphorus as P_2O_5 (%)	0.66	Sulphur SO_3 (%)	0.027

First objective: assessing genotypic variation in the RSA of okra genotypes

Experimental design and treatments

This was a greenhouse experiment using the completely randomised design (CRD), and with four biological replications. The sole treatment applied was 60 okra genotypes. The study was conducted twice to ensure reproducibility.

Rhizobox and rhizobox-stand design

Custom-made soil-filled rhizoboxes (root observation chambers) were employed for the study. These rhizoboxes were constructed using a design adapted from Bengough et al. (2004). Each rhizobox consisted of two Perspex plates, one

at the front and the other at the back. The front plate was transparent, facilitating root imaging, while the back plate was opaque. Both plates had dimensions of 40 x 30 x 1.5 cm. The two plates were separated by spacers made of Perspex, with dimensions of 1.5 × 1.5 × 40 cm for the long edges and 1.5 × 1.5 × 30 cm for the short edges to create the rhizobox structure. This arrangement provided a 3 cm separation between the two plates. Both plates were firmly held together using 5 cm clips to secure them in place. Three clips were positioned along each vertical edge, one at the middle-top and one at middle-bottom, resulting in eight clips per rhizobox. To ensure proper aeration and unhindered shoot growth, four gaps, each measuring 4.5 cm, were maintained on the surface of the rhizobox. Additionally, a sufficient number of perforations were made at the bottom for efficient drainage.

A rhizobox stand serves as a platform for aligning and growing seedlings in rhizoboxes. These stands were built using two metal components, each measuring 247 and 3.95 cm in length and thickness, respectively. The two metals were positioned parallel and connected at their extreme ends with metal separators measuring 23 cm. To provide support and stability, vertical metal pieces measuring 9 cm were affixed 6 cm equidistant from each other along the surface where each rhizobox was positioned. These vertical supports allowed each rhizobox to lean at an angle of 37^0, and the entire structure was elevated 15 cm above the ground surface.

Soil preparation, filling of rhizoboxes, sowing of seeds and cultural practices

The soil extracted was subjected to the following procedures: it was pulverized, air-dried over 14 days, and sieved through a 2 mm mesh size to

eliminate coarse particles and debris. Each rhizobox, with a volume of 3100 cm^3, was then filled with the unamended soil at a bulk density of 1.3 gcm^{-3} and arranged on the rhizobox stands. The filling of the rhizoboxes was done up to 2 cm below the apex to allow for efficient watering. To shield the transparent Perspex plate from direct sunrays and mold growth, black polyethylene sheets measuring 40 cm x 30 cm were cut and placed over the plate before securing them with the clips. The rhizoboxes were then organized in a portrait orientation on the rhizobox stands, as depicted in Figure 1A. Subsequently, the rhizoboxes were watered once daily for three days before the sowing of seeds.

Seeds from the 60 okra genotypes underwent priming in pipe-borne water for 30 minutes. Following this, two healthy seeds were sown in November 2022 at a depth of 2 cm in each of the two middle gaps on the top surface of the rhizobox. The seedlings were thinned to 1 per stand ten days after sowing (DAS), two plants per rhizobox, and four biological replications per genotype. The seedlings received irrigation three times a week using pipe-borne water, and hand-weeding was performed as needed.

Harvesting of genetic materials and data collection

The plants were harvested at 21 DAS. First, each rhizobox and the intact plant were transported to an imaging room and placed flat on a smooth platform with the transparent side facing upward. Following the removal of the black polyethene cover, high-resolution images were captured using a Nikon Digital Camera (D5600, Nikon Incorporation, Japan), which was mounted at a fixed height of 50 cm on a tripod stand positioned above the rhizobox. Subsequently, each

rhizobox was opened, and the roots were carefully washed under running pipe-borne water. Due to the delicate nature of the roots, great care was taken during the washing process to prevent root breakage and losses. After washing, the roots were separated from the shoots at the collar region using a sharp knife. The detached roots were uniformly floated in a dark and spacious basin filled with water to about half its volume and imaged as previously described. The floating process was conducted meticulously to minimise root overlap. The shoots and roots were placed in labeled envelopes and dried in an oven at 64.5 ^{0}C until a constant weight was achieved. The oven-dried samples were allowed to cool in a desiccator, following which the dry weights of both the roots and shoots were determined using an electronic weighing balance. The root-to-shoot ratio was computed as the quotient of each dried root weight and its corresponding dried shoot weight.

The root images were subsequently subjected to analysis. The image thresholding was set at 30 (96.77%) on a black-and-white background using ImageJ software (US National Institutes of Health, Bethesda, MD, USA, https://imagej.nih.gov/ij/). Batch analysis of the thresholded images was carried out in Rhizovision Explorer (Version 2.0.2) (Seethepalli & York, 2020) using the broken root algorithm to extract various RSA traits, including total root length (Trl), volume (Vol), surface area (Sa), number of branch points (Nbp), network area (Na), perimeter (Peri), branching frequency (Bf), among other parameters. The pixel dimensions were converted to physical dimensions in units of pixels per millimeter (York, 2023). The complete Rhizovision metadata is presented in Appendix 2. Root angles and primary root lengths were measured using ImageJ and SmartRoot

(version 4.21), respectively. In total, 22 RSA parameters and 3 biomass traits were utilised to select 10 okra genotypes for the subsequent phase of the study.

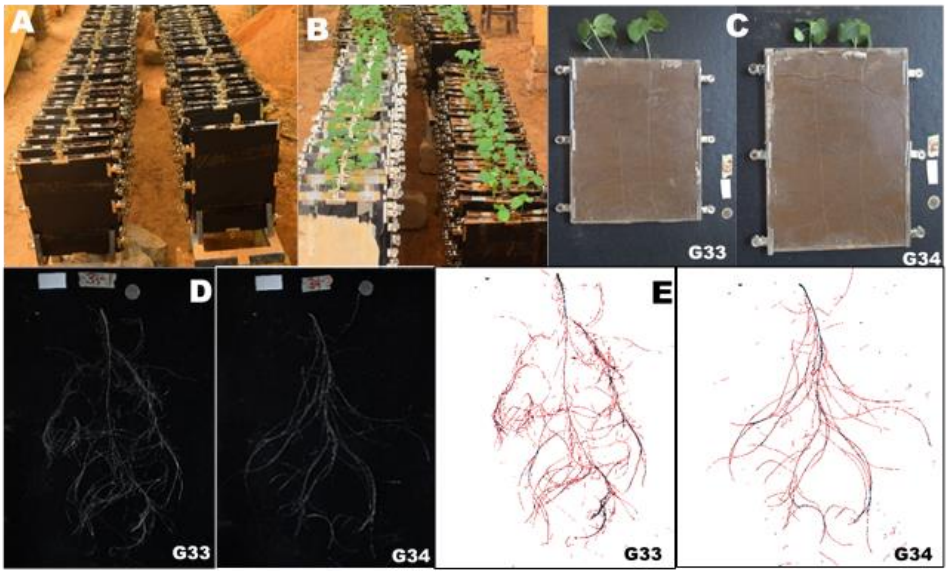

Figure 1:(A) Soil-filled rhizoboxes arranged at an angle of 370 on rhizobox-stands; (B) Okra seedlings growing in rhizoboxes in the greenhouse seven days after emergence; (C) Rhizoboxes with intact okra plants at harvest; (D) Corresponding floated roots after washing; (E) Corresponding feature images of floated roots from RhizoVision Explorer analysis.

Second objective: evaluating the effect of drought and the drought-mitigating potential of oil palm EFB biochar on the biochemical indices of selected okra genotypes.

Experimental design and treatments

A second evaluation was conducted based on the results obtained in the first experiment. The 2nd Experiment was a factorial laid out in completely randomised design. The experiment proceeded with three treatments, comprising ten okra

genotypes (seven from cluster 1 and three from cluster 2) selected from the first experiment, two water regimes (30% field capacity as drought and 90% field capacity as control), and three levels of biochar amendment (0 t/ha, 10 t/ha and 20 t/ha). Selecting only three genotypes from cluster 2 was due to the challenge of poor seed germination. The various treatment combinations were replicated three times, and there was a total of 180 plants.

Estimating the amount of air-dried soil required, filling of sacs and soil incubation

Sacks, with dimensions of 110 cm in length and 19 cm in diameter, were tailored to fit inside PVC pipes that measured 100 cm in length and 21 cm in diameter. Adequate space was maintained at the junction of the PVC and the sack to facilitate easy removal when excavating roots. The interior of the sack was lined with polyethene sheets to prevent root entanglement with the small pores in the sacks. The volume of the sack up to the 90 cm mark was determined to be 28,900 cm^3. The sack was filled with water up to the 90 cm mark, leaving the remaining 10 cm for watering to calculate this volume. The water was then transferred into 5000 ml measuring cylinders, and the volume was used to estimate soil mass. The soil mass required to fill up to the 90 cm mark at a bulk density of 1.3 gcm^{-3} was estimated to be approximately 37.60 kg. Pre-made EFB biochar was obtained and thoroughly mixed with the soil at 0, 10, and 20 t/ha rates. The calculations for the various biochar rates are presented in Appendix 4. After mixing the soil with the biochar, the sacks were inserted into the PVC and filled with the soil. Subsequently,

the soil was watered and left to incubate for 14 days, during which watering was done thrice per week (Figure 2A).

Sowing of seeds, cultural practices and drought imposition

Three healthy seeds were sown at a depth of 2 cm within each PVC. At 7 DAS, the seedlings were thinned to one per stand. Poly-Feed™ https://www.haifa-group.com was applied at 14 DAS and anthesis. The application rate was 10.7 grams per 2 litres of water, and the mixture was sprayed onto the leaves until water began to drip from them. At 28 DAS, the crops were subjected to two different water regimes: one group was maintained at 90% FC as the control (no-drought), while the other group experienced drought stress with soil moisture levels set at 30% FC. The calculations for determining the amount of water required to maintain the crops at various FC levels are provided in Appendix 5. The control and drought-stressed plants were watered to their respective FC levels twice weekly. Before each watering session, a moisture determination probe, specifically the Acclima Digital True TDR-315 H Sensor (Acclima, Inc. in Idaho, USA), was inserted into the soil to assess the current soil moisture content, and the deficit was applied to attain the respective FC.

Data Collection

At anthesis, 30g of leaves were harvested from each plant for the biochemical analysis of Carb, Pro, SA, AsA and SOD content. The fresh leaves were stored in a refrigerator until analyses were completed. The leaf Carb, Pro, SA and AsA contents were measured as described by the National Standardization

Agency (1992), while SOD content was determined following the recommendations of (Leonowicz et al., 2018).

Proline determination

To prepare the crude extract for Pro analysis, 25 mg of fresh leaf sample and 3% sulphosalicylic acid were weighed into a mortar and ground thoroughly using a pestle. The leaf extract was centrifuged at 3000 rpm for 10 minutes, and 2 ml of the supernatant was transferred into a fresh test tube. In this test tube, 2 ml of Acid Ninhydrin, 2 ml of Glacial acetic acid, and 2 ml of 6M orthophosphoric acid were added. The solution was kept in a water bath at 100°C for 1 hour. Afterwards, the solution was transferred to a separation funnel, and 4 ml of toluene was added and shaken thoroughly. The lower layer was discarded, and the upper layer was collected. The spectrophotometer was set at 520 nm, and the absorbance values were read. For the Proline stock solutions, 10 mg of pure Proline was dissolved in 100 ml of distilled water to create a 100 ppm solution. Different concentrations were prepared by diluting the stock solution to achieve 0, 20, 40, 60, 80, and 100 ppm concentrations. To each solution, 2 ml of Acid Ninhydrin, 2 ml of Glacial acetic acid, and 2 ml of 6M orthophosphoric acid were added. These solutions were kept in a water bath at 100°C for 1 hour, and then their absorbance values were read at 520 nm using a spectrophotometer. Pro content was estimated using the formula:

Amount of proline $= x \div 2 \times 10 \div 250 \times 1000$ in a unit of µg/g Equation (1)

Carbohydrate determination

In estimating the Carb content, 1 gram of finely ground fresh leaf material was placed into a flask, and a 3% HCl solution (40 ml) was added for refluxing

over one hour. After refluxing, the solution was allowed to cool and then neutralized with a 30% NaOH and 3% acetic acid solution, each at 5 ml. Following neutralization, the solution was transferred into a 100 ml volumetric flask and diluted with deionized water to reach the mark. The resultant solution was subsequently filtered using qualitative filter paper. 10 ml of the filtrate was measured into an Erlenmeyer flask and mixed with 25 ml of Luff Solution and 15 ml of deionized water. This mixture was brought to a boil and allowed to cool. It was then treated with 15 ml of a 20% KI solution and 25 ml of a 25% H_2SO_4 solution. The subsequent titration, employing a 0.1N $Na_2S_2O_7$ solution, was aided by a 1% starch indicator. The endpoint was recorded, marked by the disappearance of the purplish-blue colour. The quantification of the Carb content was done using the formula:

$$\text{Carbohydrate (g/100 mg)} = \frac{\text{weight of glucose} - \text{Dilution}}{\text{sample weight}} \qquad \text{Equation (2)}$$

Ascorbic acid determination

The determination of AsA involved weighing 1 gram of the leaf sample and grinding the sample with a small amount of water to create a homogenized leaf extract. This leaf extract was transferred into a conical flask, and 10 ml of a 10% metaphosphoric acid (MPA) solution was added. The solution was thoroughly mixed using a magnetic stirrer and left to stand for 10 minutes to ensure the precipitation of proteins and the stabilization of AsA. A burette was prepared and filled with a solution of DCPIP (2,6-dichlorophenolindophenol). An Erlenmeyer flask was set up against a white background, and 5 ml of the stabilized leaf extract was transferred into the Erlenmeyer flask. A few drops of the DCPIP solution were

added, and the mixture was gently swirled to ensure thorough mixing. As the DCPIP reacted with the AsA in the leaf extract, its initial blue colour gradually faded. The dropwise addition of the DCPIP solution continued until the blue colour persisted for at least 10 to 15 seconds, indicating the complete reaction of ascorbic acid with DCPIP. A blank determination was performed using the same procedures described above but without adding the leaf extract. Subsequently, a standardized solution of sodium ascorbate with a known concentration was prepared, and a standard curve was constructed. This standard curve determined the Vitamin C concentrations of various samples.

Salicylic acid determination

The determination of SA content began with measuring 0.1 gram of fresh leaf sample, which was then ground in a mortar using a pestle. Then, 15 ml of hot deionized water was added to the sample, and the resulting mixture was transferred into a centrifuge test tube. The sample was placed in a centrifuge and spun at 8000 rpm for 10 minutes, after which the supernatant was carefully stored on ice. Afterwards, 100 µl of the supernatant was mixed with 200 µl of a 0.1% Ferric Chloride solution to assess the SA content. The resulting mixture was then adjusted to a total volume of 3.0 ml with deionized water and allowed to stand for 10 minutes. A violet complex was formed during this period, and its absorbance was measured at 540 nm and recorded. A standard solution of SA was prepared, and serial dilutions were made to establish a standard curve at various concentrations, including 0, 20, 40, 60, 80, and 100 ppm. This standard curve served as a reference for determining the SA content in the samples.

Superoxide dismutase determination

The basal (B), middle (M), and tip (T) sections of the leaf were cut. One gram of fresh leaf material was homogenized in 2.5 cm^3 of homogenization medium at 4°C using a mortar. The non-soluble materials were removed through centrifugation for 1 minute at 12000 g. The protein fraction was rapidly frozen in liquid nitrogen and stored at -75 °C until further analysis. The protein concentration was quantified using Bradford Reagent, where Coomassie Brilliant Blue (100 mg) was dissolved in 50 ml of 95% ethanol. To this solution, 100 ml of 85% (w/v) phosphoric acid was added and diluted to a final volume of 1 litre. The reagent contained final concentrations of 0.01% (w/v) Coomassie Brilliant Blue, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid. Bovine serum albumin was used as the standard for protein quantification. Protein solutions containing 10 to 100 µg of protein in a volume of up to 0.1 ml were pipetted into 12 x 100 mm test tubes. The volume in the test tube was adjusted to 0.1 ml with an appropriate buffer. Subsequently, 3.5 ml of the protein reagent was added to the test tube, and the contents were mixed by vortexing. The absorbance at 595 nm was measured after 2 minutes and before 1 hour in 3 ml cuvettes against a reagent blank prepared from 0.1 ml of the appropriate buffer and 5 ml of the protein reagent. A standard curve was created by plotting the weight of protein against the corresponding absorbance, and this curve was utilized to determine the protein content in unknown samples.

In the subsequent steps, protein extracts were combined with glycerol in a 2:1 (v/v) ratio and stained with bromophenol blue before being applied to the gel lanes. An equal protein content of 15 µg was applied to each lane. Electrophoresis

was conducted at 4 °C, using a voltage of 180 V, and 13% polyacrylamide gels were utilized. The Laemmli buffer system was employed, except that SDS (Sodium Dodecyl Sulfate) was absent from all buffers for approximately 60 minutes. Following the electrophoresis, the gels were incubated in a staining buffer for 30 minutes in the dark at room temperature. Subsequently, the gels were exposed to daylight until the SOD activity bands became visible. The gels were stained in a buffer containing 5 mol m^{-3} H_2O_2 to inhibit specific SOD isoforms, such as Cu/Zn-SOD and Fe-SOD. Selective inhibition of Cu/Zn-SOD was achieved by incubating the gels in a buffer containing 3 mol m^{-3} KCN. The gels were washed three times with distilled water and scanned using a flatbed or TLC scanner visualizer in the transmission black-and-white mode. The intensity of the bands was calculated, and the activity of different SOD isoforms was evaluated in arbitrary units, defined as the area under the curve per µg of protein applied to each lane, reported in ng/g.

Third objective: evaluating the effect of drought and the drought-mitigating potential of oil palm EFB biochar on the yield of selected okra genotypes.

Experimental design and treatments

All experimental setups and treatments were the same as those explained for the second objective, barring the data from being collected at different stages.

Data collection

Yield data was recorded from the tender green pods f each plant. The pods were frequently harvested (thrice per week), and various yield parameters were

measured, including pod length (Pl), pod diameter (Pd), the number of pods per plant (Npp), and total pod yield (Tpy). A vernier calliper was used for Pl and Pd measurements. Pl was determined by measuring from the pod's base to the tip, while Pd was measured at two points: one near the base and the other slightly above the midpoint. These two measurements were averaged to obtain the Pd value. Npp was calculated by visually counting the total number of pods harvested from each plant during the study. After each harvest, the weight of the pods from each plant was determined using an electronic weighing balance. Thus, Tpy was estimated as the cumulative pod weight throughout the seven-week harvesting period. The data collection concluded when there was a noticeable decline in yield.

Figure 2: (A) Sacks filled with soil-biochar mixture in PVCs during incubation; (B) Harvested okra pods; Plate C and D shows okra plants at two weeks after drought imposition (C) at 30% FC (drought) (D) Corresponding okra genotype at 90% FC (control).

Statistical analysis

Data analysis was performed using the R programming Language (version 4.2.3). Data from the first and second trials of the RSA experiment were merged, and summary statistics were calculated, which included the mean, standard deviation (SD), minimum and maximum values, and the coefficient of variation (CV). An analysis of variance (ANOVA) was performed using the 'aov()' function from the R inbuilt 'stats' package to assess variations between genotypes and trials. Tukey's Honestly Significant Difference (HSD) was employed to separate means between different groups, with a significance threshold set at $P < 0.05$.

A Residual Maximum Likelihood (REML) procedure was applied to all traits for variance component estimation. This was accomplished using the 'lmer ()' function from the 'lmerTest' package. All factors were treated as random in the REML analysis to allow the determination of the proportional contribution of genotype to the overall variation in the traits (Adu et al., 2019). Equation 3 illustrates the ANOVA and REML model used for the RSA experiment. Broad-sense heritability was calculated based on Equation 4 (Adu et al., 2014). The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated using the procedures of Shabanimofrada et al. (2013), as shown in Equation (6 and 7), respectively.

$$Y_{ijk} = \mu + g_i + t_j + r_k + gt_{ij} + \varepsilon_{ij} \qquad \text{Equation (3)}$$

Where Y_{ijk} = observation from the ijk^{th} genotype, trial and replication, μ = overall mean, g_i = effect of the i^{th} genotype, t_j = effect of the j^{th} trial, r_k = effect of the k^{th}

replication, gt_{ij} = interactive effect of the i^{th} genotype with the j^{th} trial, and ε_{ijk} = experimental error.

$$H^2 = \left(\frac{\sigma_g^2}{\sigma_p^2}\right) \quad \text{Equation (4)}$$

Where σ^2_p is the phenotypic variance and σ^2_g is the genotypic variance. The phenotypic variance was estimated using Equation (5) per Kumar et al. (2012):

$$\sigma^2_p = \sigma^2_g + \frac{\sigma_g^2 \times t}{n} + \frac{\sigma_\varepsilon^2}{rn} \quad \text{Equation (5)}$$

Where r is the number of replicates, n is the number of trials, $\sigma_g^2 \times t$ is the genotype× trial variance, and σ_ε^2 is the estimated variance associated with the residual error.

$$PCV = \frac{\sqrt{\sigma_p^2}}{Grand\ mean} \times 100 \quad \text{Equation (6)}$$

$$GCV = \frac{\sqrt{\sigma_g^2}}{Grand\ mean} \times 100 \quad \text{Equation (7)}$$

To simplify the dataset that included 25 measured traits from the first objective, only traits with CVs equal to or greater than 30 % (Chen et al., 2016) were retained for further multivariate analysis.

FactoMineR (Lê et al., 2008) was employed to assess the contributions of the quantitative traits using principal component analysis (PCA), and the results of the PCA were visualized using the Factoextra package (Kassambara, 2017). The PCA was based on the correlation matrix, and the selection of significant principal components (PCs) was based on the Kaiser criterion, which involved retaining any component with an eigenvalue exceeding one (Kaiser, 1960; Tabachnick & Fidell,

1996). Only traits that contributed substantially to the selected PCs were chosen for further analysis. Pearson's correlation coefficients were calculated to assess the relationships between all possible trait combinations. This process was executed using a combination of base R packages (R Core Team, 2013) and the metan package.

Additionally, cluster analysis was performed to identify distinct groups of okra genotypes with close genetic relatedness. The clustering process utilized Ward's hierarchical approach, employing the minimum variance linking method with Euclidean distance as the similarity measure (Manschadi et al., 2008). The optimal number of clusters was determined using NbClust, an R Package designed to ascertain the most appropriate number of clusters in a dataset based on the majority rule approach (Charrad et al., 2014).

Similar statistical procedures of descriptive statistics, ANOVA and means separation were applied to the dataset for the second (Equation 8) and third (Equation 9) objectives. However, biochar and water regimes were added as additional factors (Equation 8). A simple linear regression was performed using Tpy as the dependent variable and the biochemical traits as explanatory variables to assess the relationship between each biochemical trait and Tpy at various water regimes.

$$Y_{ijk} = \mu + g_i + b_j + w_k + gb_{ij} + gw_{ik} + gbw_{ijk} + \varepsilon_{ijk} \quad \text{Equation (8)}$$

Where Y_{ijk} = observation from the ijk^{th} genotype, biochar rate, water level and trial, μ = overall mean, g_i = effect of the i^{th} genotype, b_j = effect of the j^{th} biochar rate,

w_k = effect of the k^{th} water-regime, gb_{ij} = interaction effect of the i^{th} genotype with the j^{th} biochar rate, gw_{ik} = interaction effect of the i^{th} genotype with the k^{th} water-regime, gbw_{ijk} = the interaction effect of the i^{th} genotype with the j^{th} biochar rate and the k^{th} water-regime, and ε_{ijk} = error.

$$Y_{ijkl} = \mu + g_i + b_j + w_k + t_l + gb_{ij} + gw_{ik} + gt_{il} + bt_{jl} + wt_{kl} + gbt_{ijl} + gwt_{ikl} + gbw_{ijk} + gbwt_{ijkl} + \varepsilon_{ijkl} \quad \text{Equation (9)}$$

Where Y_{ijkl} = observation from the $ijkl^{th}$ genotype, biochar rate, water level and trial, μ = overall mean, g_i = effect of the i^{th} genotype, b_j = effect of the j^{th} biochar rate, w_k = effect of the k^{th} water-regime, t_l = effect of the l^{th} trial, gb_{ij} = interaction effect of the i^{th} genotype with the j^{th} biochar rate, gw_{ik} = interaction effect of the i^{th} genotype with the k^{th} water-regime, gt_{il} = interaction effect of the i^{th} genotype with the l^{th} trial, bt_{jl} = interaction effect of the j^{th} biochar rate with the l^{th} trial, wt_{kl} = interaction effect of the k^{th} water regime with the l^{th} trial, gbt_{ijl} = the interaction effect of the i^{th} genotype with the j^{th} biochar rate and the l^{th} trial, gwt_{ikl} = the interaction effect of the i^{th} genotype with the k^{th} water regime and the l^{th} trial, gbw_{ijk} = the interaction effect of the i^{th} genotype with the j^{th} biochar rate and the k^{th} water-regime, $gbwt_{ijkl}$ = the interaction effect of the i^{th} genotype with the j^{th} biochar rate and the k^{th} water regime and the l^{th} trial, and ε_{ijkl} = error.

CHAPTER FOUR

RESULTS

First objective: assessing genotypic variation in the RSA of okra genotypes

Descriptive data and analysis of variance

Biomass traits

Root dry weight (Rdw) ranged from 0.06 g to 0.41 g, while shoot dry weight (Sdw) varied from 0.12 g to 0.44 g. The average Sdw (0.26 g) was 1.8-fold higher than the average Rdw (0.148 g) (Table 3). Root-to-shoot ratio (RS) ranged from 0.27 to 1.42, averaging 0.576 (Table 3). The coefficient of variations (CVs) was categorised as high (≥60%), intermediate (30% to 59%) and low (<30%) (Adu et al., 2022b). The CV, a measure of the relative variability for quantitative traits (Zanklan et al., 2018), was intermediate for two biomass traits (44 % for Rdw and 38 % for RS) but low (< 30%) for Sdw (26 %) (Table 3).

There was a significant ($p < 0.001$) difference for Rdw among the genotypes and between the trials (Table 4). Genotype by run interactions also had a significant ($p < 0.001$) effect on Rdw. Genotype VI063895 had the largest root biomass in each trial (0.388 g in the first trial and 0.40 g in the second trial), while the least was recorded for GH114 (0.063 g) in the first trial and GH144 (0.068 g) in the second trial (Figure 3A and 3B). The remaining 58 genotypes had Rdw ranging from 0.065 g (GH144) to 0.330 g (GH120) in the first trial and 0.07 g (GH114) to 0.338 g (GH154) in the second trial. Among the genotypes that differed significantly between the two trials was GH154, which measured 0.303 g in the first trial and 0.338 g in the second trial (Figure 3A and 3B).

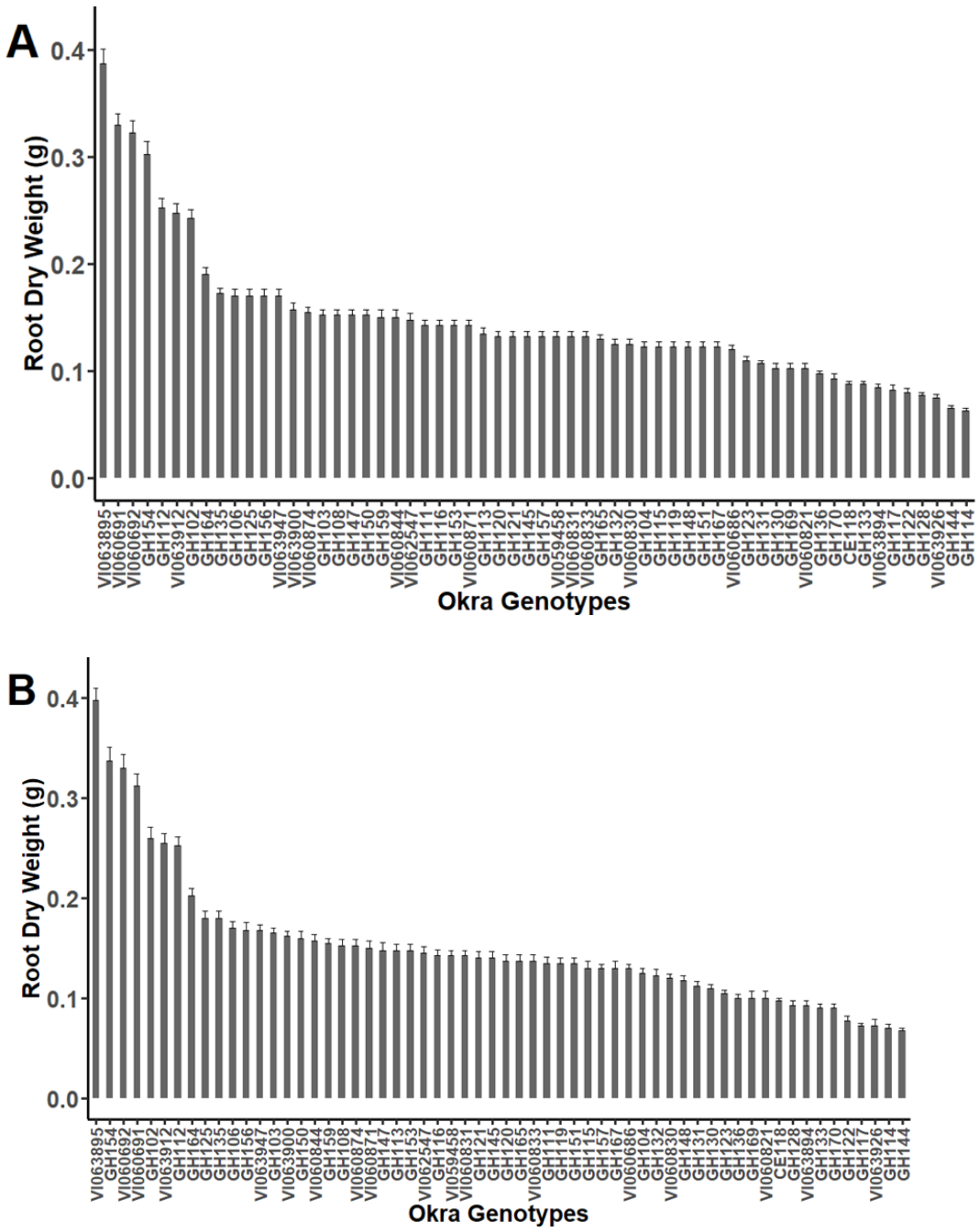

Figure 3: Variation in root dry weight. (A) First trial; (B) Second trial.

There was a significant ($p < 0.001$) difference among the okra genotypes and between the trials for Sdw (Table 4). Genotype by run interactions also had a significant ($p < 0.001$) effect on Sdw (Table 4). The top 5 % of the genotypes from

the first trial were GH159 (0.42g), GH147 (0.413g), and GH103 (0.39g), while GH148 (0.133g), GH104 and GH167 (0.15 g each) recorded the lower Sdw values, suggesting an about 93 % difference between the largest and least Sdw (Figure 4). Genotype GH159 (0.41 g), GH147 (0.408 g) and GH108 (0.40 g) were the top 5 % in the second trial, while the bottom 5 % consisted of GH148 (0.143 g), GH167 and VI060686 (0.16 g each) (Figure 5). Among the genotypes with significant differences between the trials were VI059458, VI060691, VI060830, and VI063947, with Sdw of 0.253 g and 0.235g, 0.30 g and 0.283 g, 0.28 g and 0.265 g, and 0.333 g and 0.318 g in the first and second trials respectively (Figure 4 and 5).

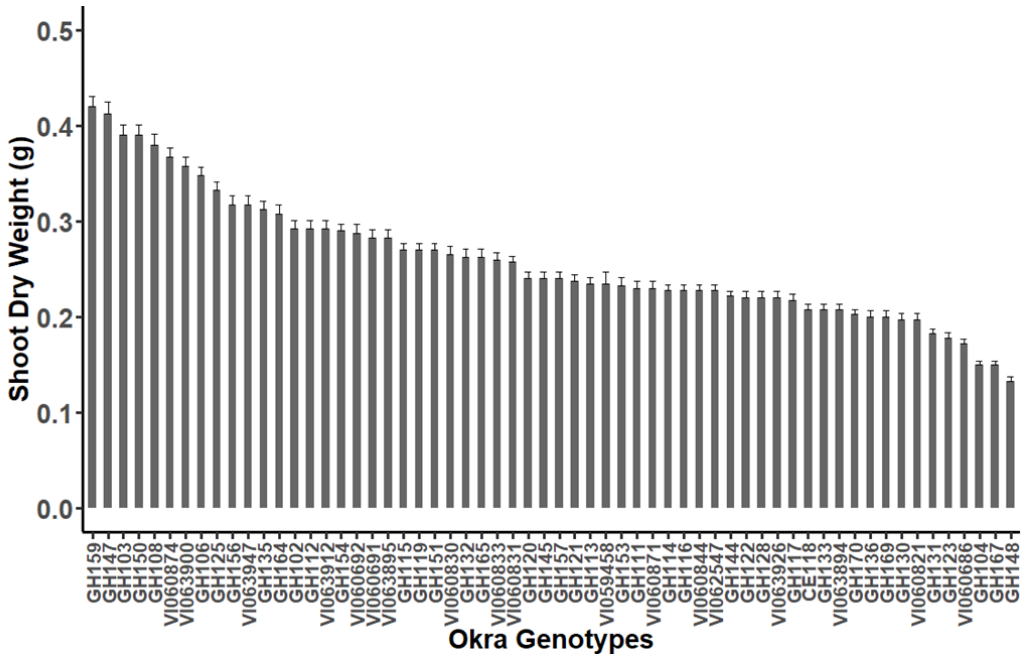

Figure 4: Variation in shoot dry weight in the first trial.

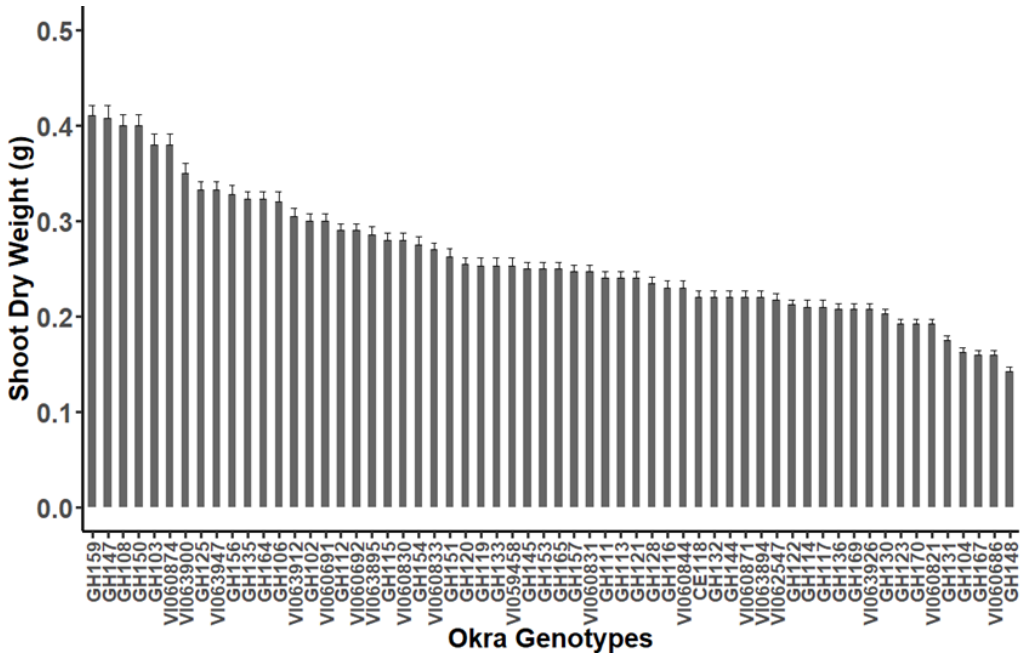

Figure 5: Variation in shoot dry weight in the second trial.

There was a significant ($p < 0.001$) difference in RS among the genotypes and between the trials (Table 4). Genotype-by-run interactions also had a significant ($p < 0.001$) effect on RS (Table 4). RS varied from 0.275 (GH114) to 1.373 (VI063895) in the first trial (Figure 6A) and 0.308 (GH144) to 1.39 (VI063895) in the second trial (Figure 6B). In that order, approximately 7 % of the genotypes had more root than shoot biomass, including VI063895, VI060692, GH154 and VI060691. Generally, it was observed that genotypes with greater root biomass had higher RS. Some genotypes for which significant ($p < 0.001$) differences were observed between the two trials included VI060686, VI060691, VI060871, and GH102, measuring 0.813 and 0.685, 1.16 and 1.033, 0.665 and 0.615, and 0.82 and 0.87 across the first and second trials respectively (Figure 6A and 6B).

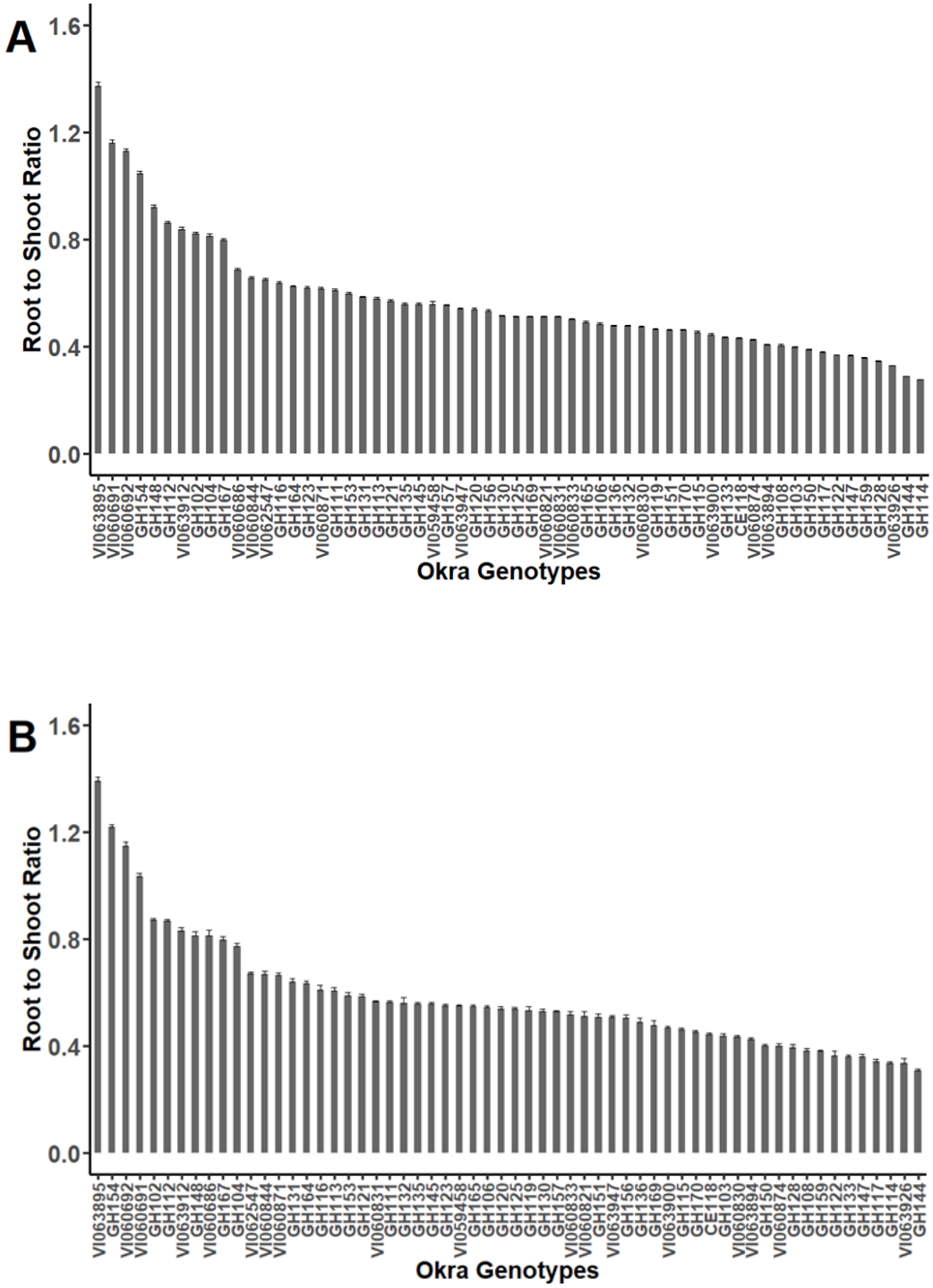

Figure 6: Variation in root-to-shoot ratio. (A) First trial; (B) Second trial.

Root system architecture traits

Root angle

The population average lateral root angle (Lra) was 69.65^0, ranging from 55^0 to 80.11^0 (Table 3). This suggested a 1.5-fold difference between the upper and lower limits (Table 3). The CV was observed to be low ($< 30\%$) among the genotypes in Lra (7 %) (Table 3).

A highly significant ($p < 0.001$) genotypic effect was observed in Lra (Table 4). However, the two trials had no significant ($p > 0.05$) difference in Lra. Genotype by run interactions also had no significant ($p > 0.05$) effect on Lra (Table 4). About 52 % and 53 % of the genotypes measured above the population mean Lra in the first and second trials, respectively. An approximately 1.3-fold difference was observed between GH169, having the largest Lra (77^0) and VI060691, with the least Lra (59.75^0) in the first trial (Figure 7A) and a similar 1.3-fold difference between VI060686, having the largest angle (77.42^0) and GH125 with the least angle (58.94^0) in the second trial (Figure 7B). The remaining okra genotypes ranged from 61^0 (GH125) to 76^0 (GH122) and 62.19^0 (GH156) to 76.56^0 (GH133) in the first and second trials each (Figure 7A and 7B)

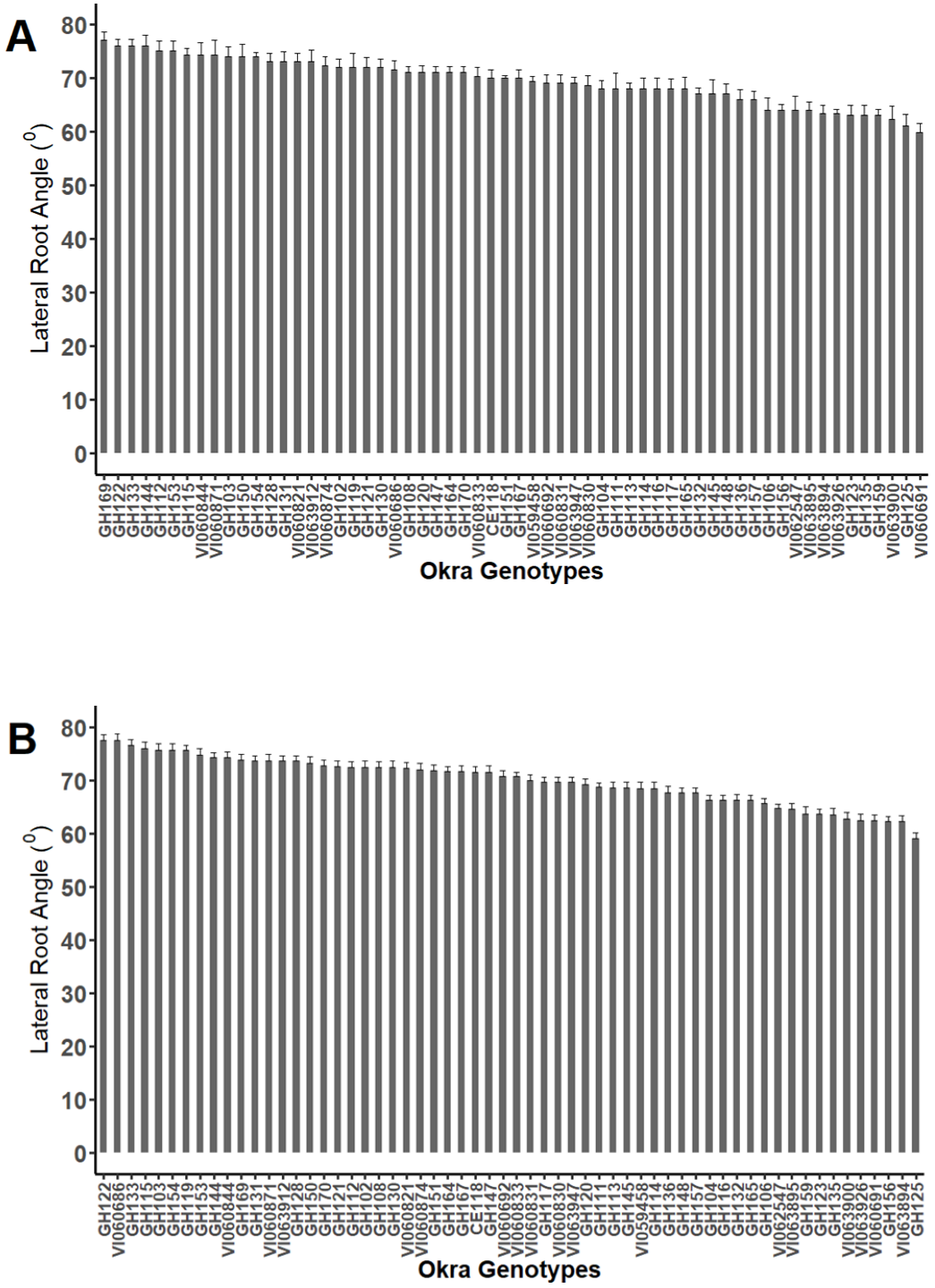

Figure 7: Variation in lateral root angle. (A) First trial; (B) Second trial.

Table 3: Descriptive statistics for all RSA and biomass traits assessed among 60 okra genotypes. The interpretation for acronyms is as follows: Min: minimum value; Max: maximum value; SD: standard deviation; CV: coefficient of variation.

Trait group	Acronym	Unit	Mean	SD	Min	Max	CV (%)
Biomass traits							
Root dry weight	Rdw	G	0.148	0.07	0.06	0.41	44
Shoot dry weight	Sdw	G	0.260	0.07	0.12	0.44	26
Root-to-shoot ratio	RS		0.576	0.22	0.27	1.42	38
Root angle trait							
Lateral root angle	Lra	Degree	69.652	5.01	55.00	80.11	7
Root number traits							
Number of first-order lateral	Nfol		28.444	9.14	6.000	58.00	32
Number of root tips	Nrt		680.879	223.41	257.00	1312.00	33
Number of branch points	Nbp		3713.858	2314.69	412.00	11335.00	62
Branching frequency	Bf		0.056	0.04	0.01	0.15	68
Root length traits							
Primary root length	Prl	Cm	41.220	2.01	35.47	48.79	5
Total root length	Trl	Cm	1103.978	396.98	174.39	2088.03	36
Root perimeter	Peri	Cm	1760.803	531.98	489.21	3680.08	30
Root length diameter range 1	RLDR1	Cm	749.041	218.04	236.85	1646.88	29
Root length diameter range 2	RLDR2	Cm	221.519	141.63	25.54	625.76	64
Root area traits							
Root network area	Na	cm^2	64.735	25.56	19.55	130.49	39
Root surface area	Sa	cm^2	265.007	119.81	64.83	645.49	45
Projected area diameter range 1	PADR1	cm^2	44.315	13.61	13.93	91.73	31
Projected area diameter range 2	PADR2	cm^2	28.999	19.25	3.35	83.68	66
Surface area diameter range 1	SADR1	cm^2	139.280	42.45	43.80	288.44	30
Surface area diameter range 2	SADR2	cm^2	91.135	60.37	10.79	263.00	66
Root diameter traits							
Average diameter	Ad	Cm	0.083	0.02	0.04	0.14	23
Median diameter	Md	Cm	0.097	0.04	0.04	0.27	44
Maximum diameter	Mxd	Cm	0.471	0.13	0.22	0.99	28
Root volume traits							
Root volume	Vol	cm^3	8.072	5.61	0.36	27.19	70
Volume diameter range 1	VDR1	cm^3	2.332	0.78	0.75	5.05	33
Volume diameter range 2	VDR2	cm^3	3.109	2.13	0.30	9.10	68

Table 4: ANOVA results for all RSA and biomass traits assessed among 60 okra genotypes. Gen: genotype.

Trait group	F-prob.	F-prob.	F-prob.
Biomass traits	**Gen**	**Trial**	**Gen x Trial**
Rdw	<0.001	<0.001	<0.001
Sdw	<0.001	<0.001	<0.001
RS	<0.001	<0.001	<0.001
Root angle trait			
Lra	<0.001	>0.05	>0.05
Root number traits			
Nfol	<0.001	>0.05	>0.05
Nrt	<0.001	>0.05	>0.05
Nbp	<0.001	>0.05	<0.001
Bf	<0.001	>0.05	<0.05
Root length traits			
Prl	<0.001	>0.05	>0.05
Trl	<0.01	>0.05	>0.05
Peri	<0.001	>0.05	>0.05
RLDR1	<0.001	>0.05	>0.05
RLDR2	<0.001	>0.05	>0.05
Root area traits			
Na	<0.001	>0.05	>0.05
Sa	<0.001	>0.05	>0.05
PADR1	<0.001	>0.05	>0.05
PADR2	<0.001	>0.05	>0.05
SADR1	<0.001	>0.05	>0.05
SADR2	<0.001	>0.05	>0.05
Root diameter traits			
Ad	<0.001	<0.01	>0.05
Md	<0.001	>0.05	>0.05
Mxd	<0.001	>0.05	>0.05
Root volume traits			
Vol	<0.001	>0.05	>0.05
VDR1	<0.001	>0.05	>0.05
VDR2	<0.001	>0.05	>0.05

Root number traits

The number of traits varied from 6 to 58, 257 to 1312, 412 to 11335, and 0.01 to 0.15 for the number of first-order lateral (Nfol), number of root tips (Nrt), number of branch points (Nbp), and branching frequency (Bf), respectively (Table 3). The range was greatest for Nbp (26-fold) followed by Bf (15-fold), Nfol (10-fold) and Nrt (5-fold) in that order. The population averages were 28.444, 680.897, 3713.858 and 0.056 in Nfol, Nrt, Nbp and Bf, respectively (Table 3). The number

of traits recorded intermediate to high CVs. Larger CVs were obtained in Bf (68 %) and Nbp (62 %), while Nrt (33 %) and Nfol (32 %) recorded intermediate CVs (Table 3).

There was a significant ($p < 0.001$) genotypic effect on Nfol (Table 4). However, there was no significant ($p > 0.05$) difference in Nfol between the two trials. Genotype-by-trial interactions also had no significant ($p > 0.05$) effect on Nfol (Table 4). About 43 % and 47 % of the genotypes were measured above the population average in Nfol across the first and second trials, respectively. In the first trial, the top 5 % genotypes were VI063895 (55.25), VI060691 (48.75), and GH156 (48.25), while the bottom 5 % consisted of GH114 (8.5), GH144 (14.25), and VI063926 (15.5) (Figure 8A). The second trial had similar genotypes in the top and bottom 5 % as the first trial, with Nfol of 55.5 (VI063895), 49.5 (GH156), and 48.5 (VI060691) in the top 5 %, and 9.0 (GH144), 15.0 (GH114) and 16.0 (VI063926) in the bottom 5 % (Figure 8B). These posited a 147 % difference between the highest Nfol (VI063895) genotype and the least Nfol (GH114) in each trial.

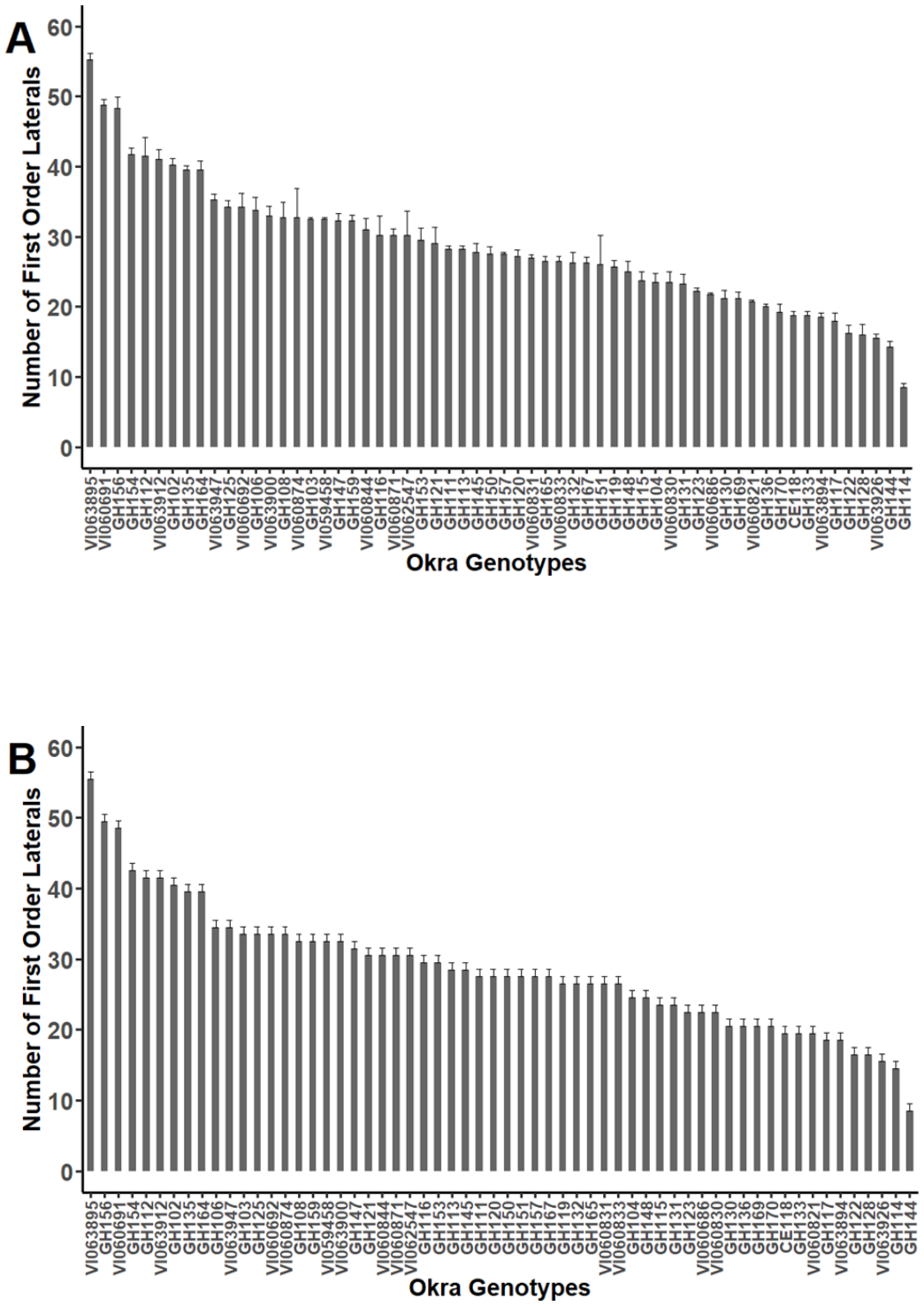

Figure 8: Variation in number of first order laterals. (A) First trial; (B) Second trial.

The okra genotypes varied significantly ($P < 0.001$) in Nrt, but no significant (P > 0.05) difference was observed between the two trials (Table 4). Genotype by run interactions had no significant (p > 0.05) effect on Nrt. Only 47 % of the genotypes were superior to the population average in Nrt in each trial. A 4.6-fold difference was observed between GH106, which had the highest Nrt (1261.75), and GH128, with the least Nrt (273.75) in the first trial (Figure 9). In the second trial, GH108 measured the highest Nrt (1251.75) and the least by GH128 (276.5), with a difference of about 4.5-fold between the two extremes (Figure 10). The remaining genotypes had Nrt ranging from 316.75 (VI060686) to 1226.8 (GH108) and 320.3 (VI060871) to 1217.0 (GH106) in the first and second trials, respectively (Figures 9 and 10).

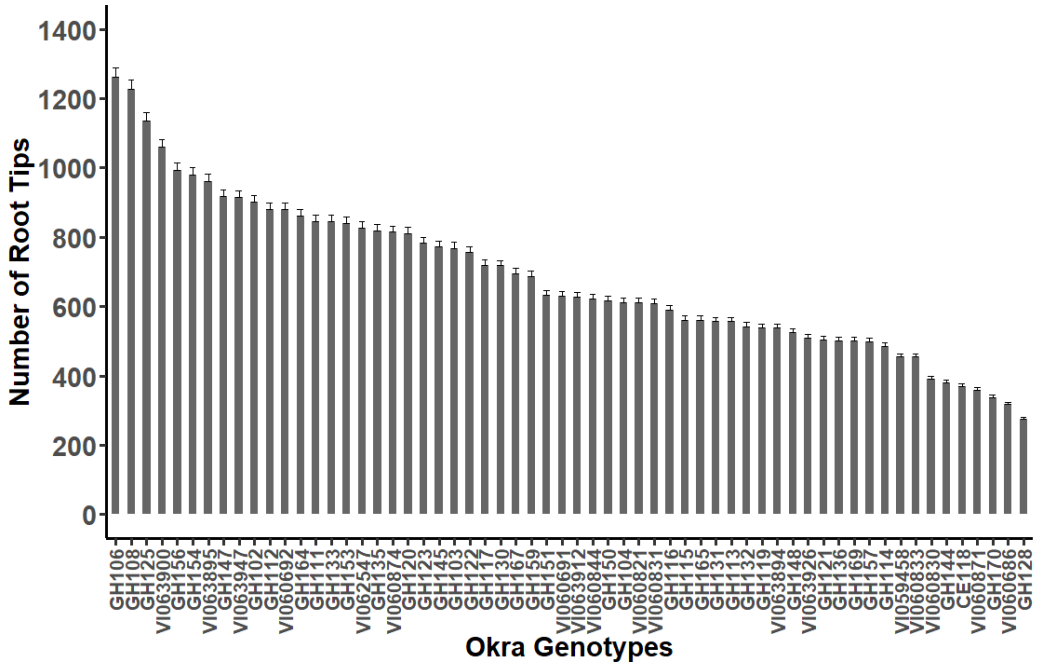

Figure 9: Variation in the number of root tips in the first trial.

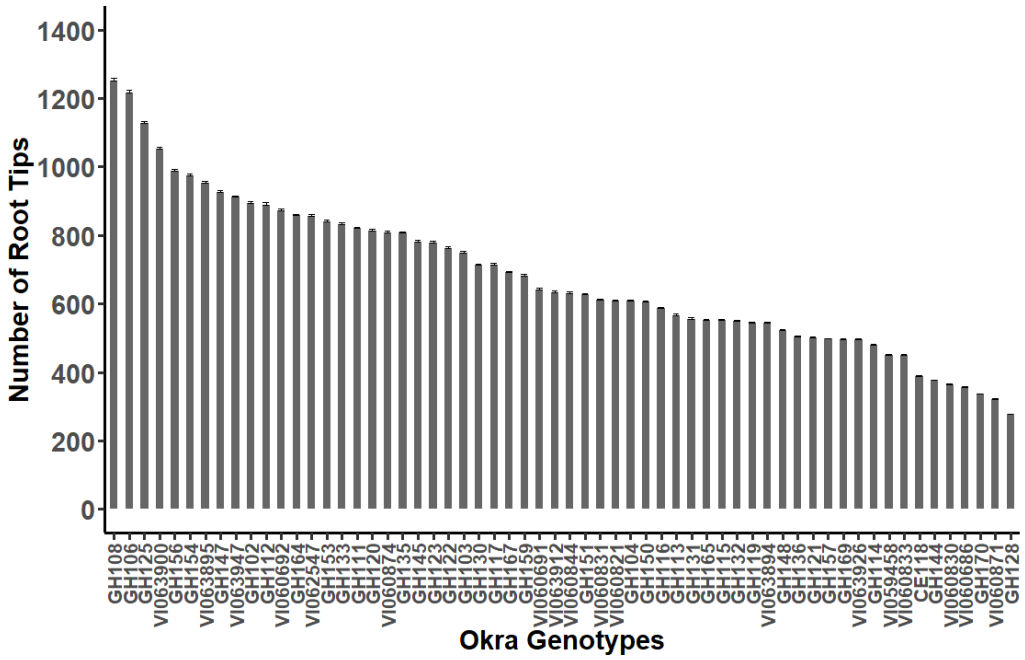

Figure 10: Variation in the number of root tips in the second trial.

There was a significant ($p < 0.001$) genotypic effect on Nbp (Table 4). The single effect of the trial did not significantly ($p > 0.05$) influence Nbp, but the interaction between genotype and trial was significant ($p < 0.001$) for Nbp (Table 4). Genotype VI063895 measured the most superior Nbp in each trial (10899.0 and 10872.75 in the first and second trials, respectively). In contrast, GH144 had the least (502 and 503 in the first and second trials each), giving a difference of about 182 % between the two genotypes (Figure 11A and 11B). Among the genotypes that varied significantly across the trials were VI060871, VI063912, GH103, and GH106, with Nbp of 7284 and 6784.25, 8540.25 and 8089.25, 7266.25 and 6773, 8560.75 and 8112.75 in the first and second trials, respectively (Figure 11A and 11B).

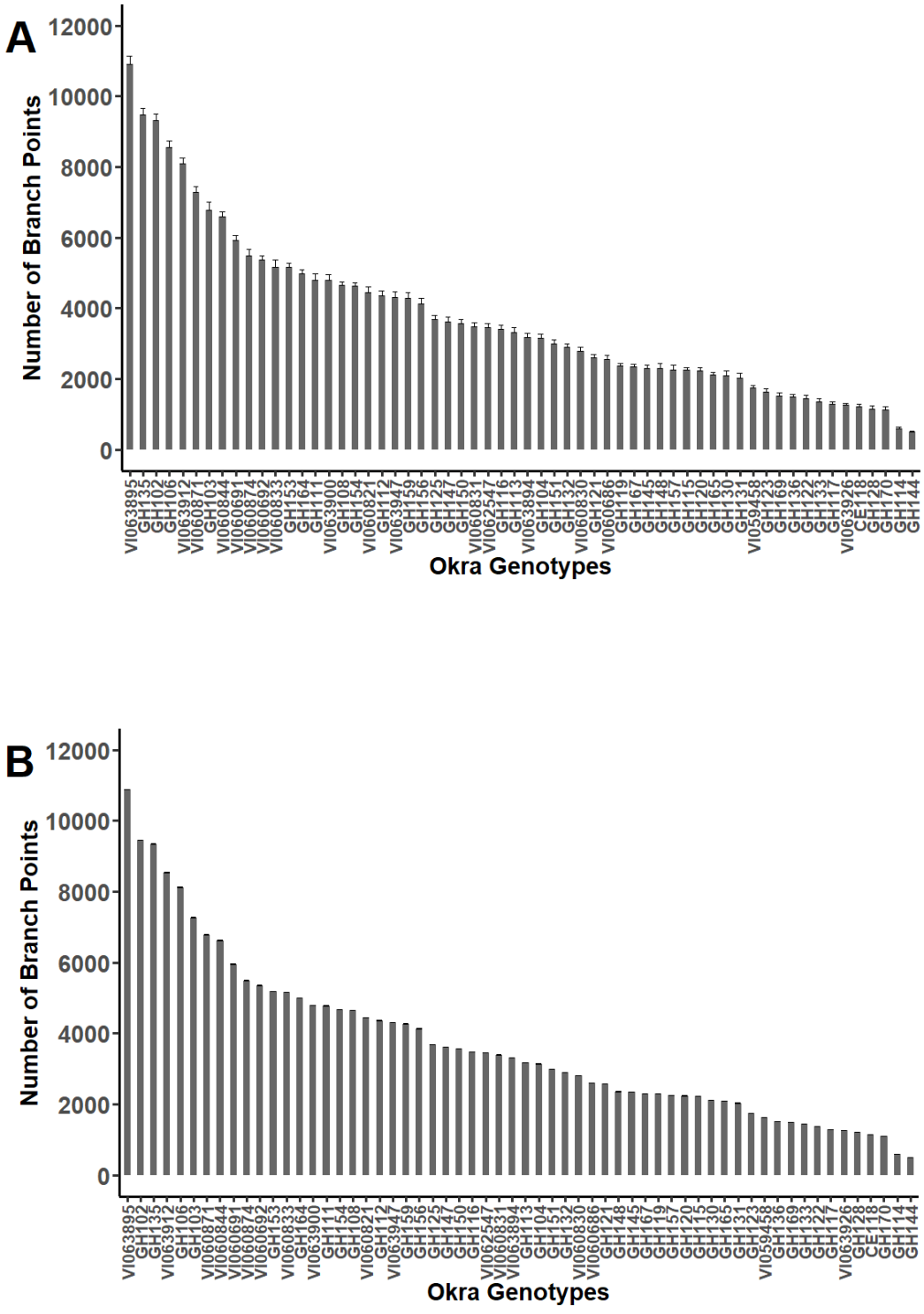

Figure 11: Variation in number of branch points. (A) First trial; (B) Second trial.

Branching frequency

The okra genotypes varied significantly ($p < 0.001$) in Bf (Table 4). The two trials had no significant ($p > 0.05$) difference. Still, the interaction between genotype and trial had a significant ($P < 0.05$) effect on Bf (Table 4). 28 % and 27 % of genotypes recorded higher than the population average Bf in the first and second trials, respectively. The top-ranked genotypes were GH112 (0.138), GH154 (0.135), VI060691, VI063912, VI063895, and GH102 (0.133 each), while genotypes with lower values consisted of GH170 (0.01), GH144, GH128, CE118, GH117, GH111, VI063894, and VI060686 (0.02 each) in the first trial (Figure 12A). Similar top-ranked genotypes were observed in the second trial, including genotype GH102 (0.145), VI060691 (0.143), VI063912 and GH112 (0.138 each), while the lower Bf were measured in GH170 (0.01), GH144, GH136, GH128, GH117, GH114, GH111, VI060821 and VI060686 (0.02 each) (Figure 12B). Among the genotypes that differed significantly ($p < 0.05$) across the trials was VI063900, which recorded 0.10 and 0.09 in the first and second trials, respectively (Figure 12A and 12B).

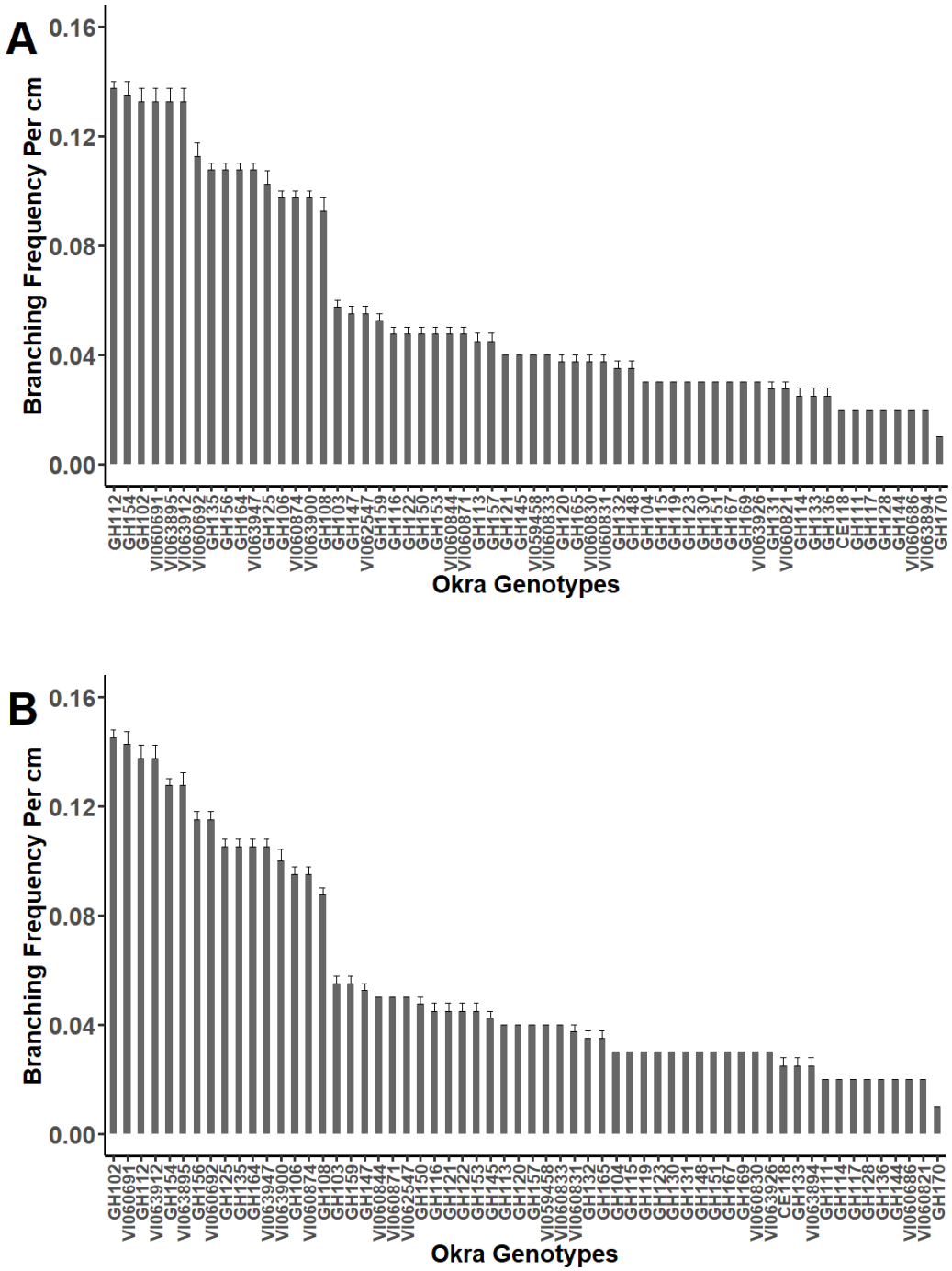

Figure 12: Variation in branching frequency per cm (A) First trial; (B) Second trial.

Root length traits

The ranges for primary root length (Prl), total root length (Trl), and root perimeter (Peri) were 35.47 cm to 48.79 cm, 174.39 cm to 2088.03 cm and 489.21

cm to 3680.08 cm, averaging 41.22 cm, 1103.978 cm and 1760.803 cm each (Table 3). The ranges between the root length diameters were 236.850 cm to 1646.88 cm for RLDR1 and 25.540 cm to 625.76 cm for RLDR2. The highest mean was recorded by RLDR1 (749.041 cm). High CV was observed in RLDR2 (64 %), whereas intermediate CVs were recorded for Trl (36 %) and Peri (30 %). On the contrary, the CVs were low for RLDR1 (29 %) and Prl (5 %) (Table 3).

A significant ($p < 0.001$) difference was observed among the genotypes in Prl (Table 4). However, the single effect of the trial and the interaction between genotype and trial did not significantly ($P > 0.05$) influence Prl. The longest Prl were observed in VI060692 (45.11 cm) and GH154 (46.59 cm) in the first and second trials each, about 15 % and 20 % longer than the shortest Prl obtained in VI063894 (38.79 cm) in the first trial and CE118 (37.97 cm) in the second trial (Figure 13A and 13B). Other genotypes had Prl varying from 39.1 cm (GH170) to 43.96 cm (GH154) in the first trial (Figure 13A) and 38.32 cm (GH114) to 44.755 cm (VI063947) in the second trial (Figure 13B).

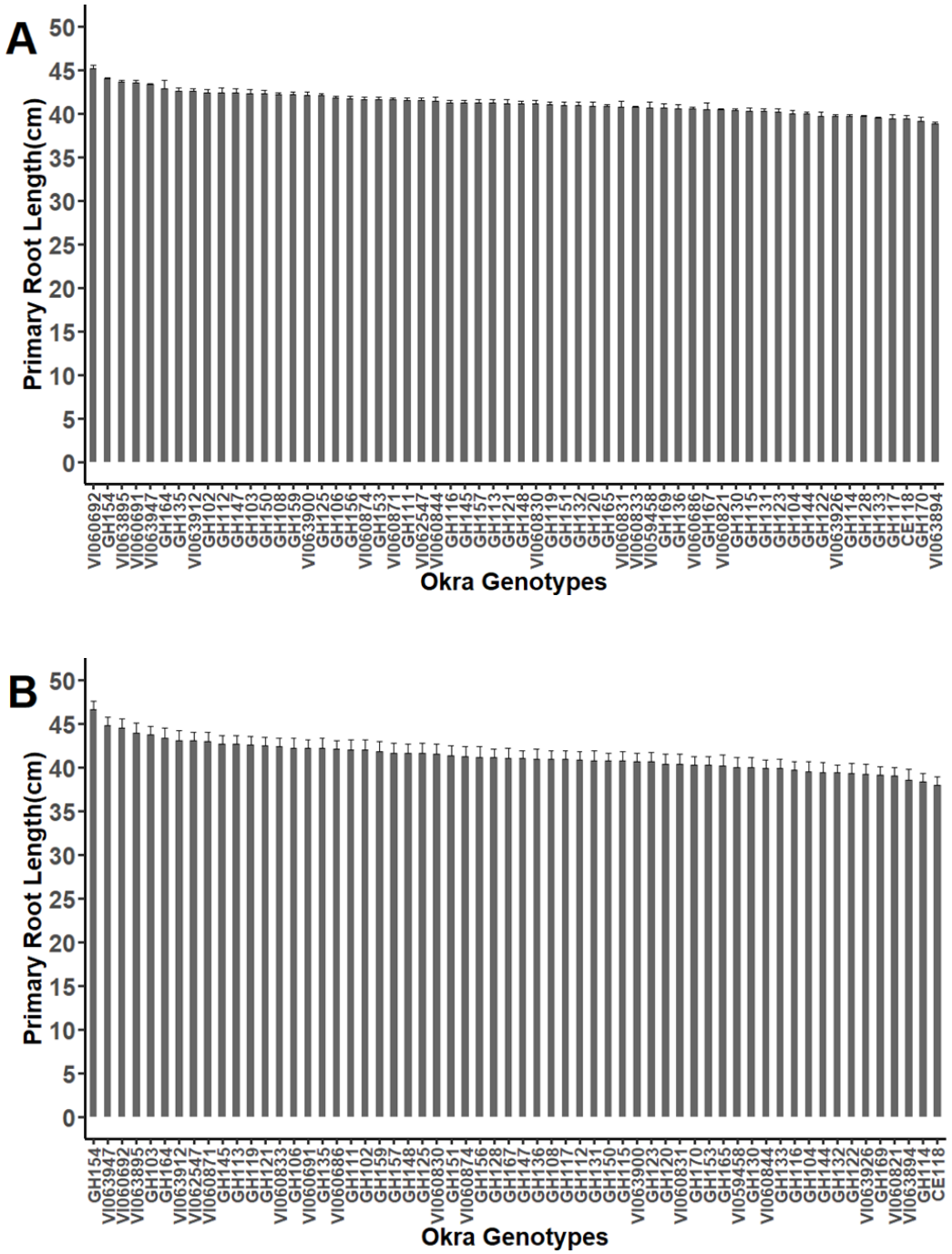

Figure 13: Variation in primary root length. (A) First trial; (B) Second trial.

A significant ($p < 0.001$) genotypic effect was observed on Trl, but without a significant ($p > 0.05$) trial and genotype-by-trial interaction effect (Table 4). The top 5 % genotypes were GH108 (2032.28 cm), VI063900 (1837.31 cm) and GH125

(1815.94 cm), whereas GH136 (481.77 cm), GH144 (274.68 cm) and VI060821 (217.98 cm) ranked within the bottom 5 % in the first trial (Figure 14). In the second trial, GH108 (1895.138 cm) and VI063900 (1835.025 cm) maintained their spots within the top 5 %, joined by VI063912 (1678.315 cm), while the bottom 5 % remained unchanged from the first trial (Figure 15). Thus, there was about a 9-fold difference between the genotype with the greatest Trl and that with the lowest Trl in both trials.

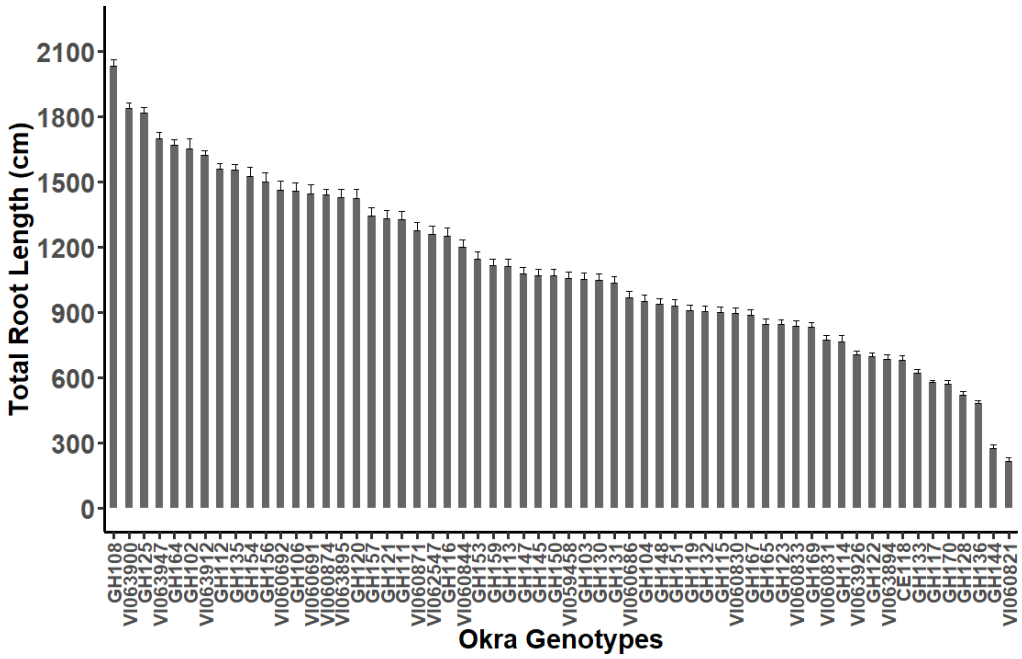

Figure 14: Variation in total root length in the first trial.

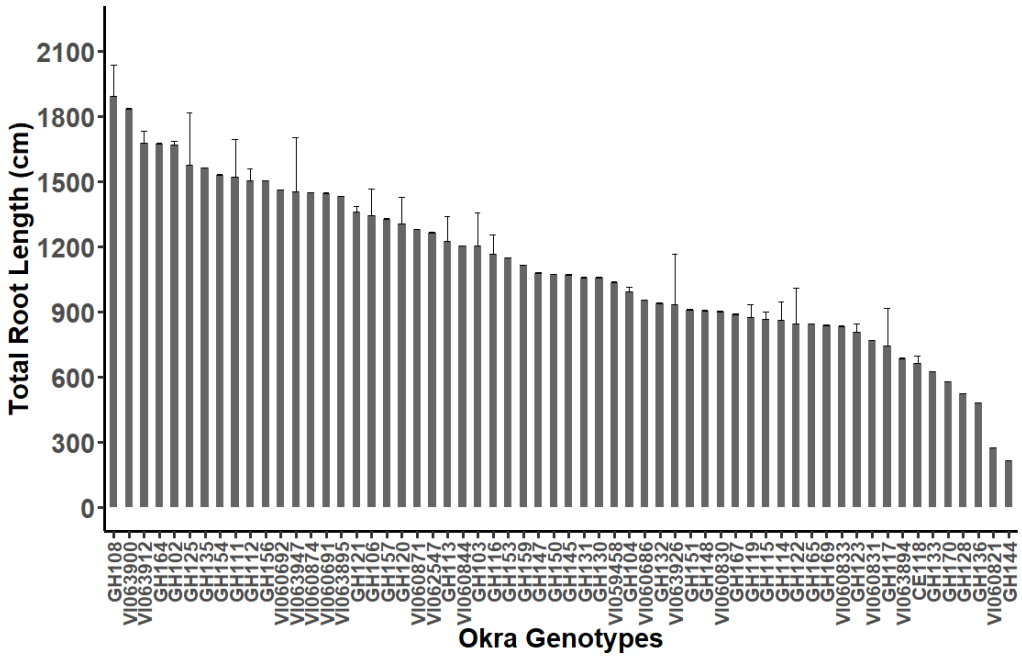

Figure 15: Variation in total root length in the second trial.

The okra genotypes differed significantly ($p < 0.001$) in root perimeter (Peri), but no significant ($p > 0.05$) trial as well as genotype and trial interaction effect were observed (Table 4). An equal number of genotypes (40 %) recorded Peri superior to the population average across the trials. The top 5 % genotypes were VI063895, GH106 and GH147 in both trials, with Peri measuring 2912.48 cm and 3057.3 cm, 2830.83 cm and 2752.29 cm, and 2761.17 cm and 2948.118 cm in the first and second trials respectively (Figure 16 A and 16B). Among the bottom 5 % were VI060871 (1101.283 cm), GH144 (988.09 cm) and GH128 (698.873 cm) in the first trial (Figure 16A) and GH115 (1083.62 cm), GH144 (946.098 cm) and GH128 (660.435 cm) in the second trial (Figure 16B). This indicated an approximately 4-fold difference between the genotype with the largest Peri and the genotype with the least Peri in both trials.

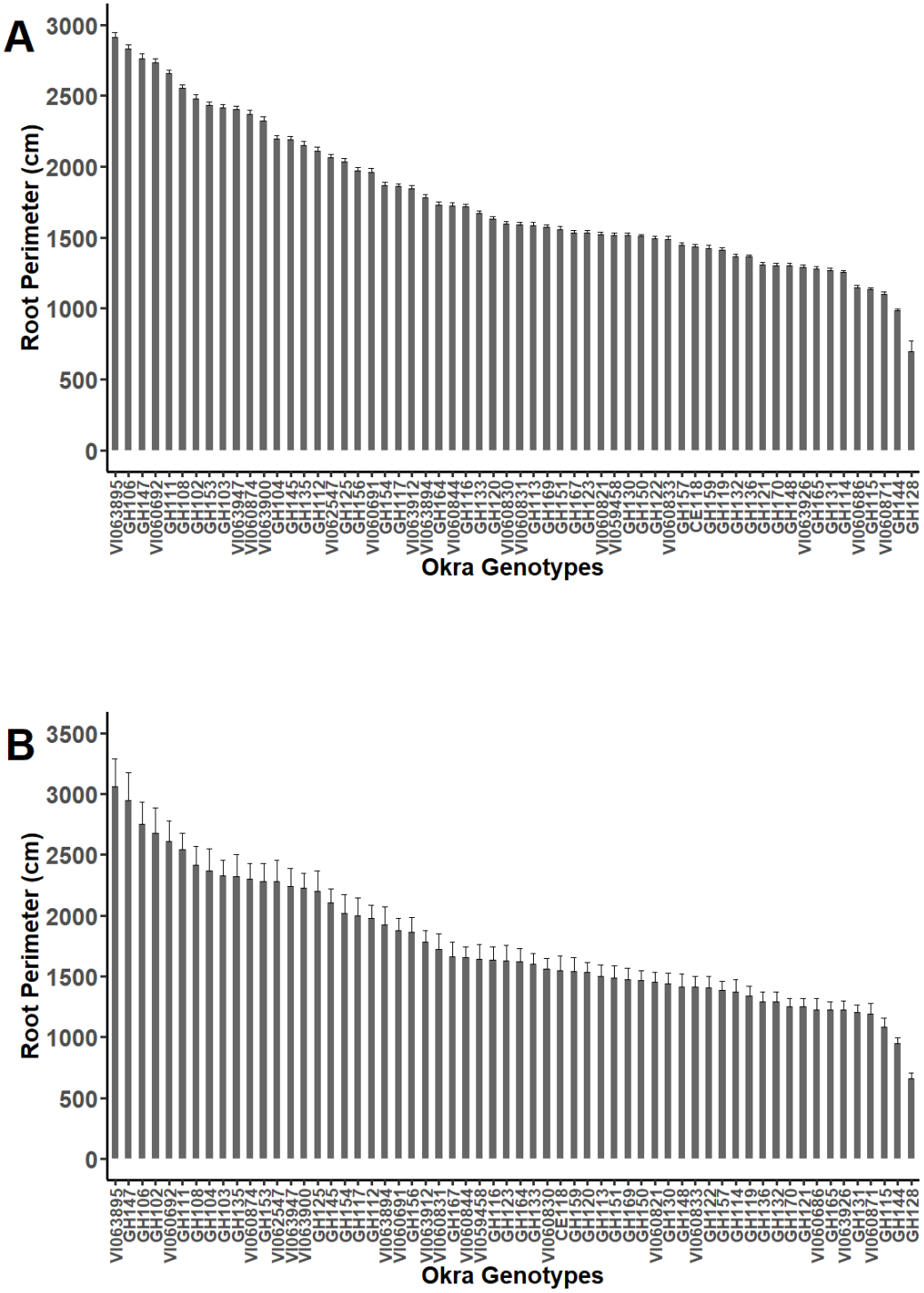

Figure 16: Variation in root perimeter (A) First trial; (B) Second trial.

There was a significant ($p < 0.001$) difference among the genotypes in RLDR1, but no significant ($p > 0.05$) trial as well as genotype and trial interaction effect were observed (Table 4). Root length diameters greater than the population average were obtained by 40 % of the genotypes in each trial. While the top 5 % genotypes ranged from 1151.77 cm (GH111) to 1266.83 cm (GH106), the bottom 5 % ranged from 296.06 cm (VI060686) to 428.23 cm (VI060871) in the first trial (Figure 17). Similarly, the top and bottom 5 % ranged from 1102.83 cm (GH147) to 1368.18 cm (GH106) and 279.78 cm (GH128) to 441.39 cm (VI060871), respectively, during the second trial (Figure 18). The result revealed 4-fold and 5-fold differences between the highest and the least RLDR1 genotype in the first and second trials.

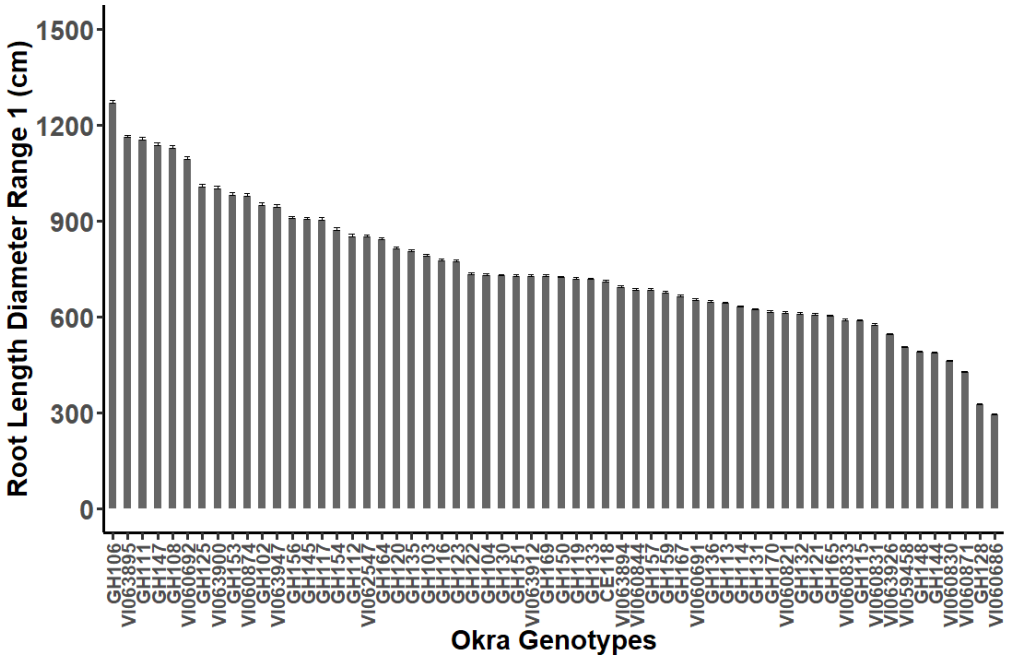

Figure 17: Variation in root length diameter range one in the first trial.

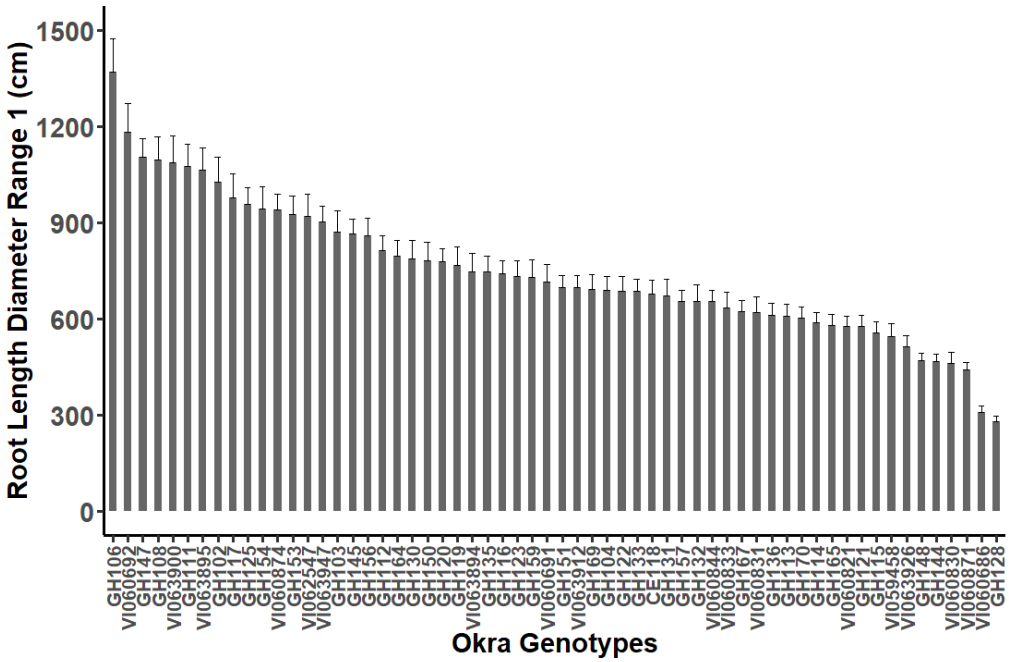

Figure 18: Variation in root length diameter range one in the second trial.

The genotypes varied significantly ($p < 0.001$) in RLDR2, while the trials and the interaction between genotype and trial did not have significant ($P > 0.05$) effects on RLDR2 (Table 4). An equal number of genotypes (47 %) measured above the population average of RLDR2 in the first and second trials. The top 5 % genotypes recorded 516.40 cm (GH103), 488.23 cm (GH104), and 485.56 cm (VI063895) in the first trial (Figure 19A), and 519.86 cm (GH104), 488.00 cm (GH103), and 473.50 cm (VI060691) in the second trial (Figure 19B). The bottom 5 % consisted of GH115 (30.0 cm), GH123 (40.46 cm) and GH128 (43.73 cm) in the first trial (Figure 19A), and GH128 (32.39 cm), GH123 (38.74 cm) and GH115 (41.322 cm) in the second trial (Figure 19B). Comparatively, the largest RLDR2 was 17-fold and 16-fold more than the least RLDR2 in the first and second trials each.

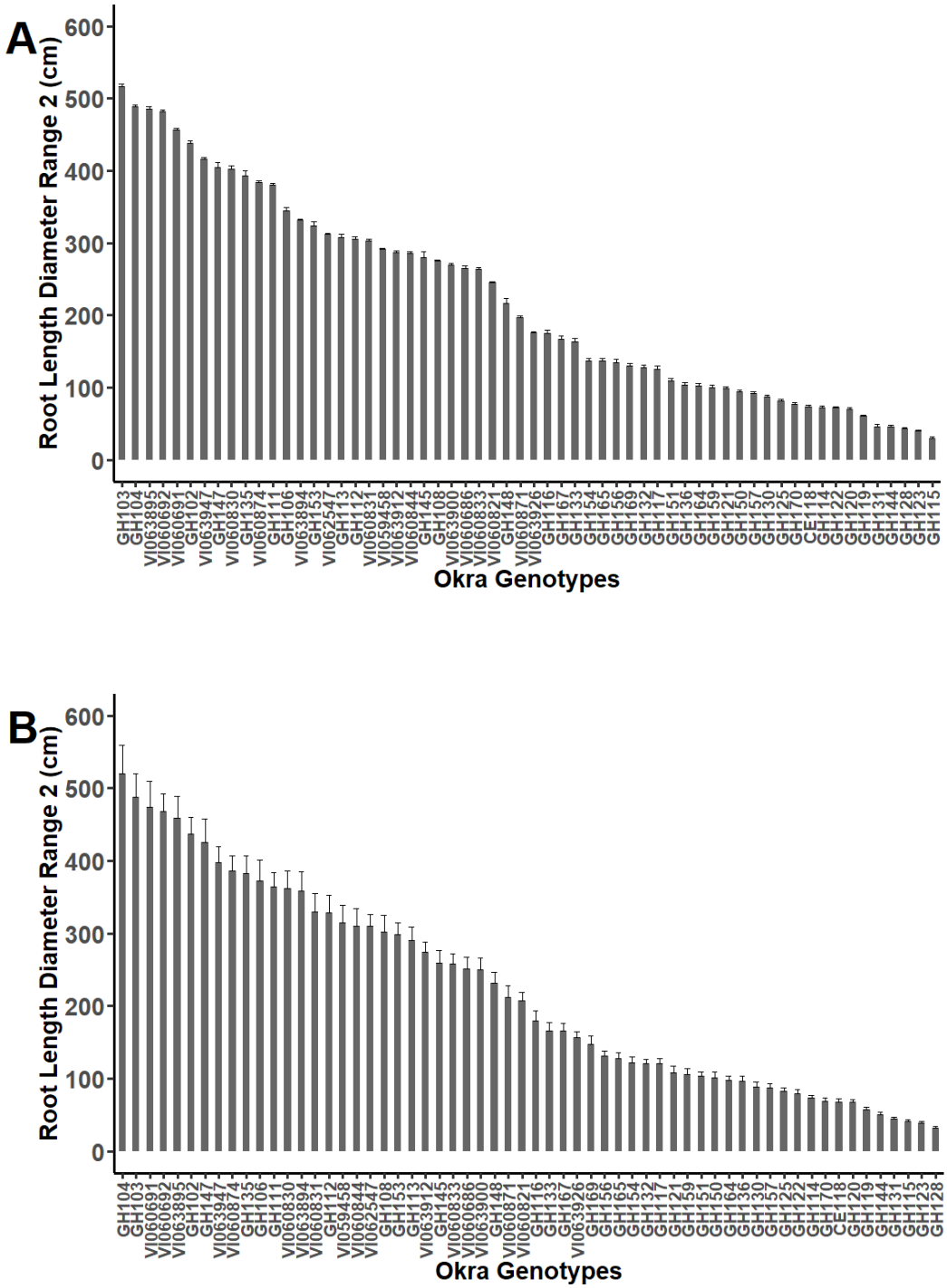

Figure 19: Variation in root length diameter range two. (A) First trial; (B) Second trial.

Root area traits

Root network area (Na) and root surface area (Sa) ranged from 19.55 cm^2 to 130.49 cm^2 and 64.830 cm^2 to 645.49 cm^2 respectively (Table 3). The average Na was 64.735 $cm^{2,}$ while Sa was 265.007 cm^2 (Table 3). Between the projected area diameters, the traits varied from 13.93 cm^2 to 91.73 cm^2 for the projected area diameter range 1 (PADR1) and 3.35 cm^2 to 83.68 cm^2 for the projected area diameter range 2 (PADR2) (Table 3). The surface area diameters varied from 43.8 cm^2 to 288.44 cm^2 for surface area diameter range 1 (SADR1) and 10.79 cm^2 to 263.00 cm^2 for surface area diameter range 2 (SADR2), averaging 139.280 cm^2 and 91.135 cm^2 respectively. Thus, the average PADR1 was 1.5-fold more than the PADR2. All area traits had intermediate to high CVs, ranging from 30 % to 66 %.

The okra genotypes varied significantly ($p < 0.001$) in Na, but the trial and genotype interaction with the trial did not have a significant ($p > 0.05$) effect on Na (Table 4). Among the genotypes, 43 % obtained above the average root Na in each trial, some of which were VI063895 (123.10 cm^2), VI060692 (115.35 cm^2), GH111 (114.81 cm^2), GH104 and GH103 (112.9 each) from the first trial (Figure 20A), and genotype GH111 (124.40 cm^2), GH104, VI063895 (114.40 cm^2 each), GH103 (113.27 cm^2) and VI060692 (112.33 cm^2) in the second trial (Figure 20B). The genotypes with lower values for root Na were GH114 (35.58 cm^2), GH131 (34.40 cm^2), GH144 (28.69 cm^2), GH115 (26.01 cm^2) and GH128 (21.72 cm^2) in the first trial (Figure 20A), and GH123 (35.38 cm^2), GH131 (34.12 cm^2), GH144 (28.54 cm^2), GH128 (25.80 cm^2) and GH115 (21.95 cm^2) in the second trial (Figure 20B). The highest root Na differed from the least by 5.7-fold in each trial.

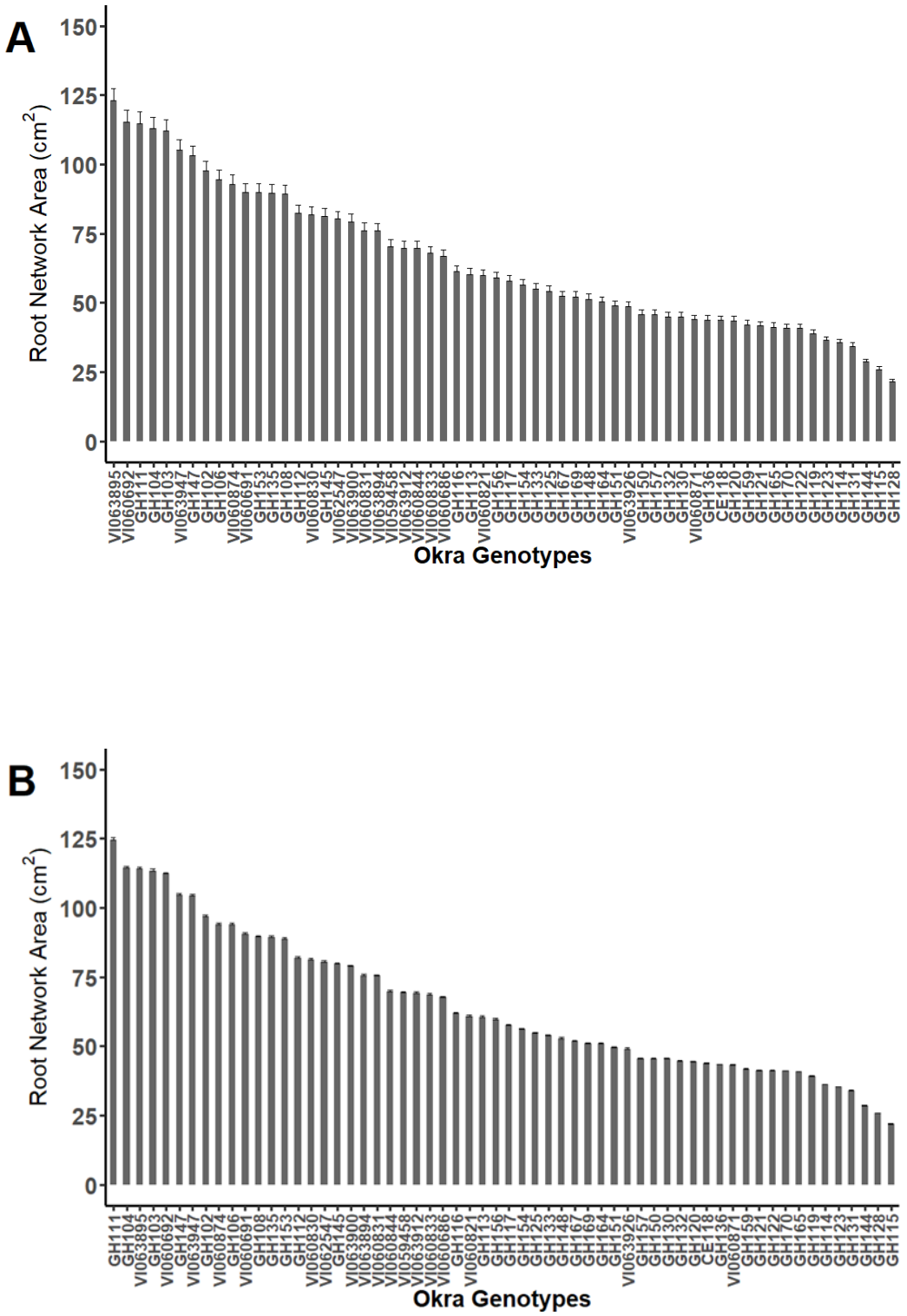

Figure 20: Variation in root network area. (A) First trial; (B) Second trial.

A significant ($p < 0.001$) difference was observed among the genotypes in root surface area (Sa), but no significant ($p > 0.05$) trial and genotype by trial interaction effect was observed (Table 4). Like root Na, 43 % of the genotypes were superior to the average root Sa population. The genotypes varied from 81.03 cm^2 (GH128) to 550.69 cm^2 (GH111) in the first trial (Figure 21), and from 76.58 cm^2 (GH128) to 536.25 cm^2 (VI060692) in the second trial (Figure 22). These revealed 6.8-fold and 7-fold differences between the highest and lowest root Sa in the first and second trials, respectively.

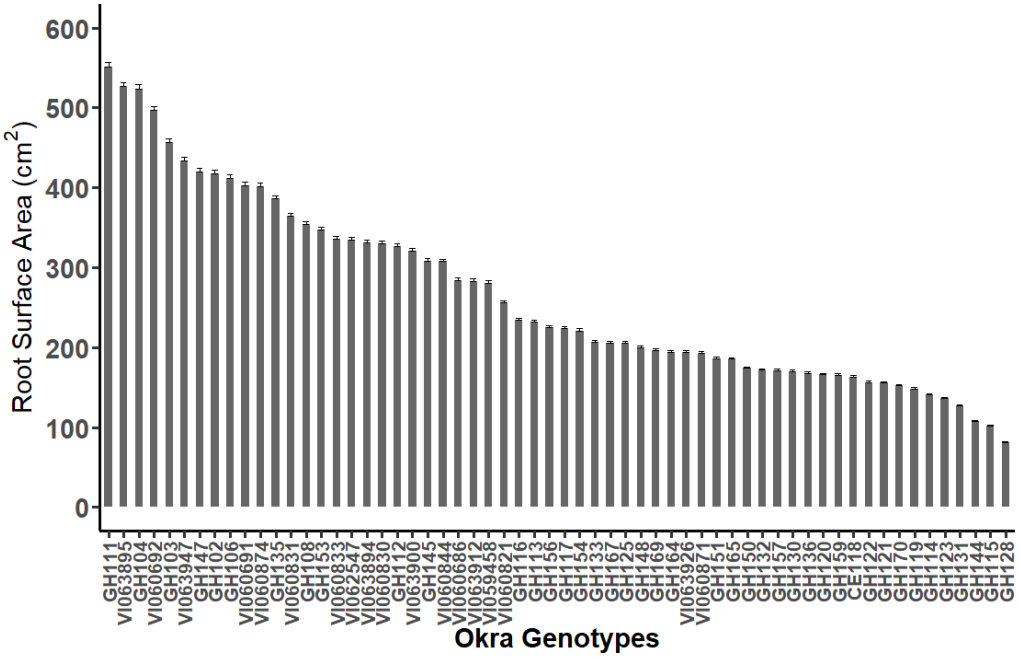

Figure 21: Variation in root surface area in the first trial.

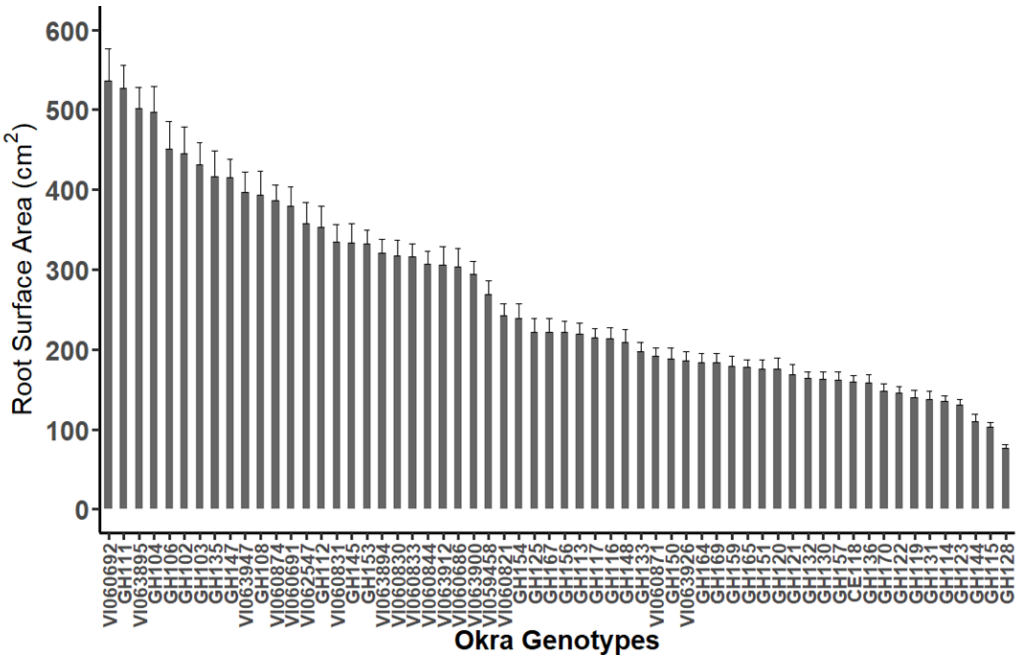

Figure 22: Variation in root surface area in the second trial.

There was a significant ($p < 0.001$) genotypic effect on PADR1. However, there were no significant ($p > 0.05$) trials and genotype-by-trial interaction effects on PADR1 (Table 4). Genotype VI063895 recorded the highest PADR1 in each trial (71.12 cm^2 in the first trial and 76.21 cm^2 in the second trial), while the least was scored for VI060686 (17.41 cm^2) in the first trial and VI060871 (16.46 cm^2) in the second trial (Figure 23A and 23B). This posited 121 % and 128 % differences between the greatest and the least PADR1 genotypes from the first and second trials, respectively.

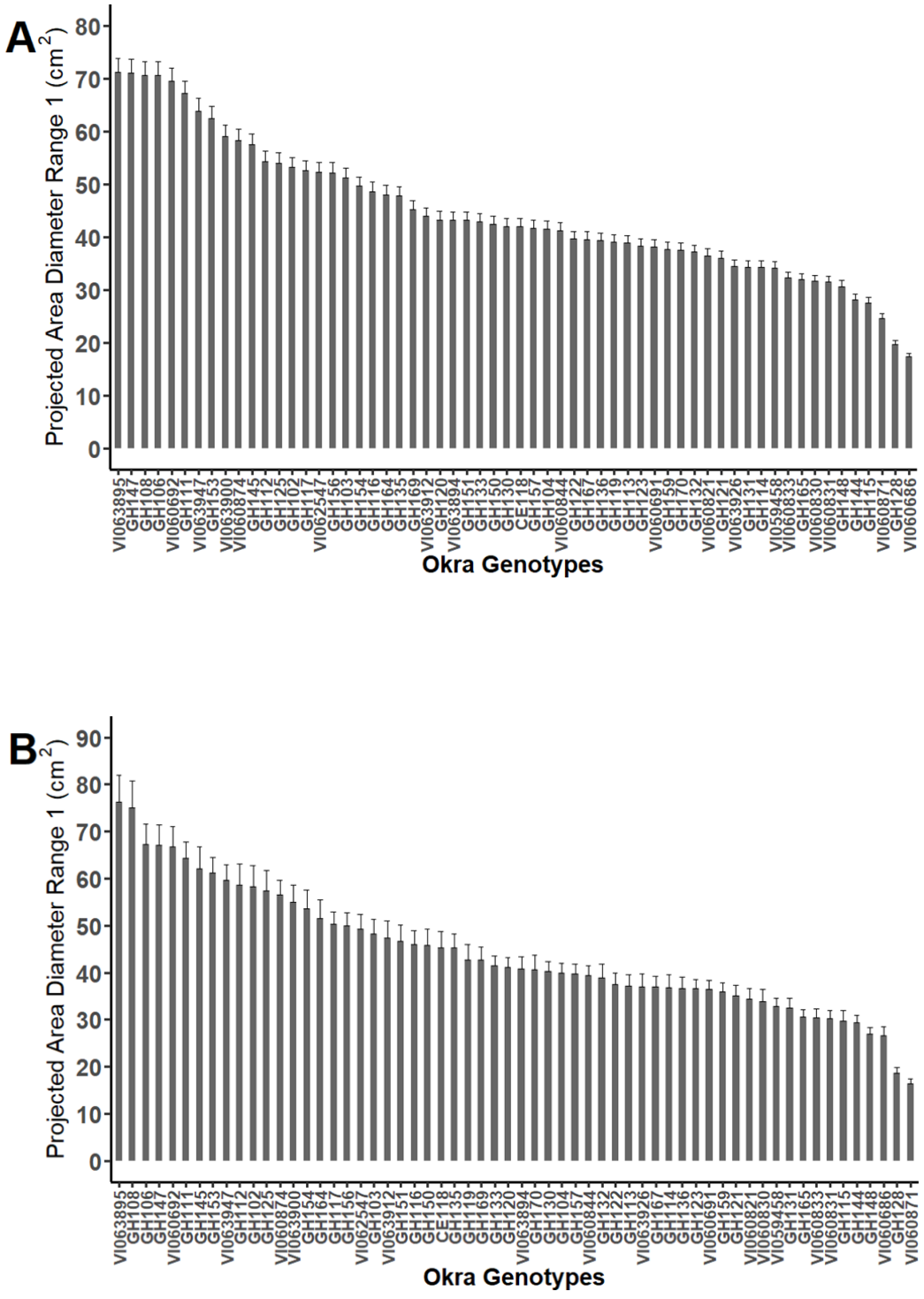

Figure 23: Variation in projected area diameter range one (A) First trial; (B) Second trial.

A significant ($p < 0.001$) difference was observed among the genotypes in PADR2, but no significant ($p > 0.05$) trial and genotype-by-trial interaction effect were detected on PADR2 (Table 4). An equal number of genotypes (46 %) measured above the population average PADR2 in each trial. There was an approximately 18.5-fold difference between the greatest PADR2 recorded for GH103 (69.025 cm^2) and the least obtained by GH115 (3.728 cm^2) in the first trial (Figure 24). Similarly, a 17.3-fold difference existed between the largest PADR2 of VI060691 (69.52 cm^2) and the lowest PADR2 of GH115 (4.028 cm^2) in the second trial (Figure 25). The remaining genotypes varied from 4.94 cm^2 (GH123) to 68.40 cm^2 (GH104) and 5.20 cm^2 (GH128) to 65.49 cm^2 (GH104) in the first and second trials, respectively (Figures 24 and 25).

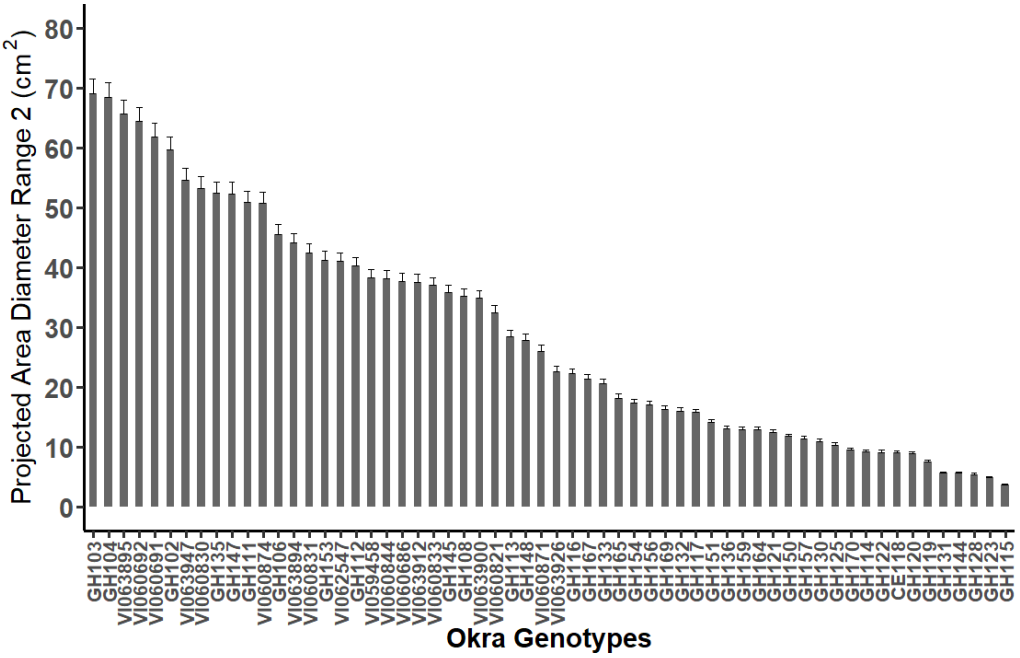

Figure 24: Variation in projected area diameter range two in the first trial.

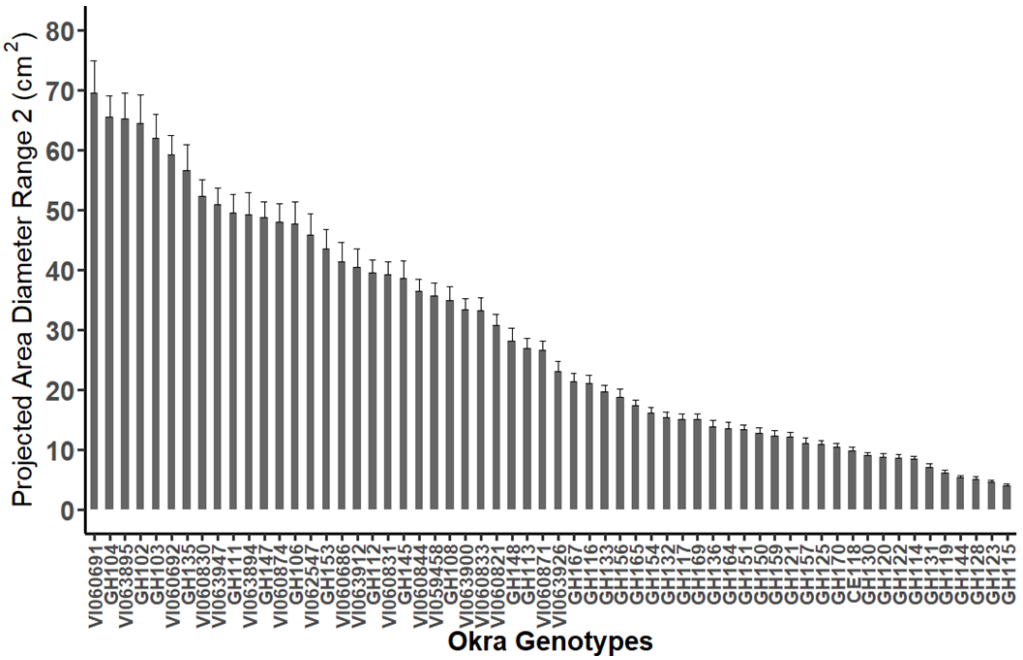

Figure 25: Variation in projected area diameter range two in the second trial.

There was a significant ($p < 0.001$) genotypic effect on SADR1, but the trial effect and the interaction between genotype and trial were not significant ($p > 0.05$) (Table 4). While the greatest SADR1 was obtained by genotype VI063895 (223.56 cm^2) in the first trial, the least was 54.748 cm^2 (VI060686), suggesting a 4.1-fold difference between the two extremes (Figure 26A). Genotype GH147 (223.338 cm^2) and GH106 (221.873 cm^2) were the other top 5 % from the first trial, while genotype GH128 (61.96 cm^2) and VI060871 (77.36 cm^2) completed the bottom 5 % (Figure 24). At the top 5 % in the second trial were genotype GH147 (239.623 cm^2), VI063947 (235.665 cm^2), and GH108 (211.263 cm^2), whereas the bottom 5 % were genotype GH128 (51.738 cm^2), VI060686 (58.555 cm^2), and VI060871 (83.55 cm^2) (Figure 26B). The difference between the greatest and the least SADR1 was 4.6-fold.

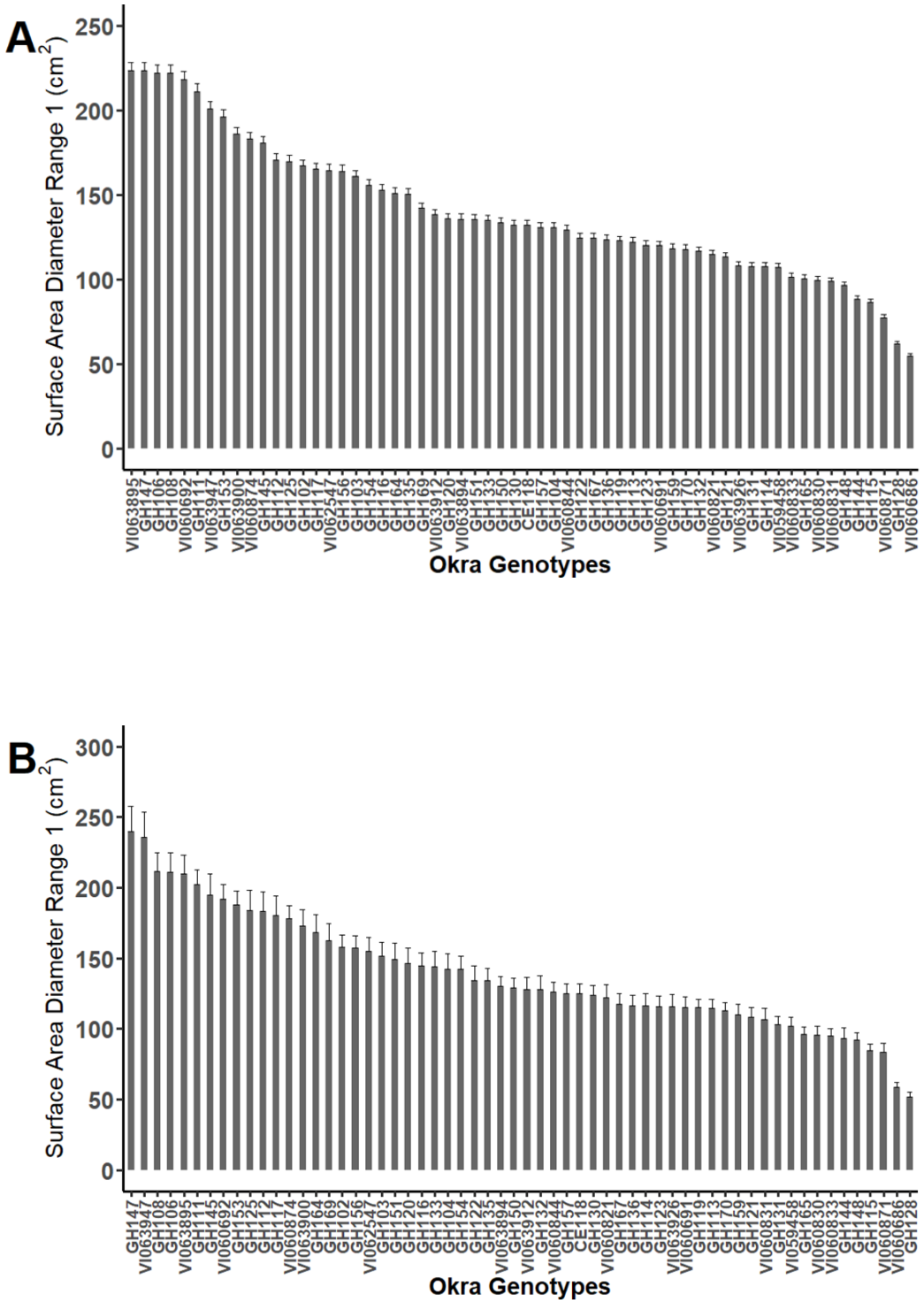

Figure 26: Variation in surface area diameter range one. (A) First trial; (B) Second trial.

The genotypes differed significantly ($p < 0.001$) in SADR2. The trials and genotype interaction with the trial did not have a significant ($P > 0.05$) effect on SADR2 (Table 4). There was approximately an 18.5-fold difference between the greatest SADR2 recorded in GH103 (216.883 cm^2) and the least recorded in GH115 (11.728 cm^2) in the first trial (Figure 27). Meanwhile, VI060692 (218.488 cm^2) measured the highest SADR2 in the second trial, about 17-fold higher than the least SADR2 observed in GH128 (12.665 cm^2) (Figure 28). The remaining genotypes within the top 5 % were GH104 (214.96 cm^2) and VI063895 (206.085 cm^2) in the first trial and GH103 (205.828 cm^2) and GH104 (204.955 cm^2) in the second trial (Figure 27 and 28).

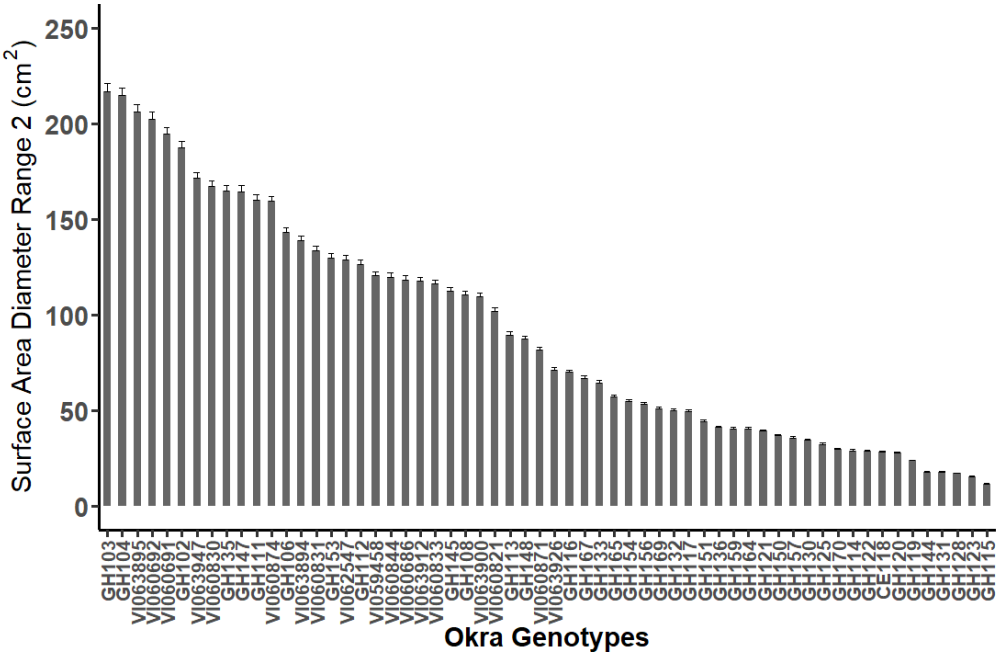

Figure 27: Variation in surface area diameter range two in the first trial.

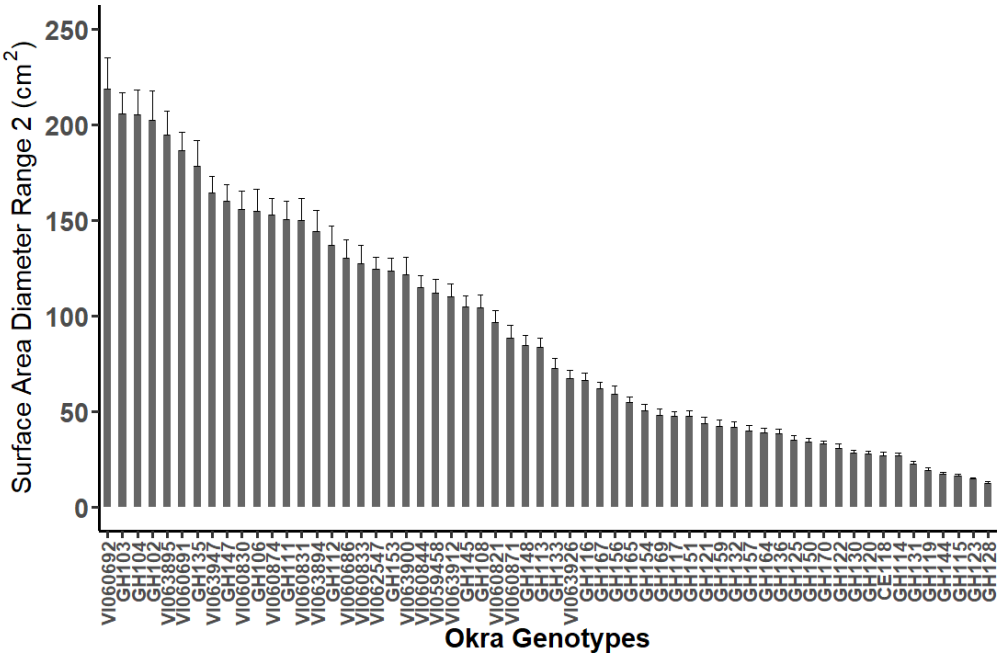

Figure 28: Variation in surface area diameter range two in the second trial.

Root diameter traits

Three diameter traits were assessed, including average diameter (Ad), median diameter (Md), and maximum diameter (Mxd) (Table 3). The diameter traits ranged from 0.04 cm to 0.10 cm, 0.04 cm to 0.27 cm, and 0.22 cm to 0.99 cm in Ad, Md, and Mxd, respectively (Table 3). The highest population average was recorded in Mxd (0.471 cm), and it was higher than Ad (0.083 cm) and Md (0.097 cm) by 140 % and 132 % respectively (Table 3). Only Md (44 %) had intermediate CVs, whereas Ad (23 %) and Mxd (28 %) scored low CVs (Table 3).

A significant ($p < 0.001$) difference was observed among the genotypes in Ad. However, no trial and genotype-by-trial interaction effect on Ad (Table 4). The top 5 % genotypes in both trials were GH111, GH121 and GH157, having a root Ad of 0.133 cm, 0.133, and 0.125 cm, respectively, in the first trial (Figure 29) and 0.135 cm, 0.128 cm, and 0.123 cm respectively, in the second trial (Figure 30). Some genotypes with lower root Ad included GH104 (0.048 cm), GH144 (0.050 cm), GH170, GH136, GH128, and GH117 (0.058 cm each) in the first trial (Figure 29), and genotype GH144 (0.045 cm), GH104 (0.050 cm), GH170 and GH117 (0.058 cm each) in the second trial (Figure 30). The difference between the highest and the least root Ad was 2.8-fold and 3-fold in the first and second trials each.

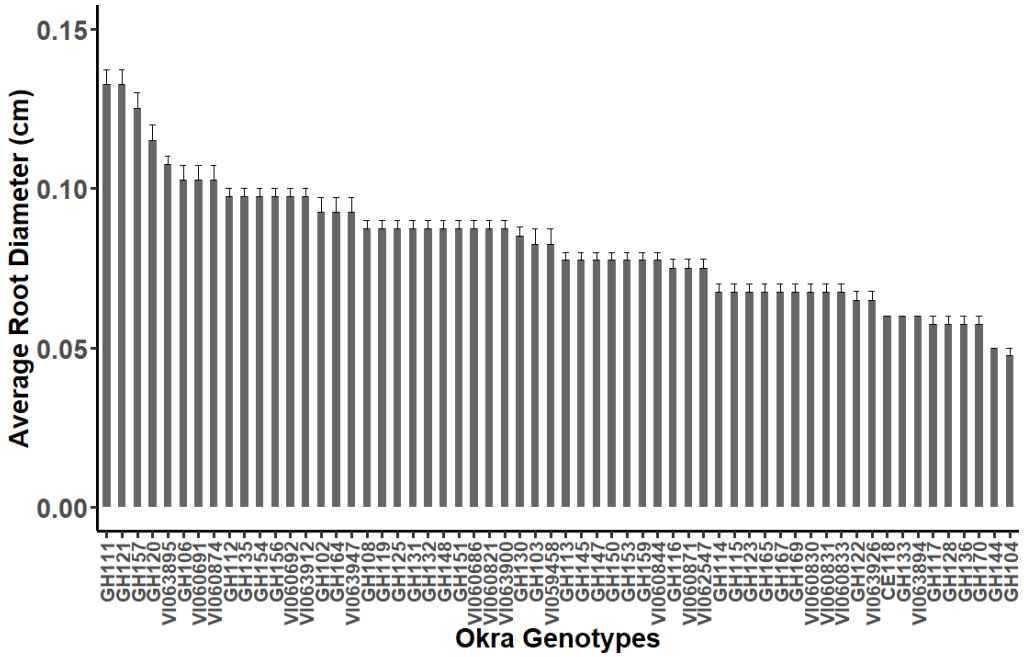

Figure 29: Variation in average root diameter in the first trial.

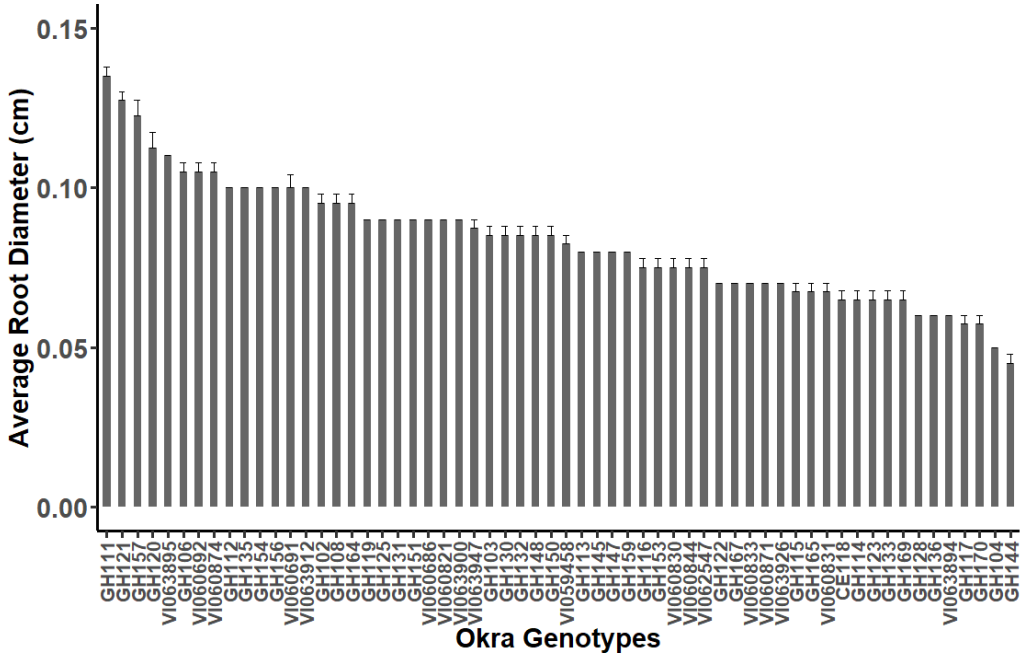

Figure 30: Variation in average root diameter in the second trial.

The okra genotypes differed significantly ($p < 0.001$) in root Md. However, no significant ($p > 0.05$) trial and genotype-by-trial interaction effects were observed (Table 4). Among the top-ranked genotypes were VI059458 (0.21 cm), GH145 (0.19 cm), GH157 and GH169 (0.18 cm each) in the first trial (Figure 31A), and genotype GH145 (0.225 cm), VI059458 (0.2025 cm), VI060692 (0.175 cm) and GH169 (0.170 cm) in the second trial (Figure 31B). Genotypes having lower root Md were GH115 (0.048 cm), GH125, GH120 (0.055 cm each), GH165, GH154, GH144 and GH119 (0.057 cm each) in the first trial (Figure 31A), and GH115 (0.05 cm), GH125 (0.053 cm), GH154, GH119 (0.055 cm), GH165 and GH120 (0.058 cm each) in the second trial (Figure 31B). There were considerable differences of about 4.4-fold and 4.5-fold between the highest and the lowest root Md in the first and second trials, respectively.

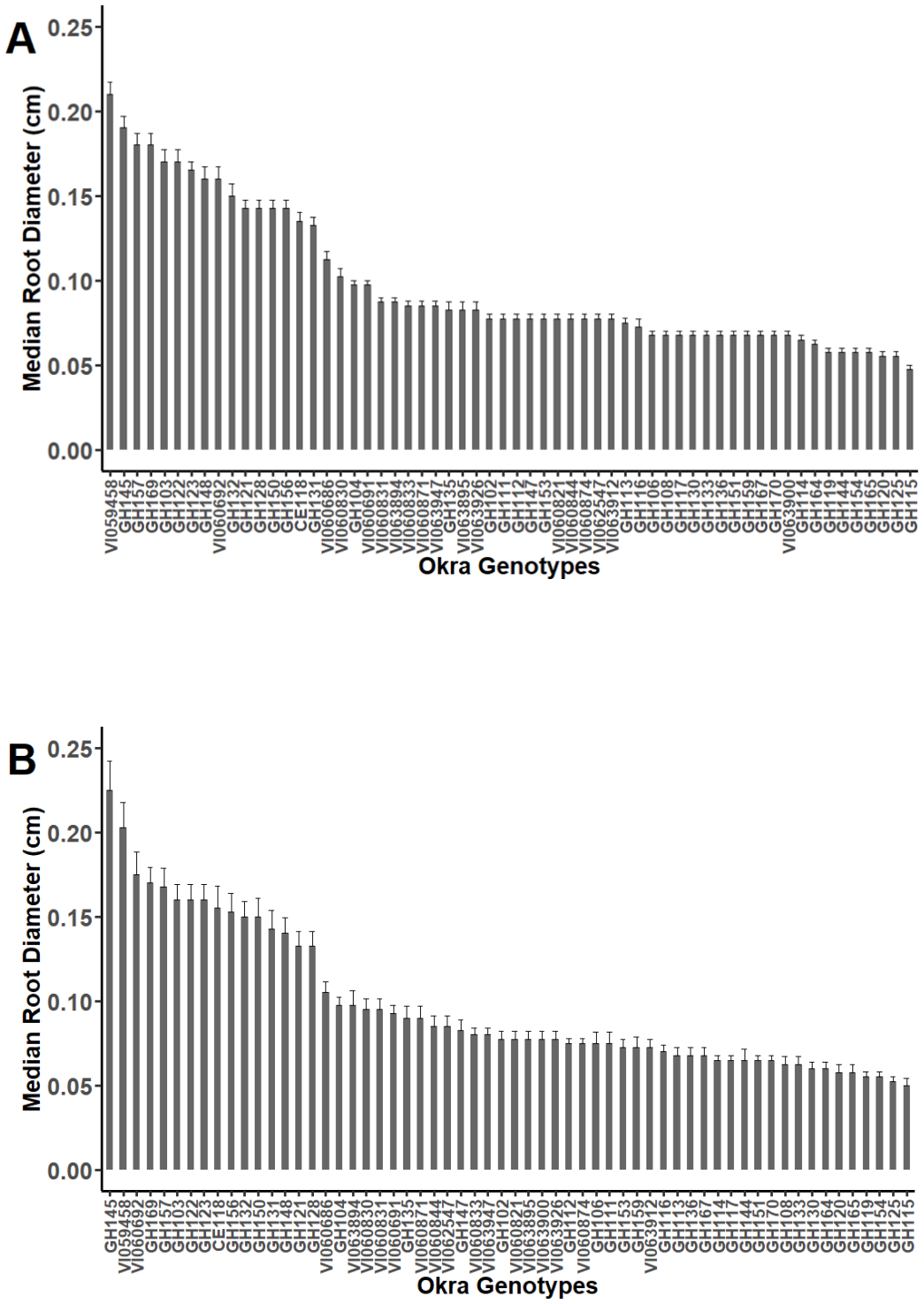

Figure 31: Variation in median root diameter. (A) First trial; (B) Second trial.

Maximum diameter

A significant ($p < 0.001$) difference existed among the genotypes in root Mxd, but no significant ($P > 0.05$) trial and genotype by trial interaction effect was found on Mxd (Figure 32). In the first trial, VI060831 (0.76 cm), VI060686 (0.713 cm), and GH108 (0.693 cm) were the top 5 %, while GH144 (0.268 cm), GH119 (0.27 cm) and GH130 (0.293 cm) were the bottom 5 % (Figure 32). This is a 2.8-fold difference between the highest and the least Mxd. The top 5 % in the second trial consisted of GH108 (0.823 cm), GH157 (0.698 cm), and VI060692 (0.678 cm), whereas the bottom 5 % were GH119 (0.255 cm), GH120 (0.258 cm), and GH144 (0.273 cm) (Figure 33). This also showed a 3.2-fold difference between the highest and least root Mxd.

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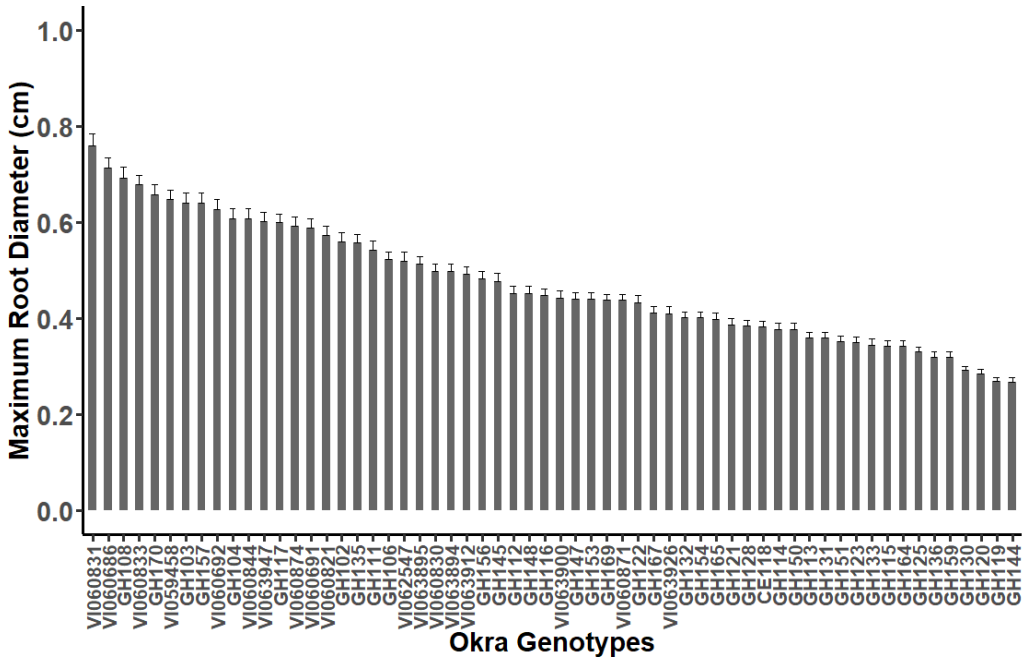

Figure 32: Variation in maximum root diameter in the first trial.

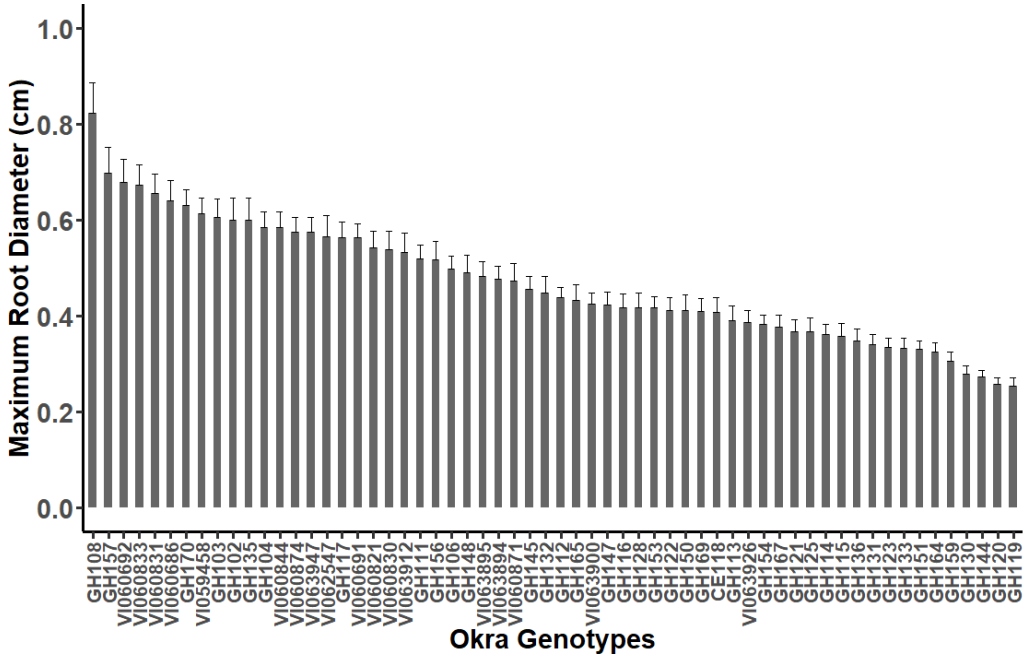

Figure 33: Variation in maximum root diameter in the second trial.

Root volume traits

Three volume traits were assessed (Table 3). Root volume (Vol) ranged from 0.36 cm^3 to 27.19 $cm^{3,}$ with a population average of 8.072 cm^3. The volume diameter ranges varied from 0.75 cm^3 to 5.05 cm^3 for volume diameter range 1 (VDR1) and 0.3 cm^3 to 9.10 cm^3 for volume diameter range 2 (VDR2). The volume traits recorded intermediate to high CVs, ranging from 33 % (VDR1) to 70 % (Vol).

The okra genotypes differed significantly ($p < 0.01$) in root Vol, but the trial and genotype interaction with the trial did not have a significant ($P > 0.05$) effect on Vol (Table 4). The top 5 % consisted of genotype GH111 (25.14 cm^3), GH121 (

23.558 cm^3) and GH157 (18.538 cm^3), while the bottom 5 % were genotype GH136 (2.045 cm^3), GH144 (1.93 cm^3) and VI060821 (1.703 cm^3) in the first trial (Figure 34). In the second trial, genotype GH121, GH111 and GH157 maintained the top 5 % spot with root volume of 24.59 cm^3, 20.80 cm^3 and 19.06 cm^3 respectively, while the bottom 5 % were genotype GH144 (0.67 cm^3), VI060821 (0.90 cm^3), and GH122 (1.83 cm^3) (Figure 35). The greatest Vol differed from the least by 175 % and 189 % in the first and second trials, respectively.

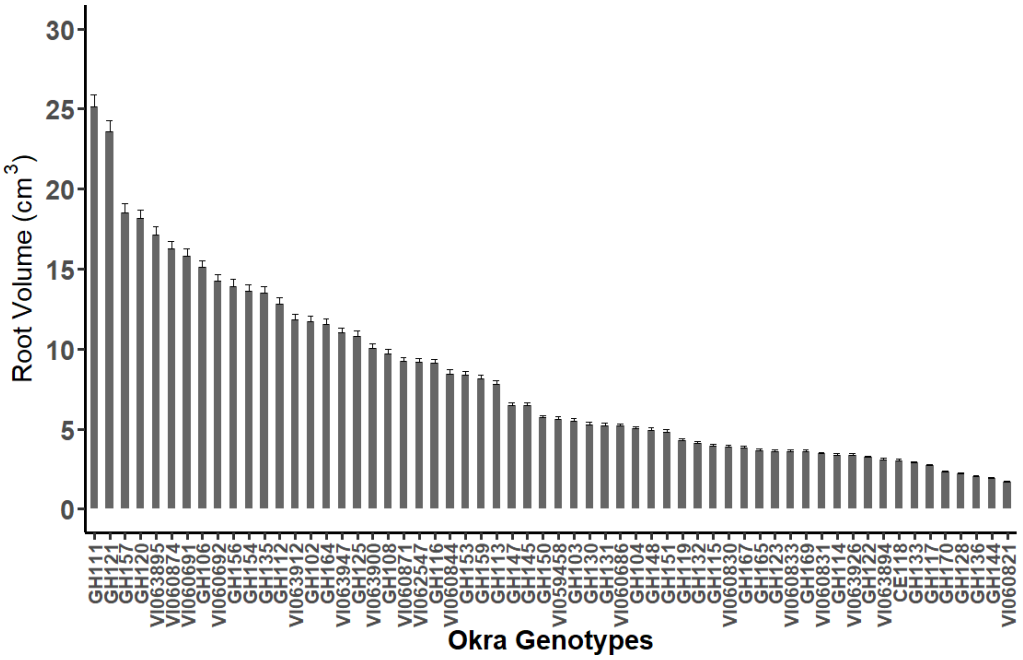

Figure 34: Variation in root volume in the first trial.

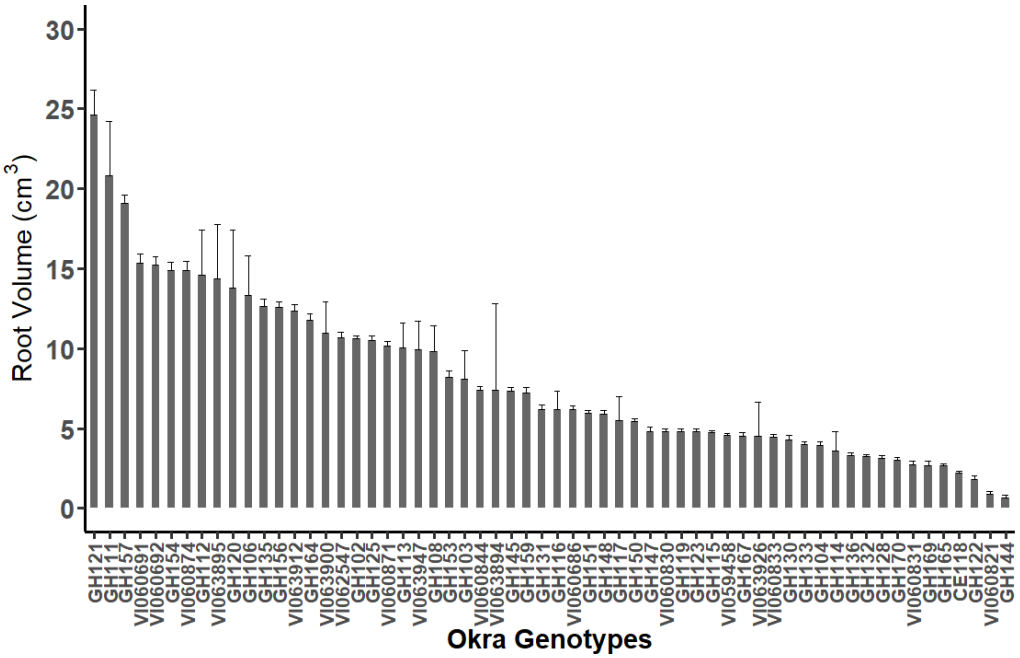

Figure 35: Variation in root volume in the second trial.

A significant ($p < 0.001$) genotypic effect was observed on VDR1, whereas trial and the interaction between genotype and trial did not significantly ($P > 0.05$) influence the trait (Table 4). Genotypes within the top 5 % in the first trial were GH147 (3.94 cm^3), VI060692 (3.885 cm^3) and GH108 (3.87 cm^3), while the bottom 5 % were genotype GH115 (1.163 cm^3), GH128 (1.03 cm^3), and VI060686 (0.937 cm^3) (Figure 36A). The second trial had GH108 (2.198 cm^3), GH106 (3.87 cm^3), and VI063895 (3.723 cm^3) within the top 5 % while GH115 (1.255 cm^3), GH144 (0.973 cm^3) and VI060871 (0.885 cm^3) were the bottom 5 % (Figure 36B). Comparatively, a 123 % and 85 % difference was observed between the highest and the least VDR1 in the first and second trials, respectively.

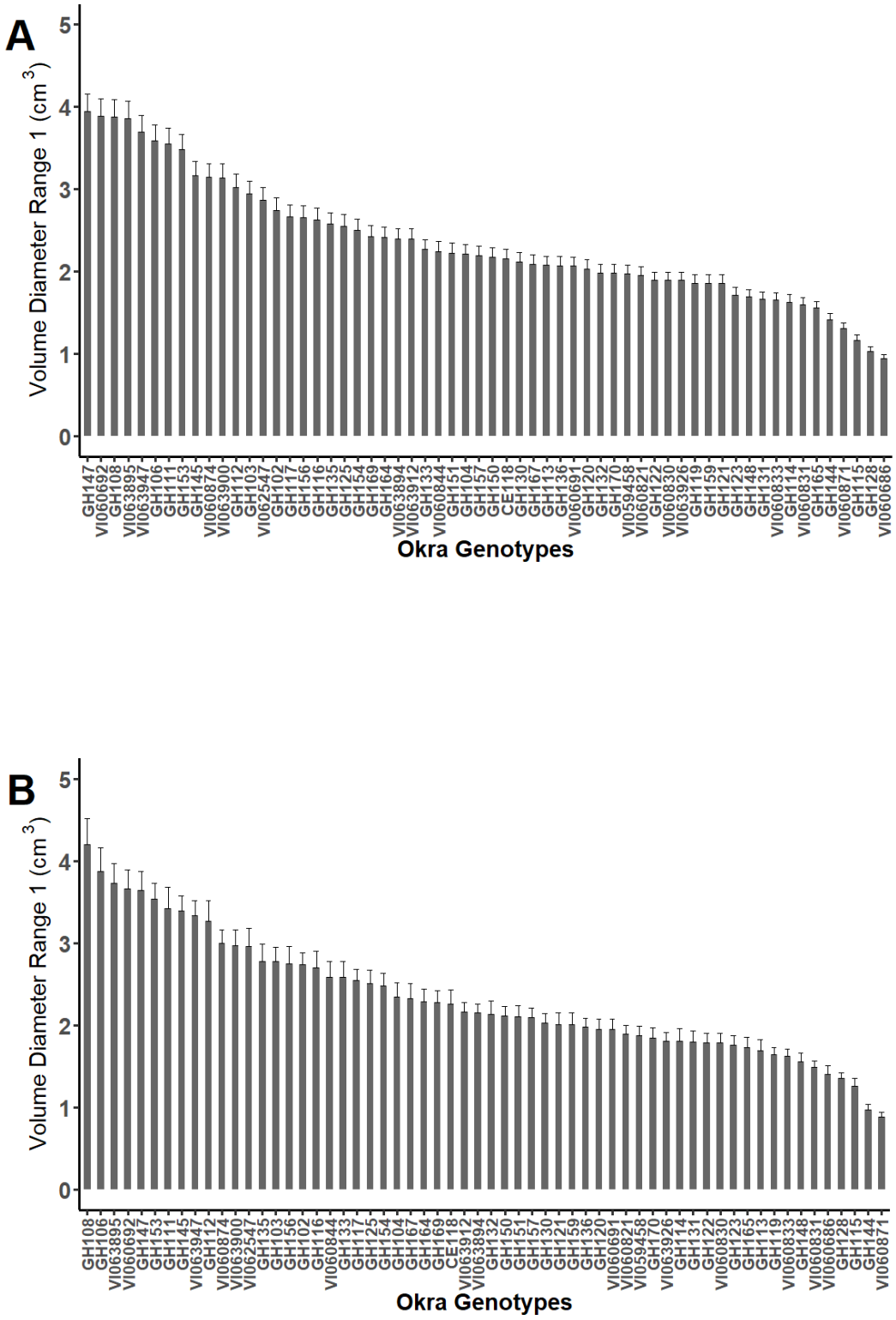

Figure 36: Variation in volume diameter range one. (A) First trial; (B) Second trial.

The okra genotypes varied significantly ($p < 0.001$) in VDR2. On the contrary, there were no significant ($p > 0.05$) trial and genotype-by-trial interaction effects on VDR2 (Table 4). In the first trial, the best 5 % genotypes were GH104 (7.798 cm^3), GH103 (7.503 cm^3), and VI063895 (7.19 cm^3), while the bottom 5 % were GH128 (0.56 cm^3), GH123 (0.4875 cm^3) and GH115 (0.378 cm^3) (Figure 37). In the second trial, genotypes GH103 (7.56 cm^3), GH104 (7.47 cm^3), VI060691, and VI060692 (7.1 cm^3 each) were the most superior, while genotype GH144 (0.41 cm^3), GH131 (0.47 cm^3), and GH128 (0.53 cm^3) were the bottom 5 % (Figure 38). In the first and second trials, the genotypes with the higher VDR2 were superior to those with the lower VDR2 values by 21-fold and 18-fold, respectively.

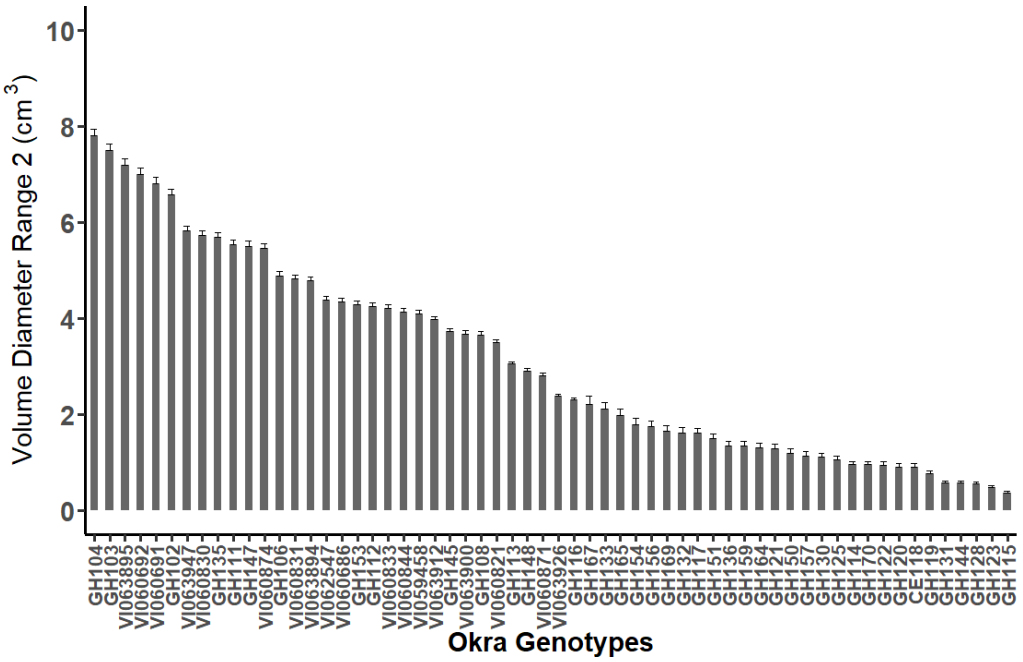

Figure 37: Variation in volume diameter range two in the first trial.

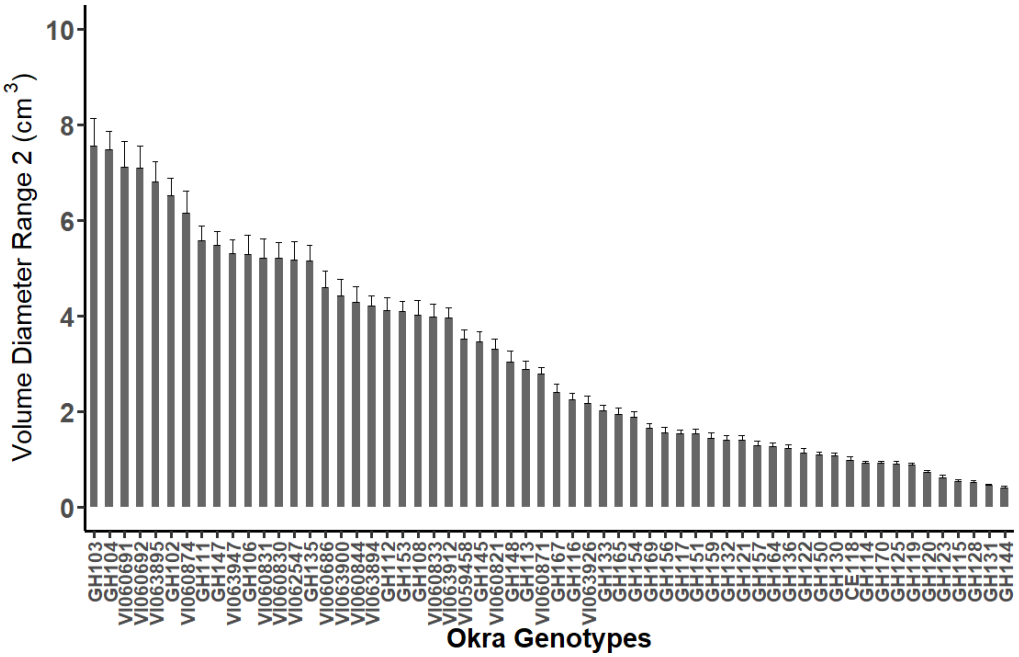

Figure 38: Variation in volume diameter range two in the second trial.

Genetic and phenotypic coefficient of variation and broad-sense heritability

The genetic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) values were classified as low (<10.00 %), moderate (10.00-20.00%) and high (>20.00%) as suggested by Sivasubramanian and Menon (1973). High GCV was observed in 92 % of the traits, ranging from 23.15 % (Ad) to 69.58 % (Vol) (Table 5). Low GCV were measured in Lra and Prl. (Table 5). A similar observation was made for PCV in which all traits recorded high values, ranging from 22.48 % (Ad) to 68.16 % (Bf), barring Lra and Prl, which recorded low PCV each. The heritability values were classified as low (<30.00%), moderate (30.00-60.00%) and high (>60.00%) as suggested by Johnson et al. (1955). Broad-sense heritability estimate was high for all traits assessed, ranging from 62.25 % (Prl) to 99.82 % (Nbp) (Table 5).

Table 5: Estimates of variance components and broad-sense heritability for the 25 traits studied among 60 okra genotypes. Gen: okra genotype; GCV: genetic coefficient of variation; PCV: phenotypic coefficient of variation; H^2: broad-sense heritability.

Trait group	GCV (%)	PCV (%)	H^2 (%)
Biomass traits			
Rdw	44.37	44.16	99.53
Sdw	24.99	24.93	99.75
RS	37.36	37.28	99.79
Root angle trait			
Lra	7.22	6.01	83.27
Root number trait			
Nfol	32.36	31.20	96.40
Nrt	32.98	32.90	99.74
Nbp	62.70	62.58	99.82
Bf	68.65	68.16	99.29
Root length traits			
Prl	4.89	3.04	62.25
Trl	36.14	34.58	95.67
Peri	30.33	28.81	94.97
RLDR1	29.25	27.64	94.51
RLDR2	64.35	63.43	98.56
Root area traits			
Na	39.63	39.42	99.46
Sa	45.48	44.37	97.56
PADR1	30.58	28.91	94.55
PADR2	66.71	65.67	98.44
SADR1	30.40	28.83	94.84
SADR2	66.65	65.71	98.60
Root diameter traits			
Ad	23.15	22.48	97.07
Md	44.27	42.85	96.81
Mxd	27.57	25.85	93.74
Root volume traits			
Vol	69.58	65.29	93.83
VDR1	33.01	31.18	94.46
VDR2	68.74	67.81	98.65

Principal component analysis

The first three principal components (PCs) with an eigenvalue >1 explained 84.7 % of the variations observed in the RSA and biomass traits among the 60 okra genotypes (Table 6). The first PC had an eigenvalue of 11.4 and explained 60 % of the observed variations (Table 6 and Figure 39A). More than half (58 %) of the traits accounted for PC1 (with above-average total contribution), consisting of traits from length, area, volume and number groups (Figure 39C), each contributing positively (Figure Table 6 and Figure 39B). Higher loading scores were obtained by Peri (0.93), Na (0.92), and Sa (0.91) (Table 6 and Figure 39B). The second PC had an eigenvalue of 2.6 and accounted for 13.7 % of the variations (Table 6 and Figure 39A). Eight traits contributed above average to this PC (Figure 39D), four of which had positive contributions (Nrt, Trl, Bf, and Vol). In contrast, the remaining four contributed negatively (VDR2, SADR2, PADR2, and RLDR2) (Table 6 and Figure 39B). The variation explained by PC3 was 11 %. Seven traits had large contributions to this PC, with at least a trait from biomass, area, volume, and number groups (Figure 39E). While three of the traits had large positive contributions (RS, Rdw, and Nfol), four had negative contributions (SADR1, VDR1, PADR1, and Nrt) (Table 6 and Figure 39B). Among all traits included in the PCA, only the diameter trait, Md, failed to contribute above average to any of the significant PCs and was eliminated from subsequent analysis.

Table 6: Loading scores, eigenvalues, percent explained variance and percent cumulative variance for the first five PCs, the first three of which had eigenvalues greater than one.

Trait group	PC1	PC2	PC3	PC4	PC5
Biomass traits					
Rdw	0.77	0.26	0.49	0.02	0.26
RS	0.56	0.06	0.62	0.09	0.42
Root number traits					
Nfol	0.78	0.35	0.40	0.04	-0.01
Nrt	0.70	0.48	-0.36	-0.07	0.06
Nbp	0.80	0.04	0.31	-0.20	-0.12
Bf	0.76	0.41	0.32	-0.04	0.03
Root length traits					
Trl	0.73	0.48	0.16	0.05	-0.33
Peri	0.93	0.02	-0.32	0.01	0.07
RLDR2	0.84	-0.52	0.03	-0.03	-0.05
Root area traits					
Na	0.92	-0.35	-0.15	0.01	-0.02
Sa	0.91	-0.37	-0.09	-0.03	-0.03
PADR1	0.79	0.31	-0.48	0.07	0.13
PADR2	0.84	-0.53	0.07	-0.04	-0.04
SADR1	0.77	0.28	-0.54	0.07	0.10
SADR2	0.84	-0.53	0.06	-0.04	-0.05
Root diameter traits					
Md	-0.08	-0.23	0.06	0.95	-0.01
Root volume traits					
Vol	0.62	0.40	0.22	0.16	-0.44
VDR1	0.82	0.18	-0.48	0.11	0.09
VDR2	0.83	-0.54	0.06	-0.05	-0.05
Eigenvalues	11.4	2.6	2.1	1.0	0.6
Variance (%)	60.0	13.7	11.0	5.4	3.2
Cumulative variance (%)	60.0	73.7	84.7	90.1	93.3

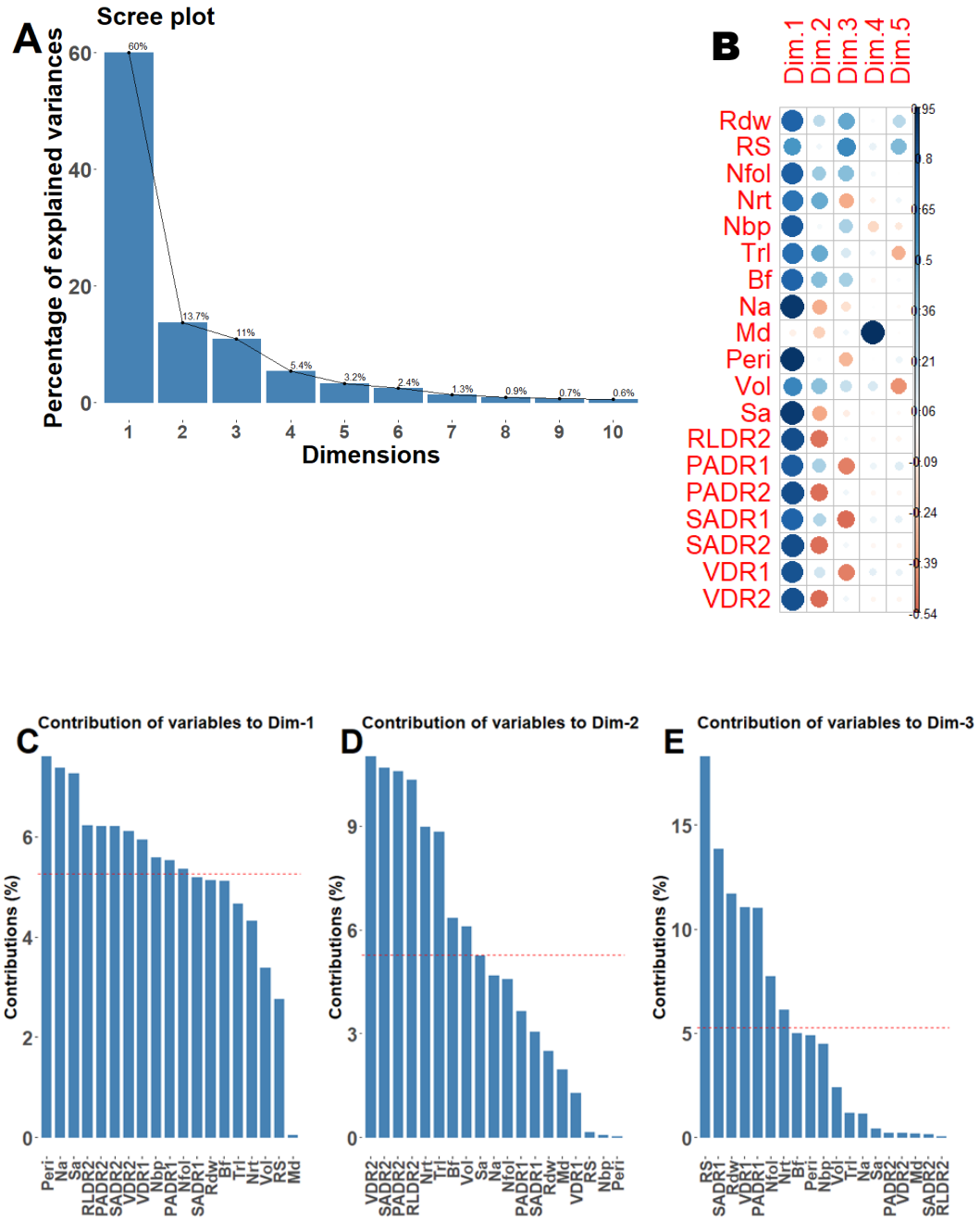

Figure 39: (A) Scree plot of the first ten PCs and their percentage variances; (B) Loading traits scores on the first five PCs, the first three of which had eigenvalues greater than one and were considered significant in PCA. Plots (C) to (E) show the total contribution of variables in accounting for the variability in (C) PC1, (D) PC2, and (E) PC3. The red dashed line on the graph indicates the expected average contribution, and variables with a contribution greater than this expected average were considered important.

The cos^2 (squared coordinates), which connotes the quality of representation of variables, are shown in Figure 40A. Nearly all traits were well represented on PC1, with the top 6 having cos^2 values ranging from 0.71 (SADR2, PADR2, and RLDR2) to 0.87(Peri) (Figure 40A and Appendix 3). However, while RS was best represented on PC3 (cos^2 of 0.38), Md was poorly represented in all three significant PCs (cos^2 of 0.00 to 0.05) (Figure 40A and Appendix 3).

In the biplot of PC1 and PC2, two main groups of positively correlated traits were revealed (Figure 40B). The first group is on the first quadrant and consists of eigenvectors such as Trl, Bf, Rdw, Nfol, Vol, etc. This group also has a positive association with PC1. The second group of positively correlated traits are on the fourth quadrant, including Sa, Na, PADR2, SADR2, VDR2, etc. They are negatively associated with PC2. However, the biplot alluded to a strong negative correlation between the diameter trait, Md, and the majority of traits, especially those on the first quadrant (Figure 40B).

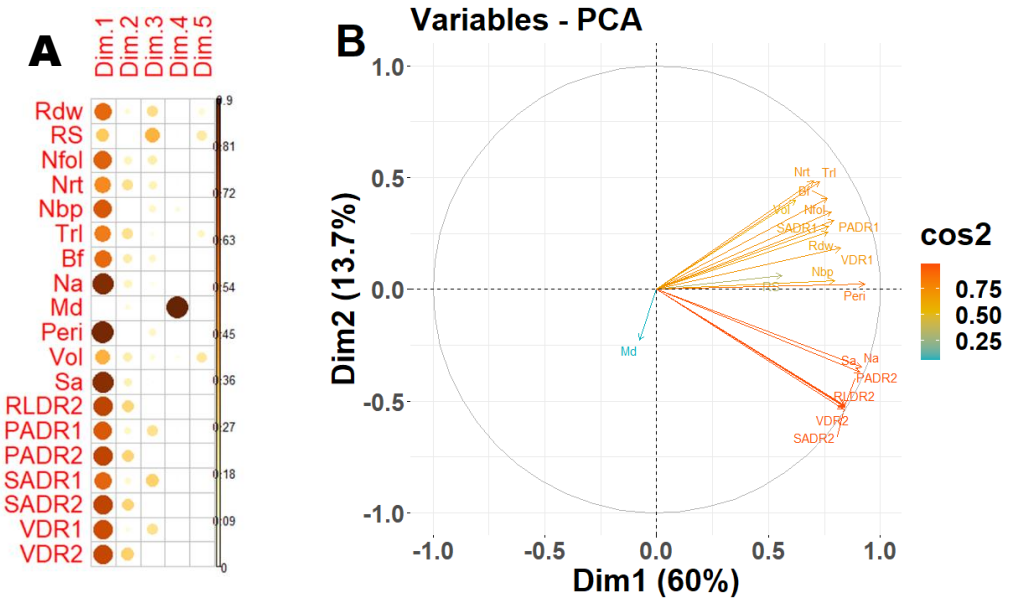

Figure 40: (A) Quality of representation of variables on the factor map for the first five PCs; (B) Variable correlation showing relationships between variables for PC1 and PC2. Variables are coloured by their representation quality on the factor map.

Relationship between measured traits

There was a significant positive correlation between many traits (Figure 41). Among the biomass traits, Rdw strongly and positively (r = 0.83; *P < 0.001*) correlated with RS. Significant ($p < 0.01$ to $p < 0.001$) positive correlation was observed between Trl and all other RSA traits, such as Nrt, Peri, Sa, Na, Nfol, Bf, Nbp, etc., ranging from weak (r = 0.38) to strong (r = 0.8). Similarly, traits such as Rdw, PADR2, Sa, RLDR2, Nfol, Vol, etc. had significant ($p < 0.05$ to $p < 0.001$)) positive (r = 0.32 to 0.99) correlation with all traits that significantly and positively correlated with Trl (Figure 41). Although RS positively correlated with Nrt, VDR1, PADR1, and SADR1, these were weak (r = 0.17 to r = 0.24) and insignificant ($p > 0.05$) (Figure 41).

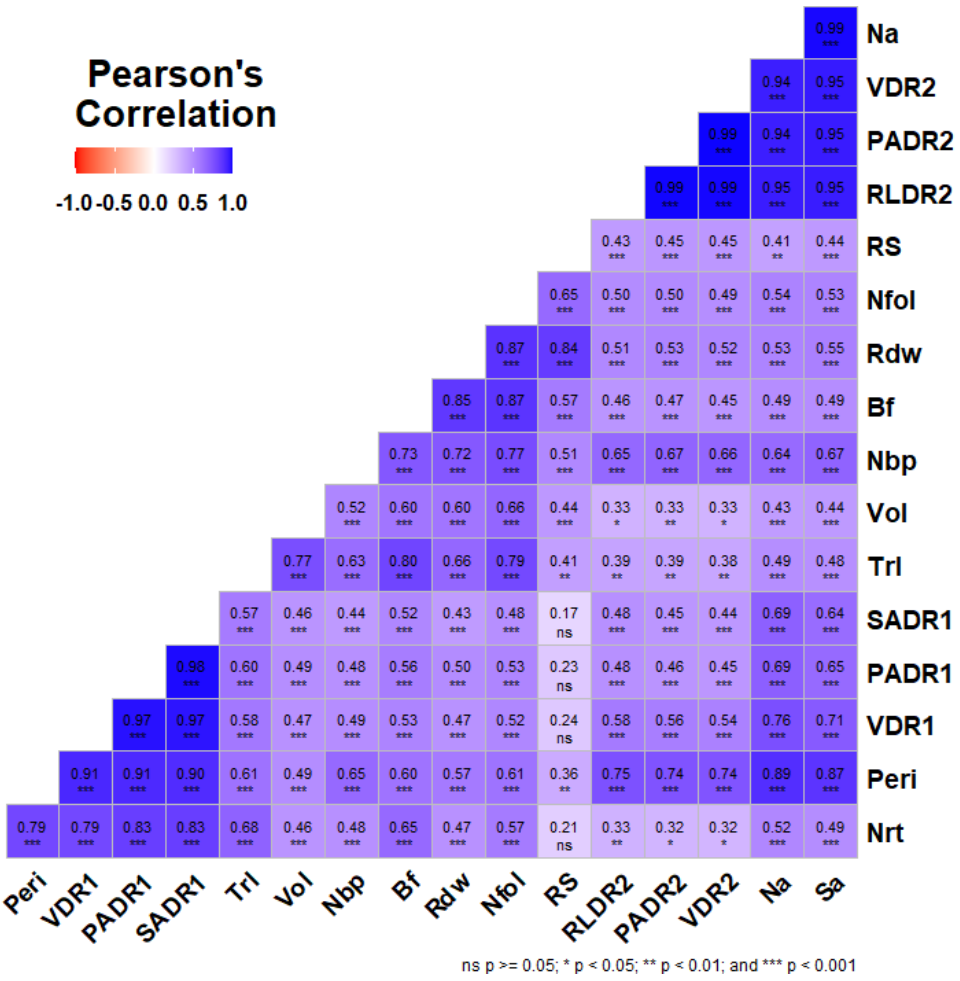

Figure 41: Correlations between RSA and root biomass traits among 60 okra genotypes grown in a soil-filled rhizobox. The scale of colour codes and the box numbers indicate the correlation coefficients between the two traits. The scale is indicated in the bar at the top left corner. A description of "ns, *, **, and ***" is at the matrix's bottom.

Hierarchical clustering

The dendrogram from the cluster analysis suggested a two-cluster solution, with 42 and 18 genotypes in clusters 1 and 2, respectively (Figure 42A). The dendrogram revealed that the okra genotypes were not clustered according to geographical origin, as genotypes from neighbouring African countries were nearly evenly distributed between the two clusters, with nine genotypes resolving in

cluster 1 and seven genotypes in cluster 2. Superimposing the okra genotypes on the PC map suggested that cluster 1 membership was largely negative to PC1, while cluster 2 was largely positive to PC1 (Figure 42B).

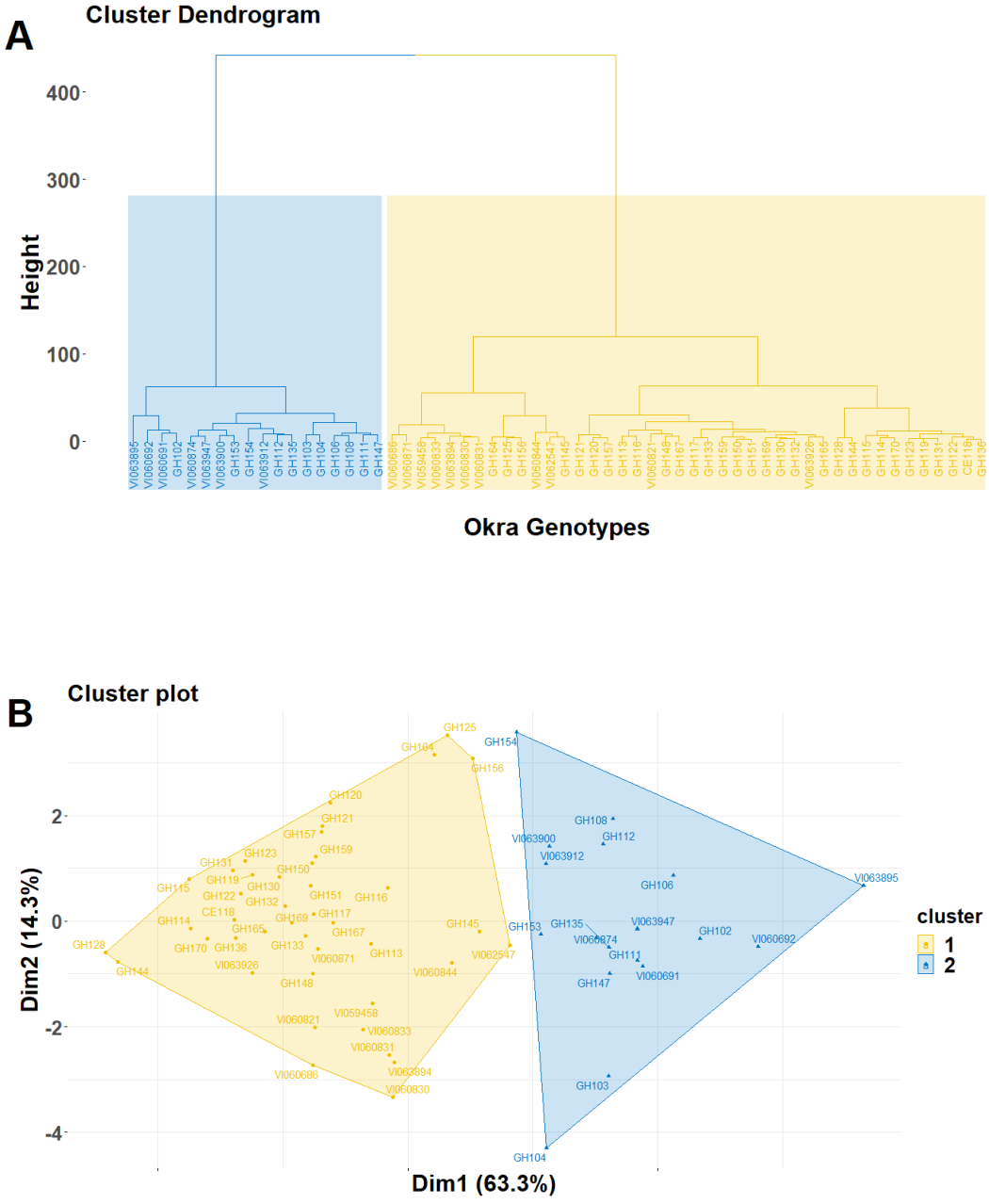

Figure 42: (A) Dendrogram showing clustering patterns of traits among 60 okra genotypes; (B) The different okra genotypes on the PC map grouped and coloured according to their assigned group following cluster analysis.

Second objective: evaluating the effect of drought and the drought-mitigating potential of oil palm EFB biochar on the biochemical indices of selected okra genotypes.

Descriptive data and analysis of variance

The five biochemical indices assessed were proline (Pro), carbohydrate (Carb), salicylic acid (SA), ascorbic acid (AsA), and superoxide dismutase (SOD). These varied from varied from 53.91 ug/g to 972.07 ug/g (Pro), 9.09 mg/100mg to 91.09 mg/100mg (Carb), 1796.67 ppm to 13357.17 ppm (SA), 56.36 mg/100g to 208.37 mg/100g (AsA), and 7.37 ng/g to 125.31 ng/g (SOD), averaging 345.16 ug/g, 51.71 mg/100mg, 5129.96 ppm, 127.92 mg/100g, and 44.12 ng/g each (Table 7). The biochemical traits had low to high CVs. The least CV was observed for AsA (28 %), intermediate for Carb (38 %) and SA (44 %) and high for Pro (63 %) and SOD (65 %).

Table 7: Descriptive statistics for the biochemical traits. Min: minimum value; Max: maximum value; SD: standard deviation; CV: coefficient of variation.

Trait	Acronym	Unit	Mean	SD	Min	Max	CV (%)
Proline	Pro	ug/g	345.16	216.79	53.91	972.07	63
Carbohydrate	Carb	mg/100mg	51.71	19.48	9.09	91.09	38
Salicylic acid	SA	Ppm	5129.96	2233.83	1796.67	13357.17	44
Ascorbic acid	AsA	mg/100g	127.92	35.18	56.36	208.37	28
Superoxide dismutase	SOD	ng/g	44.12	28.48	7.37	125.31	65

Table 8: ANOVA results for the biochemical traits measured among ten selected okra genotypes grown under water deficit conditions and biochar amendment. Gen: genotype; Bio: Biochar; WR: Water-regime.

Trait	F-prob. Gen	F-prob. WR	F-prob. Gen x WR	F-prob. Bio	F-prob. Gen x Bio	F-prob. Gen x Bio x WR
Pro	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
Carb	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
SA	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p>0.05
AsA	p<0.001	p<0.001	p=0.010	p<0.001	p>0.05	p>0.05
SOD	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001

Proline content

The genotypes differed significantly ($p < 0.001$) in Pro production (Table 8). The water regime had a significant ($p < 0.001$) impact on Pro accumulation (Table 8). Pro accumulation was more pronounced under water stress (515.14 ug/g) than ample water (175.18 ug/g), with a 98 % difference between the two water regimes (Figure 43A). Similarly, a significant ($p < 0.001$) difference was observed among the biochar rates in Pro production (Table 8). The highest Pro content was recorded at 0 t/ha (476.38 ug/g) and differed from the 10 t/ha (330.94 ug/g) and 20 t/ha (228.16 ug/g) by 36 % and 70 % each (Figure 43B).

There was a significant ($p < 0.001$) genotype and water regime interaction effect on Pro production (Table 8). At ample water, the greater Pro content was observed in GH112 (261.5 ug/g) and GH121 (229.34 ug/g), while the least was in genotype GH122 (140.81 ug/g) (Figure 43C). With drought imposition, higher Pro accumulation was observed in GH112 (724 ug/g), VI060692 (573.18 ug/g), GH144 (549.50 ug/g), and GH103 (537.23 ug/g) and lower values in GH120 (389.2 ug/g), GH122 (430.36 ug/g), GH121 (467.51 ug/g) and GH150 (479.07 ug/g) (Figure 43C). Comparing each water-stressed genotype with its corresponding well-

watered counterpart, the water-stressed genotypes accumulated more Pro than their well-watered counterparts, ranging from 68 % to 115 % (Figure 43C).

A significant ($p < 0.001$) genotype and biochar interaction effect was observed for Pro content (Table 8). Without biochar amendment (0 t/ha), the greatest Pro content was observed to be 642.45 ug/g in GH112. This was 1.2-fold and 2.1-fold higher than the greatest Pro measured at 10 t/ha (533.31 ug/g in GH112) and 20 t/ha (303.46 ug/g in GH112) respectively (Figure 44A). Overall, GH112 and VI060692 maintained higher Pro content across each biochar rate, whereas GH120 and GH122 consistently measured lower amounts of Pro.

The three interactions of genotype, water regime and biochar had a significant ($p < 0.001$) effect on Pro accumulation (Table 8). Pro content measured at well-watered conditions varied from 182.70 ug/g in GH122 to 338.0 ug/g in GH112 (Figure 44B). The effect elicited more Pro accumulation in all genotypes, with values ranging from 512.81 ug/g in GH120 to 946.90 ug/g in GH112 (Figure 44B). It was observed that Pro content was higher among the drought-stressed crops by a range of 2.3-fold to 3.5-fold (Figure 44B). However, Pro accumulation declined with an increasing biochar application rate during the water deficit.

With 10 t/ha biochar amendment at water deficit, the Pro accumulation varied from 367.03 ug/g in GH122 to 749.52 ug/g in GH112 (Figure 44B). Pro content measured under water deficit with 10 t/ha biochar was observed to be greater by a range of 1.5-fold to 2.6-fold, unlike the 2.3-fold to 3.5-fold observed without biochar amendment. With 20 t/ha biochar amendment at water deficit, Pro content varied from 261.36 ug/g in GH122 to 477.52 ug/g GH112 (Figure 44B).

When each genotype is compared to its well-watered unamended counterpart, the difference in Pro accumulation ranges from 1-fold to 1.9-fold. Again, this was much less than the 2.3-fold to 3.5-fold observed without biochar amendment.

The well-watered crops treated with biochar also recorded lower Pro content than those untreated (Figure 44B). GH112 consistently maintained high Pro content across all water regimes and biochar levels. GH120 was ranked bottom in most instances, while inconsistencies were observed in other genotypes.

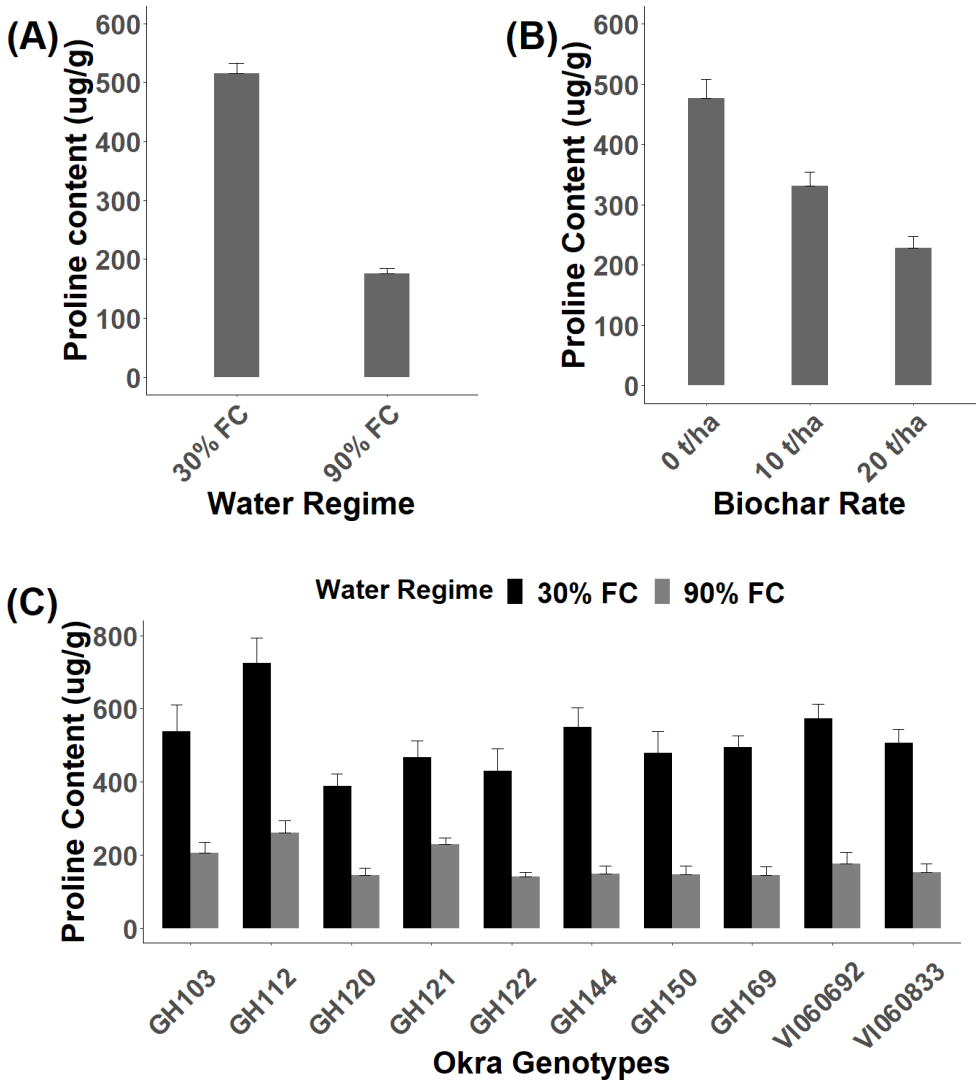

Figure 43: Variation in leaf proline content. (A) Single effect of water regime; (B) of biochar rates; (C) Interaction effect of genotype and water regimes.

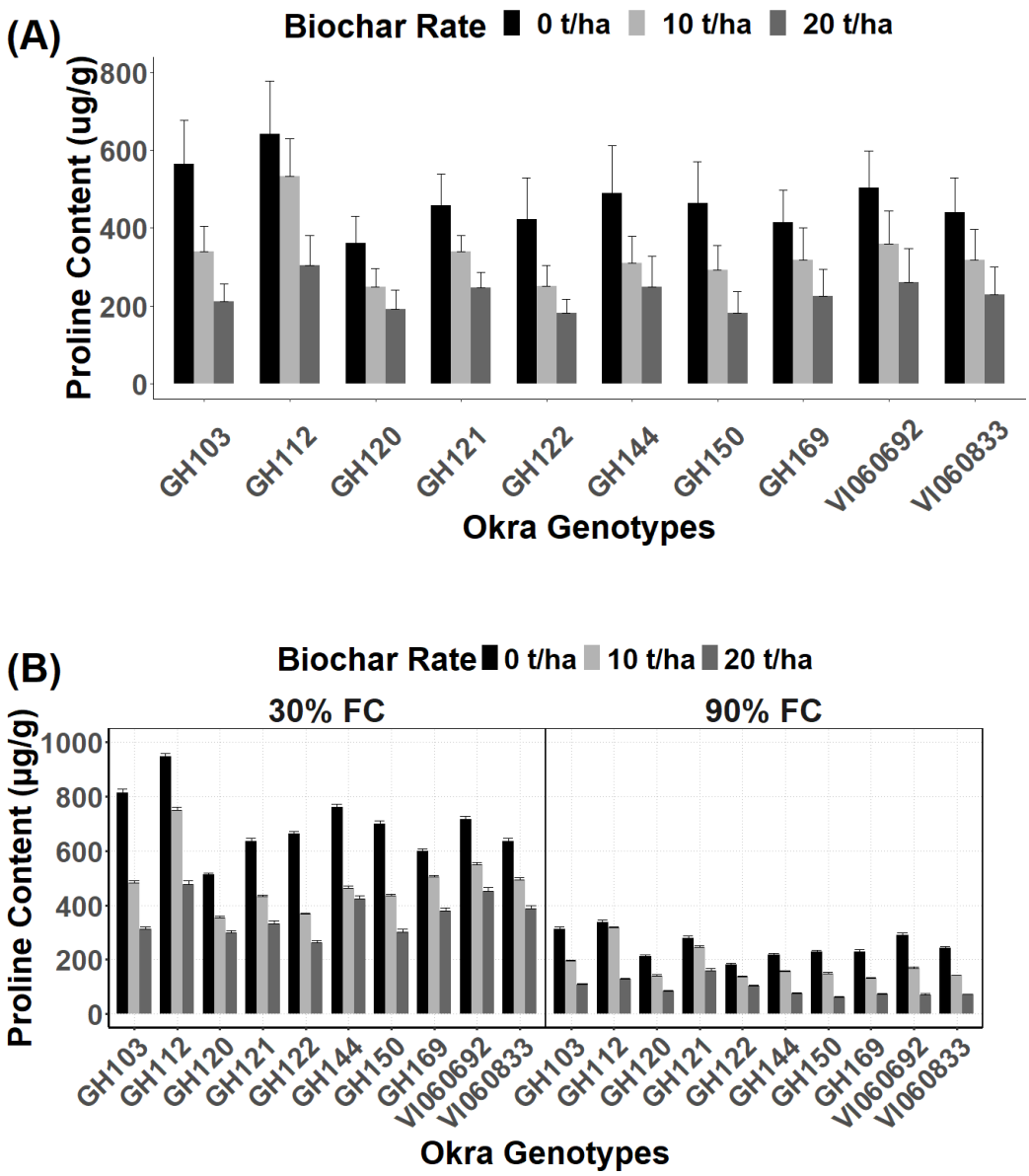

Figure 44: Variation in leaf proline content. (A) Interaction effect of genotype and biochar rates; (B) Interaction effect of genotype, water regime and biochar rates.

Carbohydrate content

The okra genotypes varied significantly ($p < 0.001$) in their Carb content (Table 8). A significant ($p < 0.001$) difference was equally observed between the two water regimes (Table 8). The carb content at water deficit was 67.55 mg/100mg differed from the well-watered (35.86 mg/100mg) treatment by 61 % (Figure 45A).

Biochar amendment significantly ($p < 0.001$) influenced Carb accumulation (Table 8). The largest amount of Carb was measured at 0 t/ha (63 mg/100g) biochar rate, nearly 20 % and 42 % higher than the 10 t/ha (51 mg/100g) and 20 t/ha (41 mg/100g) respectively (Figure 45B).

The interaction between genotype and water regime was significant ($p < 0.001$) for Carb content (Table 8). Crops subjected to water stress accumulated more Carb than their well-watered counterparts, varying from 46 % to 87 % (Figure 45C). The genotype that measured the greatest Carb at water deficit was GH103 (75.25 mg/100mg), while the least was GH122 (61.91 mg/100mg). However, in an ample-water condition, GH112 (43.97 mg/100mg) measured the highest carb content and the lowest carb content, GH169 (27.97 mg/100mg) (Figure 45C).

Genotype and biochar interaction produced a significant ($p < 0.001$) effect on Carb content (Table 8). The 0 t/ha recorded the greatest Carb amount in all genotypes and differed from the 10 t/ha and 20 t/ha by margins ranging from 1.1-fold to 1.4-fold and from 1.3-fold to 2-fold, respectively (Figure 46A). Genotypes which generally maintained high Carb content in response to biochar application were GH103 (56 mg/100mg at 10 t/ha and 52 mg/100mg at 20 t/ha), GH112 (55 mg/100mg at 10 t/ha and 47 mg/100mg at 20 t/ha) and VI060692 (54 mg/100mg at 10 t/ha and 46 mg/100mg at 20 t/ha). In comparison, GH120 (45 mg/100mg at 10 t/ha and 31 mg/100mg at 20 t/ha) and GH122 (46 mg/100mg at 10 t/ha and 34 mg/100mg at 20 t/ha) consistently measured low Carb (Figure 46A).

The three interactions of genotype, water regime and biochar had a significant ($p < 0.001$) effect on Carb accumulation (Table 8). In well-watered

conditions, the highest carb content was obtained by GH121 (58.17 mg/100mg) and the lowest by GH122 (38.65 mg/100mg) (Figure 46B). The remaining genotypes had Carb content in the 42.42 mg/100mg range in GH144 to 51.31 mg/100mg in GH122 (Figure 46B). These values obtained at well-watered conditions markedly increased under drought effect, ranging from 68.72 mg/100mg GH150 to 85.36 mg/100mg VI060833 (Figure 46B). When matched with their respective well-watered genotypes, drought increased Carb in the magnitudes of 1.3-fold to 2-fold. However, the biochar effect reduced Carb accumulation under water deficit at an increasing application rate (Figure 46B).

At 10 t/ha, the Carb content under drought varied from 63.7 mg/100mg in GH144 to 75.1 mg/100mg in GH103 (Figure 46B). Juxtaposing each genotype with its respective unamended well-watered counterpart revealed a difference of about 1.1-fold to 1.7-fold in Carb content, unlike the 1.3-fold to 2-fold observed without biochar application. When biochar was applied at 20 t/ha under water deficit, Carb accumulated 46.98 mg/100mg in GH122 to 68.0 mg/100mg in GH103 (Figure 46B). The amount of Carb accumulated was at par with the well-watered unamended crops in GH120 (52.78 mg/100mg) but higher in the remaining genotypes by 1.1-fold to 1.4-fold, unlike the 1.3-fold to 2-fold previously recorded without biochar amendment (Figure 46B).

Also, within the well-watered crops, unamended groups recorded higher Carb than their respective genotypes amended with biochar (Figure 46B). When matched, 11 % to 69 % and 35 % to 117 % more Carb accumulation in the

unamended group than in groups treated with 10 t/ha and 20 t/ha biochar, respectively (Figure 46B).

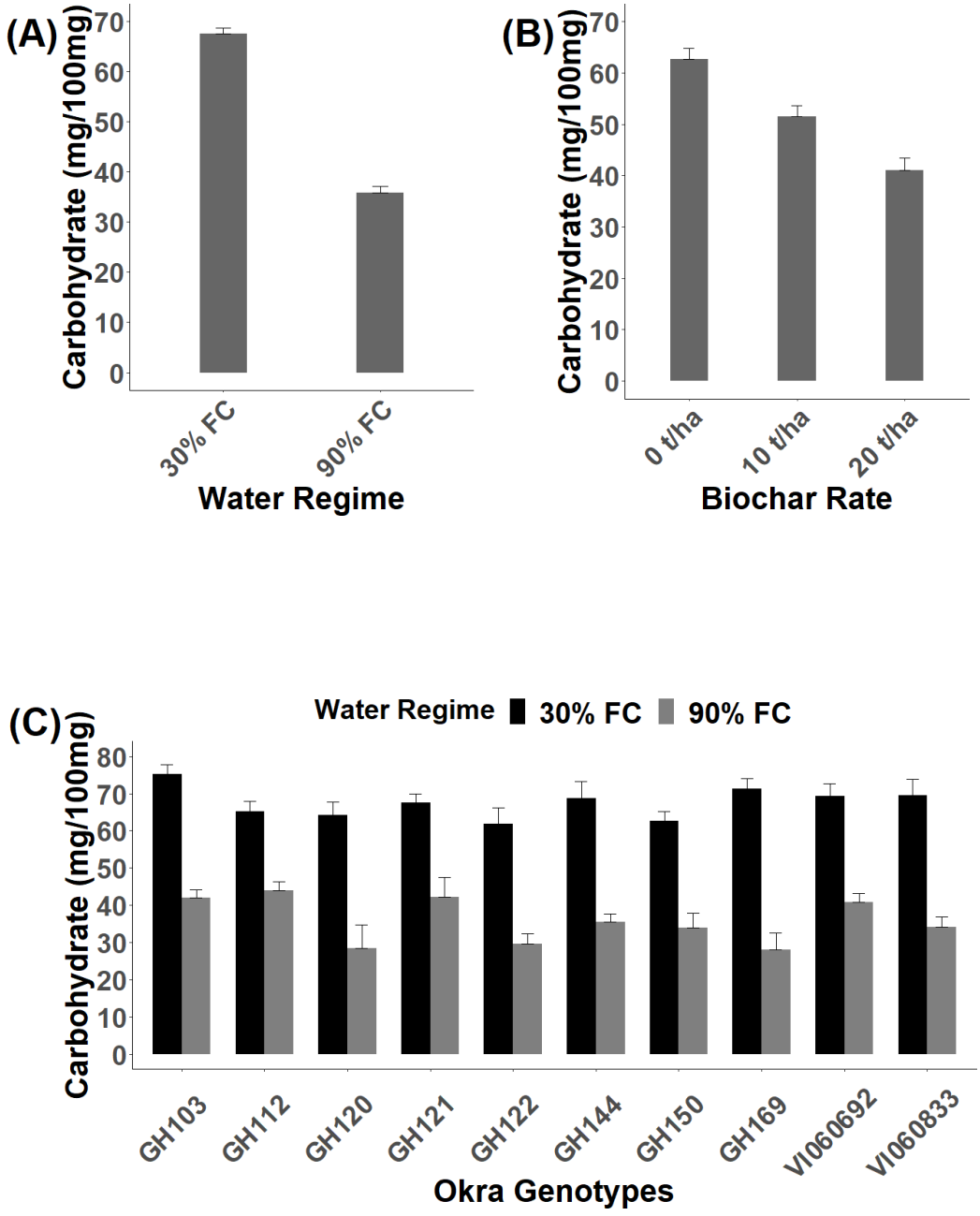

Figure 45: Variation in leaf carbohydrate content. (A) Single effect of water regime; (B) of biochar rates; (C) Interaction effect of genotype and water regimes.

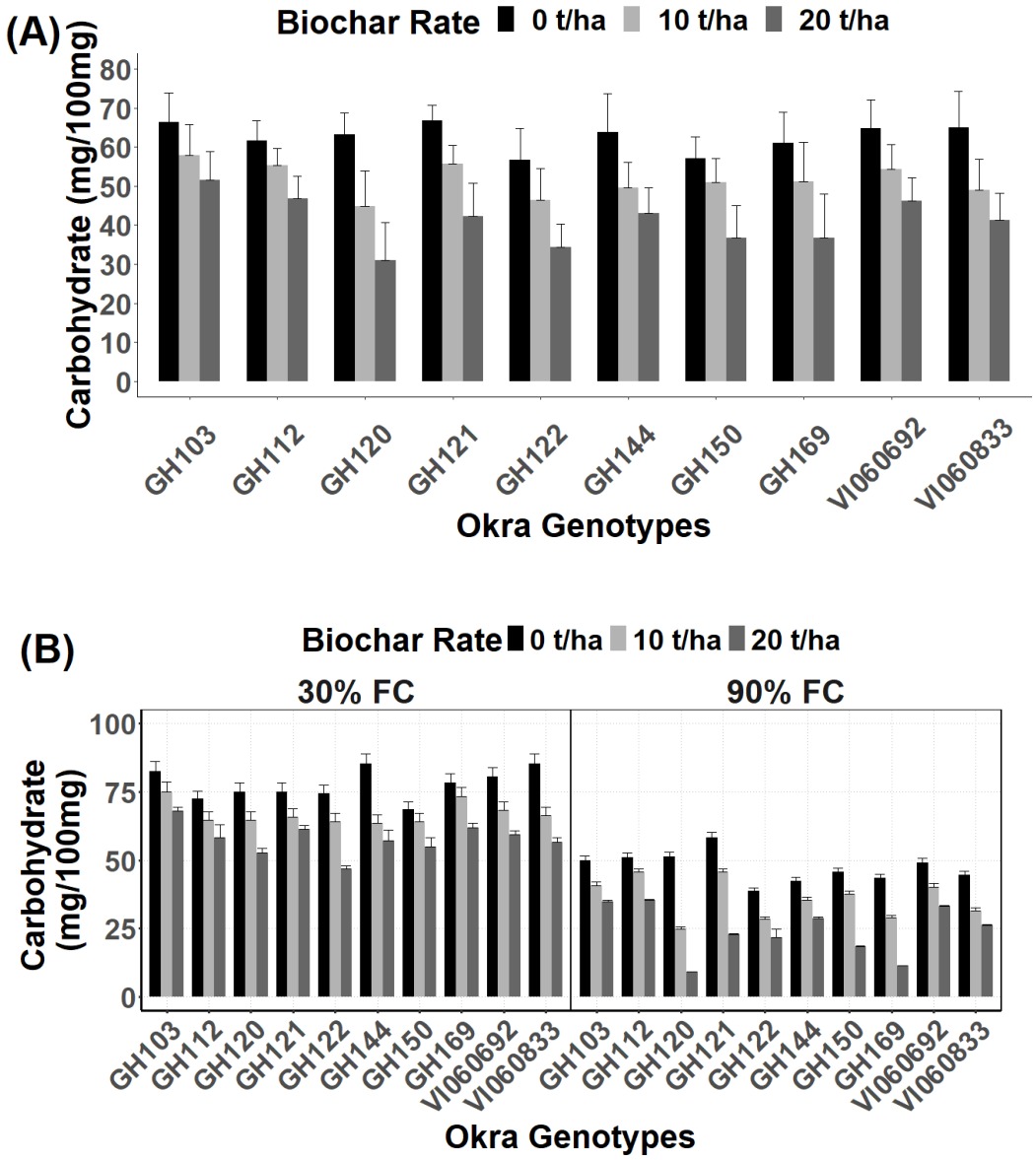

Figure 46: Variation in leaf Carbohydrate content. (A) Interaction effect of genotype and biochar rates; (B) Interaction effect of genotype, water regime and biochar rates.

Salicylic acid activity

There was a significant ($p < 0.001$) difference among the genotypes in SA activity (Table 8). The water regimes significantly ($p < 0.001$) influenced SA activity (Table 8). The highest SA activity occurred under water deficit and was measured to be 64.89 ppm, 2.8-fold greater than the well-watered (23.3 ppm)

condition (Figure 47A). A significant ($p < 0.001$) difference was also observed among the biochar rates in which SA activity at 0 t/ha (64.9 ppm) was 1.6-fold and 2.5-fold higher than the 10 t/ha (41.1 ppm) and 20 t/ha (26.3 ppm) respectively (Figure 47B).

A significant ($p < 0.001$) genotype and water regime interaction was observed for SA activity (Table 8). Each genotype had more (about 2.8-fold) SA activity at water deficit than ample-water conditions (Figure 47C). Genotype VI060692 (75.99 ppm), GH112 (73.71 ppm) and GH103 (70.67 ppm) maintained the higher SA activities, while GH169 (55.47 ppm), GH150 (57.75 ppm) and GH122 (59.27 ppm) had lower values at water deficit (Figure 47C). The remaining genotypes varied from 60.79 ppm in GH120 to 67.63 ppm in VI060833. Similar top and bottom genotypes were observed at ample water conditions, ranging from 19.4 ppm in GH169 to 27.3 ppm in VI060692 (Figure 47C).

Genotype interaction with biochar significantly ($p < 0.001$) influenced SA activity (Table 8). There was a decreasing SA activity with an increasing biochar rate. SA activity at 0 t/ha was higher than the 10 t/ha and 20 t/ha by approximately 1.6-fold and 2.5-fold, respectively (Figure 48A). The highest SA activity was recorded by VI060692 (75.97, 48.15 and 30.85 ppm at 0 t/ha, 10 t/ha and 20 t/ha, respectively), while the least was observed in GH169 (55.46, 35.16, and 22.52 ppm at 0 t/ha, 10 t/ha and 20 t/ha respectively) (Figure 48A). Salicylic acid activity in other genotypes ranged from 36.6 ppm to 46.71 ppm, 36.6 ppm to 46.71 ppm, and 23.44 ppm to 29.92 ppm at 0 t/ha, 10 t/ha and 20 t/ha correspondingly (Figure 48A).

The three interactions of genotype, water regime and biochar did not have a significant ($p > 0.05$) effect on SA activity (Table 8). Salicylic acid activity varied from 83.2 ppm in GH169 to 113.9 ppm in VI060692 among the water-stressed crops and from 27.8 ppm in GH169 to 38.02 ppm in VI060692 among the well-watered crops (Figure 48B). Overall, 3-fold more SA activity was recorded in each genotype under drought stress than in their respective well-watered counterparts (Figure 48B). Biochar application, however, reduced the SA activity across all genotypes at increasing application rates (Figure 48B). Under water deficit, SA activity varied from 46.9 ppm in GH169 to 64.2 ppm in VI060692 among crops treated with 10 t/ha biochar and from 36.4 ppm in GH169 to 49.8 ppm in VI060692 with 20 t/ha biochar amendment (Figure 48B). Compared to their well-watered unamended counterparts, each drought-stressed genotype recorded only 1.7-fold and 1.3-fold more SA activity at 10 t/ha and 20 t/ha biochar, respectively, unlike the 3-fold recorded without biochar amendment.

Also, within crops treated with ample water, more SA activity was observed in crops unamended with biochar. Salicylic acid activity ranged from 27.75 ppm in GH169 to 38.02 ppm in VI060692, 23.43 ppm in GH169 to 32.09 ppm in VI060692 and 8.67 ppm in GH169 to 11.87 ppm in VI060692 with 0 t/ha, 10 t/ha and 20 t/ha biochar amendment, respectively (Figure 48B). Comparatively, there were 1.2-fold and 3.2-fold more SA activities in the well-watered crops unamended with biochar than their corresponding genotypes treated with 10 t/ha and 20 t/ha biochar each (Figure 48B). The general trend was a high SA activity in VI060692, GH112,

GH103 and VI060833, while GH169, GH150, GH122 and GH120 consistently recorded low SA activity across all water regimes and biochar rates.

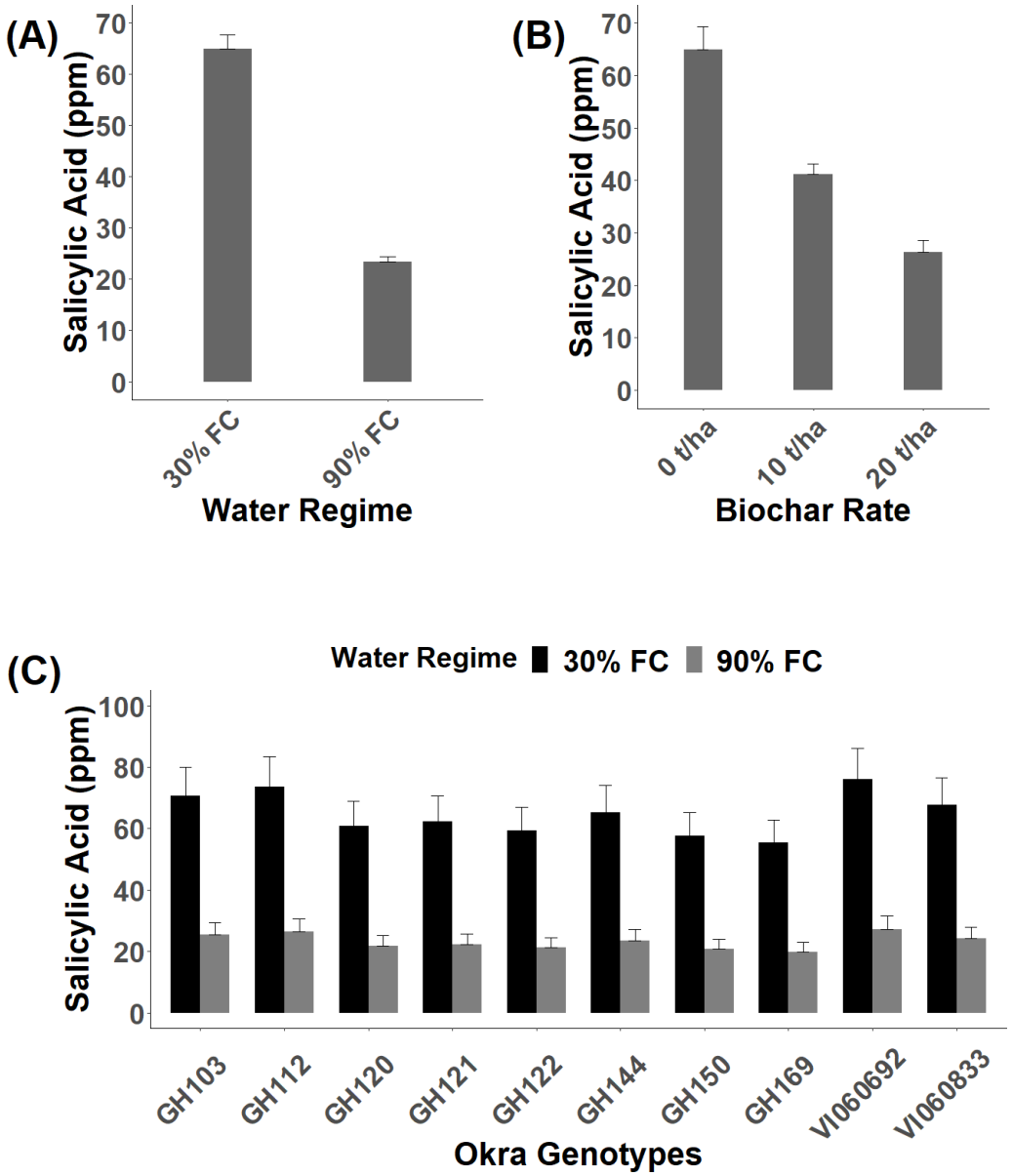

Figure 47: Variation in leaf salicylic acid activity. (A) Single effect of water regime; (B) Single effect of biochar rates; (C) Interaction effect of genotype and water regimes.

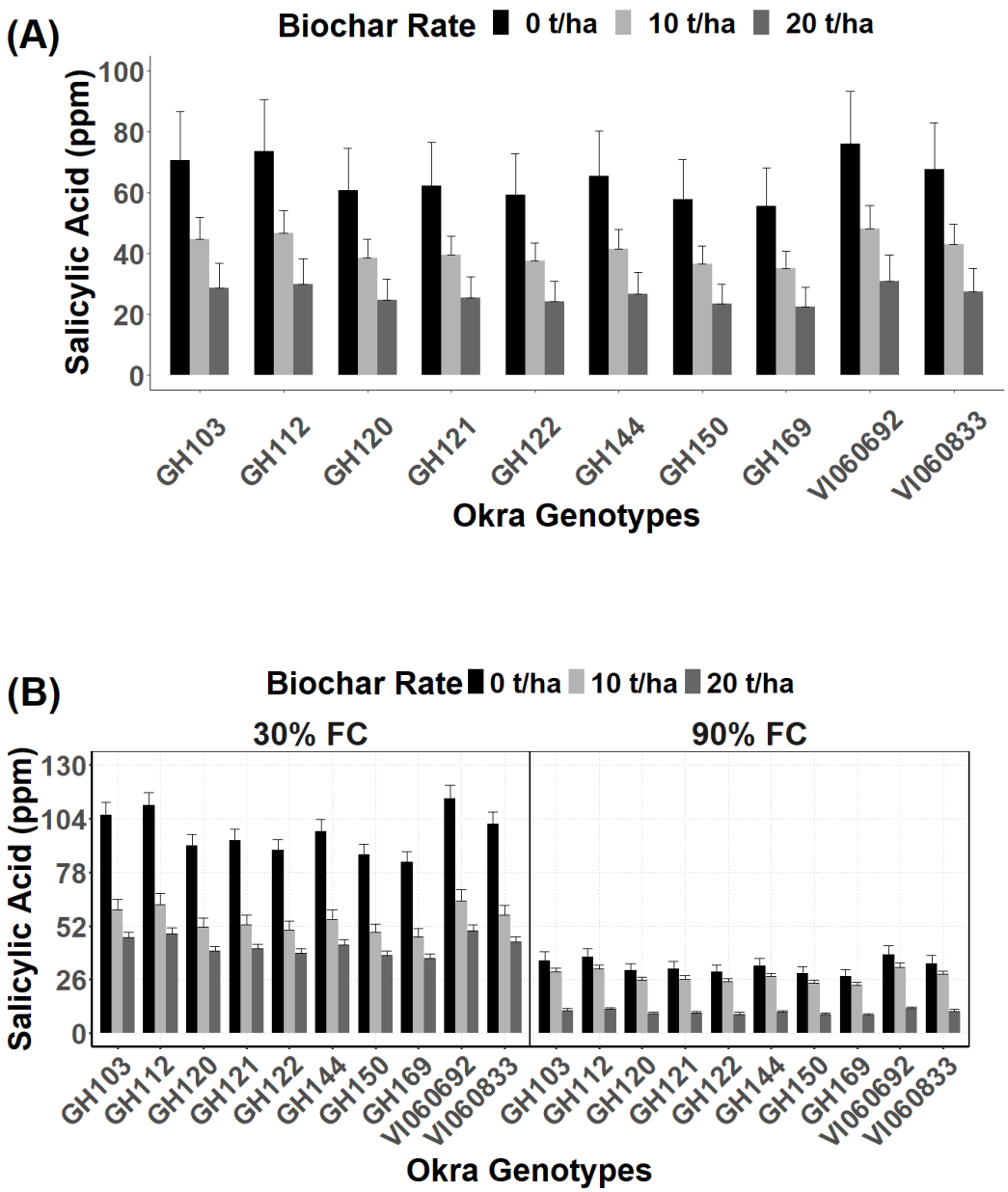

Figure 48: Variation in leaf salicylic acid activity among ten okra genotypes grown in a soil-filled PVC in a greenhouse under water deficit and biochar amendment. (A) Interaction effect of genotype and biochar rates; (B) Interaction effect of genotype, water regime and biochar rates.

Ascorbic acid activity

The genotypes varied significantly ($p < 0.001$) in AsA activity (Table 8). Water regime also had a significant ($p < 0.001$) effect on AsA activity (Table 8).

AsA activity during drought stress (151.8 mg/100g) was 1.5-fold more than in the well-watered (104.1 mg/100g) condition (Figure 49A). Similarly, the various biochar rates significantly ($p < 0.001$) differed in their effects on AsA activity (Table 8). More AsA activity was observed at 0 t/ha (149.9 mg/100g), which differed from the 10 t/ha (124.9 mg/100g) and 20 t/ha (109.0 mg/100g) by approximately 1.2-fold and 1.4-fold respectively (Figure 49B).

There was a significant ($p = 0.01$) interaction effect between genotype and water regime on AsA activity (Table 8). At well-watered conditions, GH112 (112.76 mg/100g), VI060692 (110.50 mg/100g) and GH103 (109.94 mg/100g) had higher AsA activities, while the lower values were measured for GH120 (78.93 mg/100g), GH122 (90.21 mg/100g) and GH169 (106.56 mg/100g) (Figure 49C). These increased markedly under drought stress. At water deficit, greater AsA activity was observed in GH112 (164.42 mg/100g), VI060692 (161.13 mg/100g), and GH103 (160.31 mg/100g), while lower values were observed in GH120 (115.09 mg/100g), GH122 (131.53 mg/100g), and GH169 (155.37 mg/100g) (Figure 49C). Overall, about 1.5-fold more AsA activity was observed in all genotypes under water stress than in the well-watered crops.

There was no significant ($p > 0.05$) interaction effect between genotype and biochar rates on AsA activity (Table 8). AsA activity was greatest at 0 t/ha across all genotypes, greater than the 10 t/ha and 20 t/ha by approximately 1.2-fold and 1.4-fold each (Figure 50A). Across all biochar rates, GH112 had the greatest AsA activity (162.37, 135.30, and 118.08 mg/100g at 0 t/ha, 10 t/ha and 20 t/ha,

respectively), while the least genotype was GH120 (113.66, 94.72, and 82.66 mg/100g at 0 t/ha, 10 t/ha and 20 t/ha respectively).

The three interactions of genotype, water regime and biochar rate did not have a significant ($p > 0.05$) effect on AsA activity (Table 8). The ascorbic acid activity was greatest in GH112 at both water deficit (189.4 mg/100g) and ample-water (135.3 mg/100g) but the least in GH120 at both water deficit (132.6 mg/100g) and ample-water (94.7 mg/100g) too (Figure 50B). The addition of biochar, however, elicited a decline in AsA activity across all genotypes and water regimes with an increasing application rate (Figure 50B).

After 10 t/ha biochar amendment, the highest AsA activity was observed to be 162.4 mg/100g at water deficit and 108.2 mg/100g at ample water in GH112. Meanwhile, the lowest genotype in AsA activity was GH120, measuring 113.7 mg/100g at 30% FC and 75.8 mg/100g at 90% FC (Figure 50B). Similarly, at 20 t/ha biochar rate, GH112 maintained the highest AsA activity, recording 141.5 mg/100g at water deficit and 94.7 mg/100g at ample water. The least AsA activity was 99.0 mg/100g at 30% FC and 66.3 mg/100g at 90% FC, still in GH120. Comparing each genotype with its respective well-watered unamended counterpart revealed that those at water deficit measured marginally 1.2-fold more AsA activity after 10 t/ha biochar amendment but were at par with their respective well-watered genotypes at the back of 20 t/ha biochar amendment (Figure 50B).

It was also observed that, among the well-watered crops, individuals unamended with biochar recorded higher AsA activities than their corresponding genotypes with biochar amendment. In general, in each water regime and biochar

rate, there was a trend of high AsA activity in GH112, VI060692 and GH103, while genotypes GH120, GH122 and GH169 had lower AsA activities (Figure 50B).

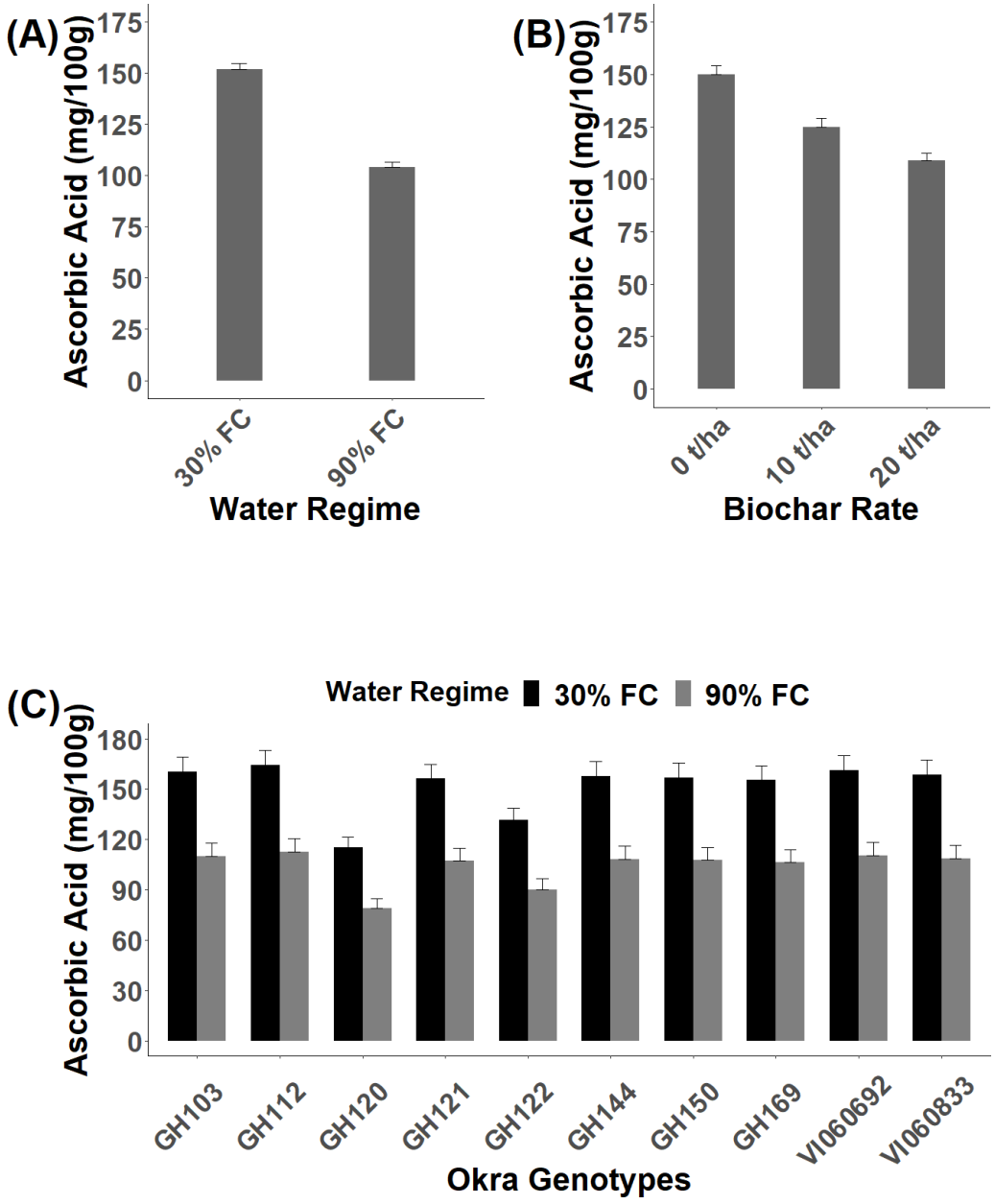

Figure 49: Variation in leaf ascorbic acid activity. (A) Single effect of water regime; (B) Single effect of biochar rates; (C) Interaction effect of genotype and water regimes.

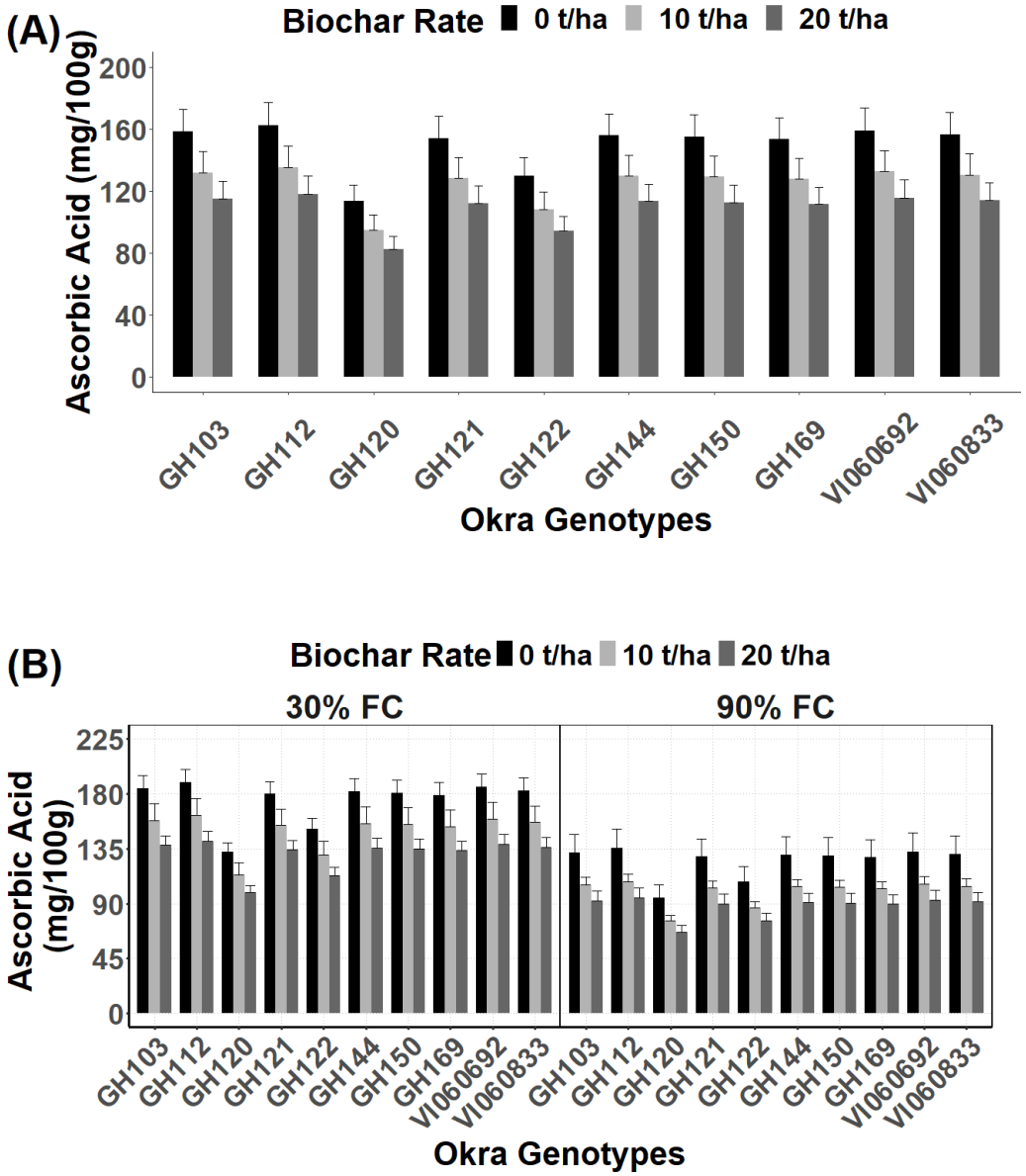

Figure 50: Variation in leaf ascorbic acid activity. (A) Interaction effect of genotype and biochar rates; (B) Interaction effect of genotype, water regime and biochar rates.

Superoxide dismutase activity

There was a significant ($p < 0.001$) difference among the genotypes in SOD activity (Table 8). A significant ($p < 0.001$) difference was also observed between the two water regimes (Table 8). The drought condition elicited more SOD activity

(6713.9 ng/g) than the well-watered condition (3546.0 ng/g) by a difference of 62 % (Figure 51A). However, biochar amendment significantly ($p < 0.001$) effected a decline in SOD activity with increasing application rate (Table 8). The 0 t/ha had the highest SOD activity (6635.5 ng/g) and differed from the 10 t/ha (4866. ng/g) and 20 t/ha (3888.1 ng/g) by 31 % and 22 % each (Figure 51B).

The interaction between genotype and water regime was observed to be significant ($p < 0.001$) for SOD activity (Table 8). More SOD activity was recorded in all genotypes at water deficit than in well-watered conditions. The extent of the difference varied from 1.5-fold to 2.3-fold. In well-watered conditions, GH103 (4287.21 ng/g) had the greatest SOD activities, while the least was recorded in GH120 (2878.92 ng/g) (Figure 58C). At water deficit, SOD activity markedly increased and was recorded to be greatest in VI060692 (8212.22ng/g), while GH122 (5495.33 ng/g) and GH120 (5862.33 ng/g) had lower values (Figure 51C).

Genotype and biochar interaction had a significant ($p < 0.001$) effect on SOD activity (Table 8). The highest SOD activity at 0 t/ha was obtained by GH112 (8469.39 ng/g). But at 10 t/ha and 20 t/ha biochar rates, the highest SOD activity was measured in VI060833 to be 5628 ng/g and 4538.33 ng/g respectively. The least SOD activity was recorded in GH122 (5322.53 ng/g) at 0 t/ha and in GH120 at both 10 t/ha (4155.17 ng/g) and 20 t/ha (2860 ng/g) (Figure 52A). Overall, More SOD activity was observed in all genotypes at 0 t/ha biochar rate and differed from the 10 t/ha and 20 t/ha by a range of 1.1-fold to 1.7-fold and 1.5-fold to 2.2-fold each (Figure 52A).

The three interactions of genotype, water regime and biochar rate were significant for SOD activity (Table 8). Water deficit produced the greatest SOD activity in GH112 (12483.3 ng/g) and was 74 % higher than the greatest SOD activity measured in VI060833 (5728.0 ng/g) at well-watered condition (Figure 52B). When compared to their respective well-watered genotypes, SOD activity was more pronounced under drought in all genotypes by margins ranging from 1.4-fold to 2.8-fold (Figure 52B). SOD activity was, however, reduced with increasing rate of biochar application.

With 10 t/ha biochar application, SOD activity was greatest in VI060692 (7610 ng/g) at 30% FC and GH121 (4596.7 ng/g) at 90% FC (Figure 52B) but the least in GH120 (5207.0 ng/g) at 30% FC and GH144 (2080.0 ng/g) at 90% FC (Figure 52B). When biochar was amended at 20 t/ha rate, SOD activity was greatest in VI060833 (6623.3 ng/g) at 30% FC and GH144 (3580.0 ng/g) at 90% FC (Figure 52B) but the least in GH120 (3520.0 ng/g) at 30% FC and GH169 (1996.7 ng/g) at 90% FC (Figure 52B). When each genotype was matched with its corresponding unamended well-watered counterpart, SOD activity at water deficit was higher by a range of 1.2-fold to 1.7-fold and 1.1-fold to 1.2-fold when biochar was applied at 10 t/ha and 20 t/ha each. This was substantially less than the 1.4-fold to 2.8-fold difference observed without biochar amendment.

Among the well-watered crops, too, the group unamended with biochar recorded more SOD activities than their corresponding genotypes treated with biochar. In all genotypes, the well-watered crops unamended with biochar recorded

7 % to 74 % and 23 % to 80 % more SOD activities than their corresponding genotypes amended with 10 t/ha and 20 t/ha biochar, respectively (Figure 52B).

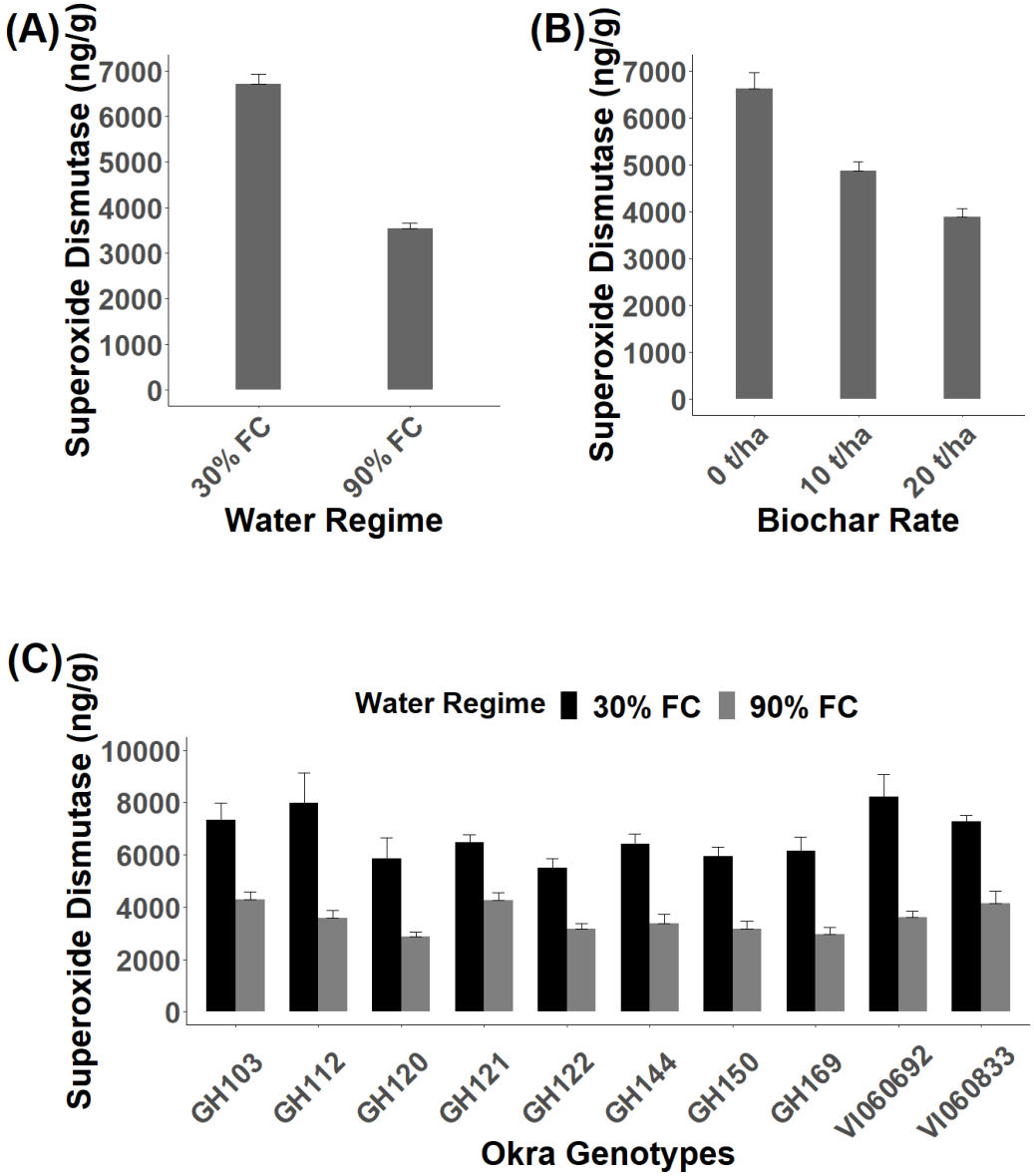

Figure 51: Variation in leaf superoxide dismutase activity. (A) Single effect of water regime; (B) Single effect of biochar rates; (C) Interaction effect of genotype and water regimes.

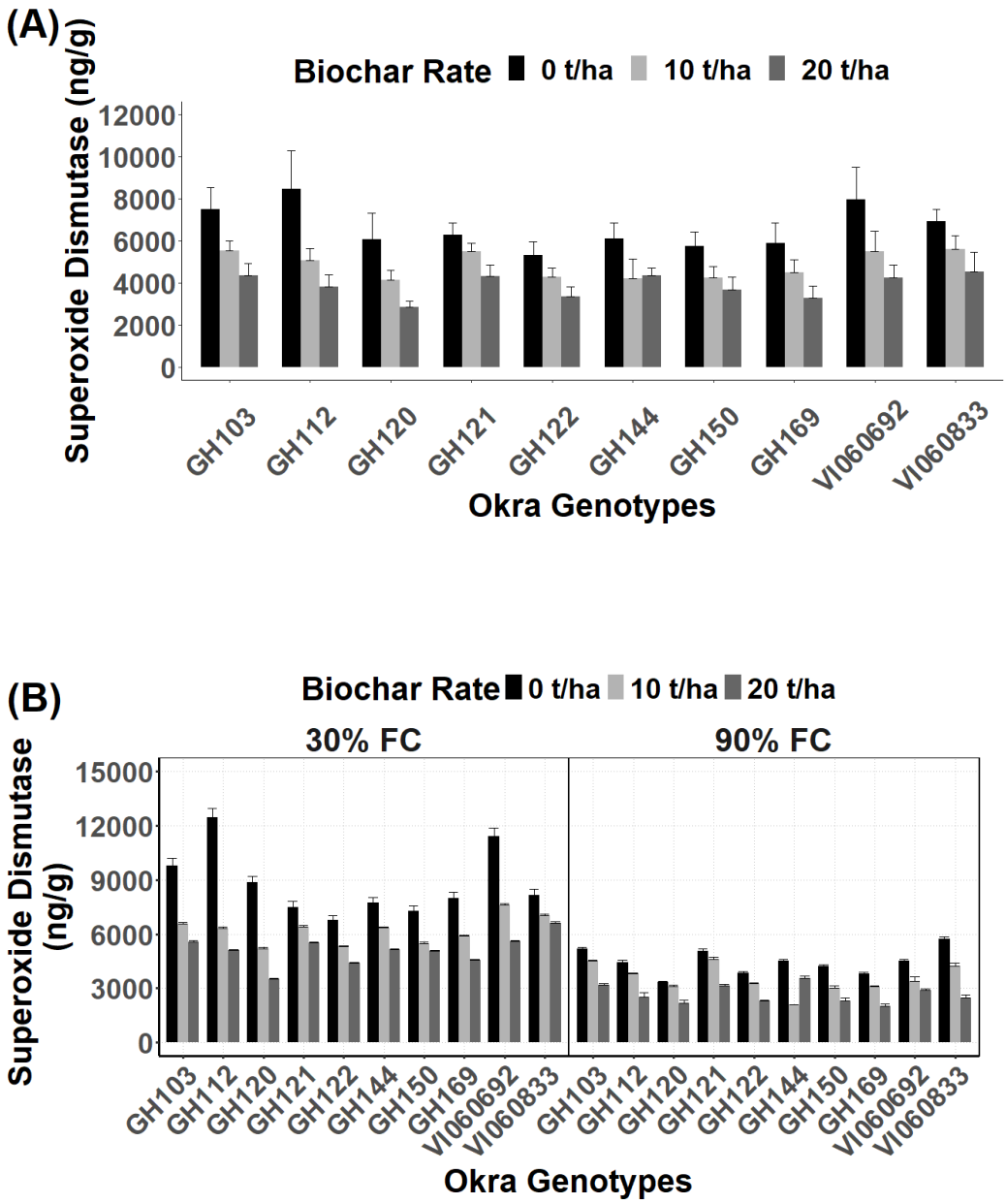

Figure 52: Variation in leaf superoxide dismutase activity. (A) Interaction effect of genotype and biochar rates; (B) Interaction effect of genotype, water regime and biochar rates.

Third objective: evaluating the effect of drought and the drought-mitigating potential of oil palm EFB biochar on the yield of selected okra genotypes.

Descriptive data and analysis of variance

Data was collected on four yield indices, including pod diameter (Pd), pod length (Pl), number of pods per plant (Npp) and total pod yield (Tpy). The range for the four yield traits was 0.23 cm to 2.73 cm for Pd, 0.90 cm to 13.27 cm for Pl, 1.00 to 20.00 for Npp, and 0.02 t/ha to 4.35 t/ha for Tpy, with an average of 1.28 cm, 5.67 cm, 5.85, and 1.25 t/ha respectively (Table 9). The CVs were intermediate (for Pd and Pl) to high (for Npp and Tpy) (Table 9).

Pod diameter

The genotype, trial, and their interaction effect were significant ($p < 0.001$) for Pd (Table 10). Also, both the single effect of the water regime and its interaction with the trial were significant ($p < 0.001$) for Pd (Table 10). The well-watered (90% FC) crops had Pd of 1.60 cm in the first trial and 1.70 cm in the second trial, whereas the water-stressed (30% FC) crops recorded 1.0 cm in the first trial and 0.89 cm in the second trial (Figure 53A). This was a 1.8-fold and 1.9-fold difference between the two water regimes in the first and second trials, respectively. Similarly, biochar had a significant ($p < 0.001$) effect on Pd, but the interaction effect of biochar and trial was not significant ($p > 0.05$) (Table 10). The 20 t/ha biochar measured the largest Pd of 1.51 cm and 1.55 cm in the first and second trials each. These were greater than the Pd measured at 10 t/ha (1.25 cm in the first trial and 1.29 cm in the

second trial) and 0 t/ha (1.02 cm in the first trial and 1.05 cm in the second trial) by about 1.2-fold and 1.5-fold, respectively (Figure 53B).

The interaction between genotype and water regime, as well as their interaction with the trial, was significant ($p < 0.001$) for Pd (Table 10). Among the well-watered crops, larger Pd were recorded in GH169 (2.23 cm), GH122 (2.12 cm) and VI060833 (1.92 cm), while lower values were measured for GH120 (1.10 cm), GH150 and GH121 (1.20 cm each) in the first trial (Figure 53C). The second trial had GH122 (2.30 cm), GH169 (2.22 cm) and VI060833 (2.02 cm) recording larger Pd while GH112 (1.3 cm), GH150 and GH120 (1.20 cm each) measured lower values (Figure 53C). These values were significantly ($p < 0.001$) reduced among the drought-stressed crops. At water deficit, the top-ranked genotypes measured 1.40 cm in GH169, 1.32 cm in GH122 and 1.12 cm in VI060833, while the bottom-ranked genotypes recorded 0.43 cm (GH120), 0.64 cm (GH112), and 0.73 cm (GH150) in the first trial (Figure 53C). A similar trend of top and bottom genotypes was observed in the second trial in which GH122 (1.40 cm), GH169 (1.30 cm) and VI060833 (1.10 cm) were the top 30 % genotypes, while GH120 (0.42 cm), GH121 (0.63 cm) and GH150 (0.71 cm) were the bottom 30 % (Figure 53C). Generally, matching each genotype at water deficit with its control counterpart revealed that the drought effect reduced Pd by 34 % to 87 % in the first trial and 43 % to 97 % in the second trial.

A significant ($p < 0.001$) genotype and biochar interaction effect was observed for Pd, but no significant ($P > 0.05$) interaction was observed between genotype, biochar and trial (Table 10). Biochar application substantially increased

Pd in all genotypes with an increasing application rate relative to their counterparts in unamended soil (Figure 54A). Genotype GH169 obtained the highest Pd across the different biochar rates in the first trial, with 18 % and 31 % differences between the largest Pd obtained in the 20 t/ha (2.10 cm) and those of 10 t/ha (1.77 cm) and 0 t/ha (1.54 cm) respectively. Similarly, in the second trial, the largest Pd was obtained by GH122 across all biochar rates, and there were 21 % and 40 % differences between the largest Pd measured at 20 t/ha (2.15 cm). The largest Pd obtained at 10 t/ha (1.81 cm) and 0 t/ha (1.57 cm) respectively (Figure 54A). The remaining genotypes had Pd varying from 0.97 cm in GH120 to 2.02 cm in GH122, 0.73 cm in GH120 to 1.68 cm in GH122, and 0.61 cm in GH120 to 1.46 cm in GH122 at 20 t/ha, 10 t/ha and 0 t/ha biochar respectively in the first trial. In the second trial, Pd in the remaining genotypes varied from 1.02 cm in GH120 to 2.07 cm in GH169, 0.77 cm in GH120 to 1.73 cm in GH169 and 0.64 cm in GH120 to 1.49 cm in GH169 at 20 t/ha, 10 t/ha and 0 t/ha respectively. When each genotype was matched with its counterparts at various biochar rates, Pd among the 20 t/ha biochar differed from the 10 t/ha and 0 t/ha by amounts ranging from 1.1-fold to 1.3-fold and 1.4-fold to 1.6-fold respectively in each trial (Figure 54A).

The interaction between genotype, water regime and biochar was significant ($p < 0.001$) for Pd, but their interaction with the trial was not significant ($p > 0.05$) (Table 10). The well-watered crops recorded longer Pd than the water-stressed crops in all genotypes by about 1.6-fold to 3.5-fold in the first trial and 1.8-fold to 4.2-fold in the second trial (Figure 54B-C). Biochar application, however, offsets

drought impact in all genotypes. The drought-mitigating potential of biochar increased with increasing application rate in the order of 20 t/ha > 10 t/ha > 0 t/ha.

At water deficit, Pd in the first trial ranged from 0.33 cm in GH120 to 1.18 cm in GH169, 0.34 cm GH120 to 1.40 cm in GH169, and 0.62 cm GH120 to 1.61 cm in GH169 when biochar was applied at 0 t/ha, 10 t/ha and 20 t/ha respectively. These observations did not differ significantly ($p > 0.05$) in the second trial as Pd varied at water deficit from 0.29 cm for GH120 to 1.14 cm for GH122, 0.34 cm for GH120 to 1.40 cm for GH122 and 0.62 cm for GH120 to 1.61 cm for GH122 with 0 t/ha, 10 t/ha and 20 t/ha biochar amendment respectively (Table 8 and Figure 54B-C). These suggested that when matched with their corresponding unamended well-watered genotypes, Pd at water deficit differed only by a marginal range of 1-fold to 1.4-fold at 20 t/ha biochar, but greatly by a range of 1.3-fold to 2.6-fold at 10 t/ha, in each trial (Figure 54B-C).

Among the well-watered crops, groups amended with biochar also recorded larger Pd than their corresponding genotypes without biochar amendment (Figure 54B-C). At well-watered condition, Pd ranged from 0.89 cm in GH120 to 1.91 cm in GH169, 1.11 cm in GH120 to 2.11 cm in GH169 and 1.31 cm in GH120 to 2.59 cm in GH169 at 0 t/ha, 10 t/ha and 20 t/ha biochar rates respectively in the first trial. The ranges were 0.99 cm in GH120 to 2.00 cm in GH122, 1.20 cm in GH120 to 2.21 cm in GH122, and 1.41 cm in GH120 to 2.69 cm in GH122 at 0 t/ha, 10 t/ha and 20 t/ha respectively in the second trial. The okra genotypes treated with 20 t/ha and 10 t/ha biochar were superior to their unamended counterparts by 1.3-fold to 1.5-fold and 1.1-fold to 1.3-fold, respectively, in each trial. Generally, irrespective

of the water regime, biochar rate and trial, GH169, GH122 and VI060833 had larger Pd, while GH120 consistently recorded the least Pd.

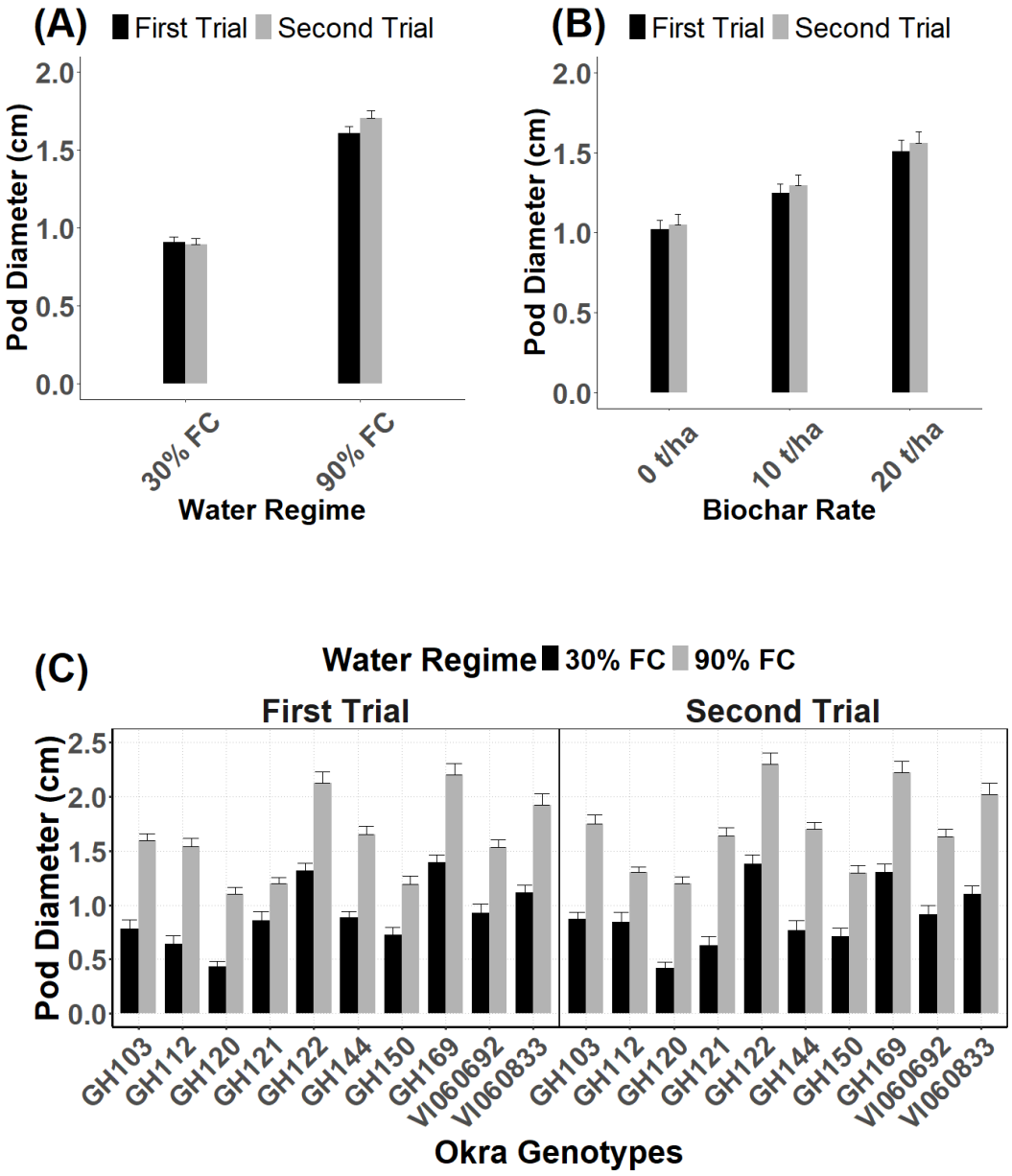

Figure 53 Variation in pod diameter. (A) Single effect of water regime across trials; (B) Single effect of biochar rates across trials; (C) Interaction effect of genotype and water regimes across trials.

Table 9: Descriptive statistics for the yield traits measured among ten selected okra genotypes grown under water deficit conditions and biochar amendment. Min: minimum value; Max: maximum value; SD: standard deviation; CV: coefficient of variation.

Trait	Acronym	Unit	Mean	SD	Min	Max	CV (%)
Pod diameter	Pd	Cm	1.28	0.55	0.23	2.73	43
Pod length	Pl	Cm	5.67	2.76	0.90	13.27	49
Number of pods per plant	Npp		5.85	4.08	1.00	20.00	70
Total pod yield	Tpy	t/ha	1.25	0.85	0.02	4.35	68

Table 10: ANOVA results for yield traits measured among ten selected okra genotypes grown under water deficit conditions and biochar amendment. Gen: genotype; Bio: Biochar; WR: Water-regime.

Trait	Gen	Trial	Gen x Trial	WR	WR x Trial	Gen x WR	Gen x WR x trial	Bio	Bio x Trial	Gen x Bio	Gen x Bio x Trial	Gen x Bio x WR	Gen x Bio x WR x Trial
Pd	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	<0.001	>0.05	<0.001	>0.05
Pl	<0.001	0.001	0.05	<0.001	<0.001	0.001	<0.05	<0.001	>0.05	<0.001	>0.05	0.001	>0.05
Npp	<0.001	<0.001	<0.001	<0.001	>0.05	<0.001	0.001	<0.001	<0.001	<0.001	<0.05	<0.001	>0.05
Tpy	<0.001	>0.05	>0.05	<0.001	<0.001	<0.001	<0.001	<0.001	>0.05	<0.001	<0.001	<0.001	>0.05

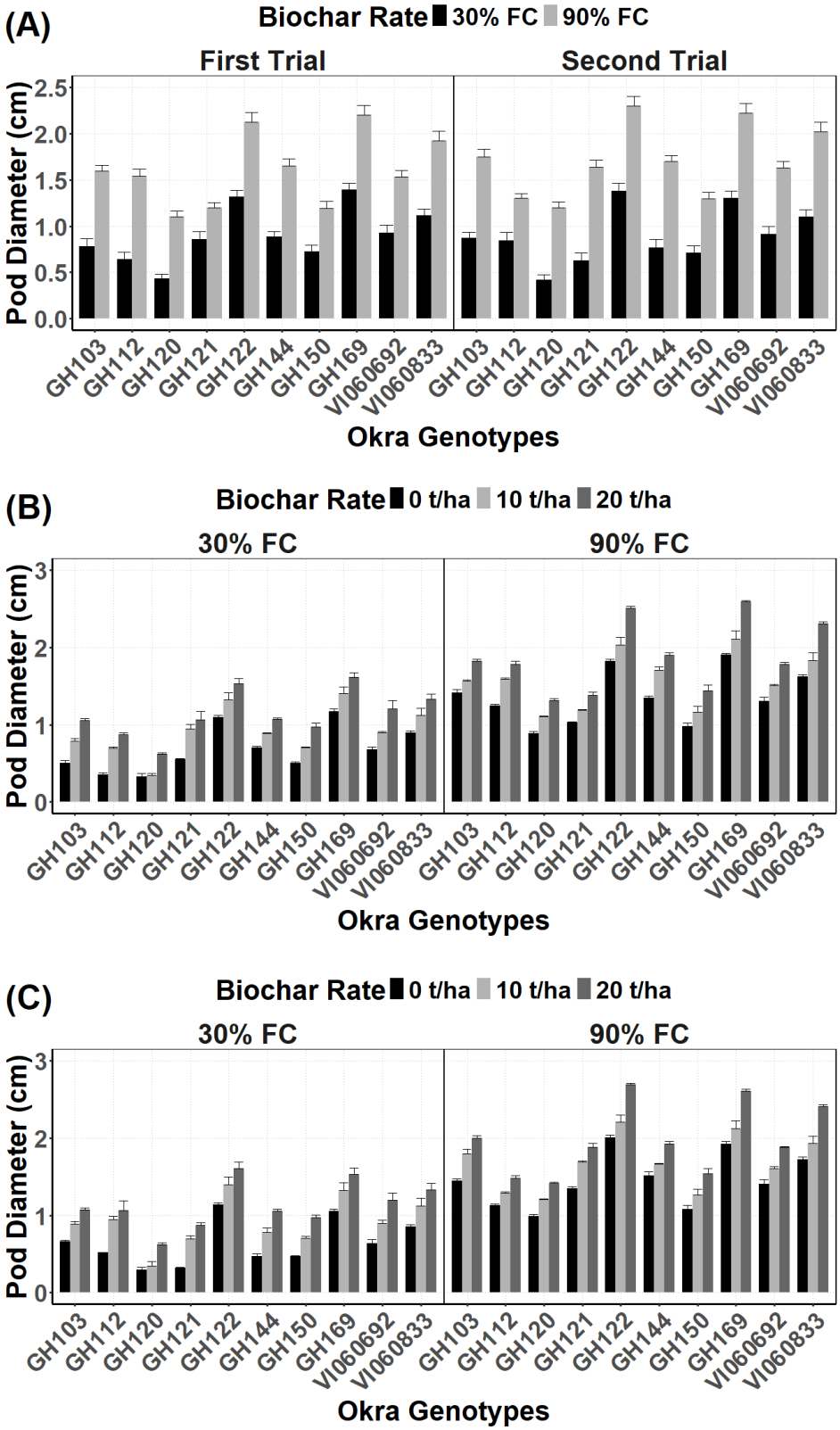

Figure 54: Variation in pod diameter. (A) Interaction effect between genotype and biochar rates across trials. Panel B and C are interaction effect of genotype, water regime and biochar rates for: (B) First trial and (C) Second trial.

Pod length

Pod length varied significantly among the genotypes ($p < 0.001$) and trials ($p = 0.001$), but no significant ($p = 0.05$) effect was observed in genotype and trial interaction (Table 10). Water regime had a significant ($p < 0.001$) effect on Pl in each trial (Table 10). In well-watered conditions, Pl was 7.22 cm in the first and 7.83 cm in the second trials. These were 1.8-fold and 2-fold longer than the Pl at water deficit in the first trial (3.94 cm) and second trial (3.68 cm), respectively (Figure 55A). Pod length also varied significantly ($p < 0.001$) among the biochar rates, but no significant ($p > 0.05$) effect was observed in biochar and trial interaction (Table 10). Crops amended with 20 t/ha biochar recorded the longest Pl in both the first trial (7.32 cm) and the second trial (7.48 cm). These were longer than the Pl measured at 10 t/ha (5.78 cm in the first trial and 6.02 cm in the second trial) by 1.3-fold but differed from the 0 t/ha (3.65 cm in the first trial and 3.77 cm in the second trial) by 2-fold in each trial (Figure 55B).

The interaction between genotype and water regime was significant ($p = 0.001$) for Pl as well as the three interactions of genotype, water regime and trial ($p < 0.05$) (Table 10). Among the well-watered crops, the longest Pl was measured in GH150 in both the first trial (9.3 cm) and the second trial (10.1 cm). These were 51 % and 48 % longer than the shortest Pl observed in GH120 (5.52 cm) in the first trial and GH122 (6.10 cm) in the second trial, respectively (Figure 55C). At water deficit, Pl were reduced in all genotypes. The longest Pl was still observed in GH150, which measured 6.8 cm and 6.5 cm in the first and second trials, respectively (Figure 55C). These differed from GH120 (2.47 cm) by 93 % and from

GH122 (2.26 cm) by 96 %, in which the least Pl were recorded in the first and second trials, respectively (Figure 55C). Pl in the remaining genotypes varied from 2.6 cm in GH122 to 5.6 cm in GH121 in the first trial and from 2.3 cm in GH120 to 5.5 cm in GH121 in the second trial (Figure 55C). Overall, the drought effect reduced Pl in all genotypes compared to their respective well-watered counterparts, and the reduction varied from 1.4-fold to 2.4-fold in the first trial and 1.6-fold to 3-fold in the second.

There was a significant ($p < 0.001$) genotype and biochar interaction effect for Pl, but no significant ($p > 0.05$) three-way interaction effect was observed between genotype, biochar and trial (Table 10). With 20 t/ha biochar amendment, Pl varied from 5.21 cm in GH120 to 10.5 cm in GH150 in the first trial and from 5.35 cm in GH122 to 10.65 cm in GH150 in the second trial (Figure 56A). Among the crops treated with 10 t/ha biochar, the Pl ranged from 4.19 cm in GH120 to 8.58 cm in GH150 in the first trial, and from 4.43 cm in GH122 to 8.82 cm in GH150 in the second trial (Figure 56A). The 0 t/ha biochar rate yielded Pl ranging from 2.60 cm in GH120 to 5.31 cm in GH121 in the first trial and 2.81 cm in GH122 to 5.39 cm in GH121 in the second trial (Figure 56A). This suggested an increasing Pl with an increasing rate of biochar application. Juxtaposing each genotype with its counterparts at various biochar rates revealed that Pl in crops treated with 20 t/ha biochar was greater than those of 10 t/ha by a range of 1.2-fold to 1.4-fold but differed from the 0 t/ha rate by 1.7-fold to 2.5-fold in each trial (Figure 56A). However, irrespective of the biochar rate, there was a general trend of long Pl in GH121 and GH150 in each trial (Figure 56A).

A significant ($p = 0.001$) interaction effect was observed between genotype, biochar and water regime for Pl. Still, the four-way interaction of genotype, water-regime, biochar and trial was not significant ($p > 0.05$) (Table 10). The longest Pl under water deficit was obtained by GH121 in both the first trials (3.96 cm) and the second trial (3.51 cm) (Figure 56B-C). These were less than the longest Pl obtained at ample water in GH121 (6.66 cm) and GH150 (7.35 cm) by 51 % and 71 % in the first trial and second trial, respectively. The remaining water-stressed crops varied from 1.47 cm in GH120 to 3.71 cm in GH150 and from 1.27 cm in GH122 to 3.26 cm in GH150 in the first and second trials. Meanwhile, the range for the remaining well-watered crops was 3.72 cm in GH120 to 6.26 cm in GH150 and 3.76 cm in VI060692 to 7.23 cm in GH121 in the first and second trials, respectively (Figure 56B-C). The drought impact was, however, lessened in all genotypes with an increasing rate of biochar amendment.

With 20 t/ha biochar amendment at water deficit, the longest Pl was 8.96 cm in GH150 in the first trial and 8.74 cm in GH150 in the second trial. This was about 29 % and 17 % longer than the longest Pl measured in GH121 (6.66 cm) and GH150 (7.35 cm) among the unamended well-watered crops in the first and second trials, respectively (Figure 56B-C). The range for the remaining water-stressed crops amended with 20 t/ha biochar was 3.45 cm GH120 to 7.37 cm in GH121 in the first trial and 3.23 cm in GH122 to 7.42 cm in GH121 in the second trial. The overall picture in the first trial was that the 20 t/ha biochar yielded longer Pl under water deficit in 40 % of the genotypes (VI060692, VI060833, GH121, and GH150) than their respective well-watered unamended counterparts but at par with their

well-watered counterparts in 20 % of the genotypes (GH112 and GH103) and inferior in the remaining 40 % of the genotype (GH144, GH169, GH122, and GH120). In the second trial, the 20 t/ha biochar produced longer Pl in 20 % of the genotypes (GH150 and VI060833) than their unamended well-watered counterparts, at par in 20 % of the genotypes (VI060692 and GH121) but inferior in the remaining 60 % of the genotypes.

Similarly, with 10 t/ha biochar amendment at water deficit, the longest Pl was obtained in GH150 in both the first trials (7.60 cm) and the second trial (7.39 cm). When juxtaposed with the well-watered unamended crops, this is superior to the longest Pl measured in GH121 (6.66 cm) by 13 % in the first trial but at par with the longest Pl recorded in GH150 (7.34 cm) in the second trial (Figure 56B0-C). The remaining water-stressed crops amended with 10 t/ha biochar varied from 2.46 cm in GH122 to 5.4 cm in GH121 in the first trial and 2.25 cm in GH120 to 5.57 cm in GH121 in the second trial. Generally, at water deficit in the first trial, the 10 t/ha biochar amendment yielded Pl superior to their unamended well-watered counterparts in 20 % of the genotypes (GH150 and VI060692), while the well-watered crops were superior in the remaining 80 %. In the second trial, however, the unamended well-watered crops had superior Pl in all genotypes except GH150, of which there was parity.

Besides the well-watered crops, groups amended with biochar yielded longer Pl than the unamended crops (Figure 56B-C). The Pl varied from 3.72 cm GH120 to 6.66 cm in GH121, 5.89 cm GH120 to 9.56 cm in GH150, and 6.97 cm in GH120 to 12.04 cm in GH150 with 0 t/ha, 10 t/ha and 20 t/ha biochar amendment

respectively in the first trial. In the second trial, the range was 4.33 cm in GH122 to 7.35 cm in GH150, 6.59 cm in GH122 to 10.25 cm in GH150 and 7.48 cm in GH122 to 12.55 cm in GH150 when 0 t/ha, 10 t/ha and 20 t/ha biochar were applied respectively.

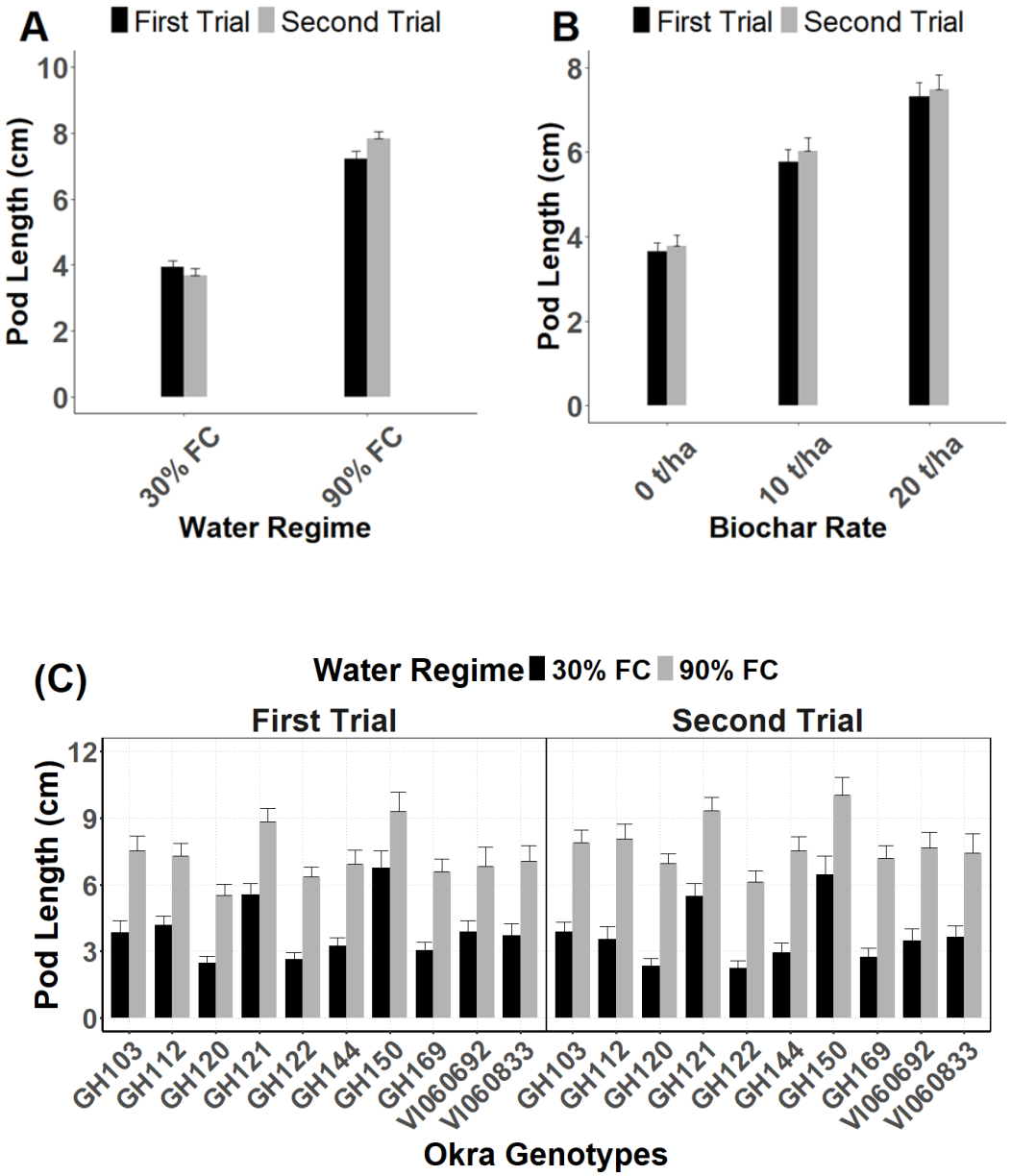

Figure 55: Variation in pod length. (A) Single effect of water regime across trials; (B) Single effect of biochar rates across trials; (C) Interaction effect of genotype and water regimes across trials.

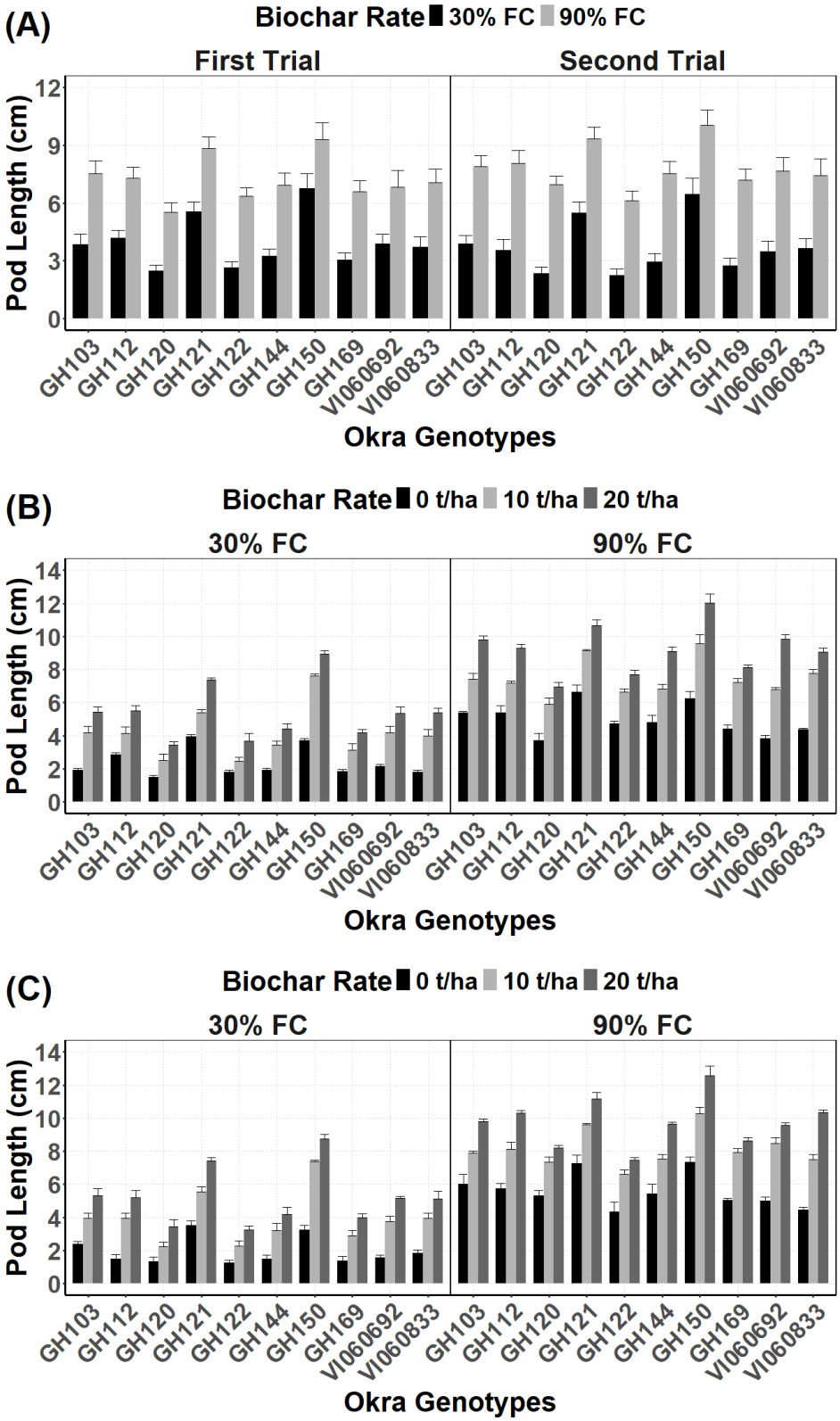

Figure 56: Variation in pod length. (A) Interaction effect between genotype and biochar rates across trials. Panel B and C are interaction effect of genotype, water regime and biochar rates for: (B) First trial and (C) Second trial.

Number of pods per plant

The single effect of genotype, trial as well as their interaction was significant ($p < 0.001$) for Npp (Table 10). Similarly, water regime had a significant ($p < 0.001$) effect on Npp, but no significant ($p > 0.05$) interaction effect was observed between water regime and the trial (Table 10). The 90% FC yielded eight and nine pods in the first and second trials each. These were higher than the three pods obtained in each trial at water deficit by 91% and 100%, respectively (Figure 57A). Biochar rate and its interaction with the trial was significant ($p < 0.001$) for Npp (Table 10). In the first and second trials, the 20 t/ha biochar yielded seven and nine pods, respectively. These differed from the Npp at 10 t/ha by 33 % in the first trial (5 pods) and 40 % in the second trial (6 pods). At 0 t/ha biochar, four pods were obtained in each trial and were about 55 % and 77 % less than the Npp at 20 t/ha in the first and second trials each (Figure 57B).

Genotype and water interaction were significant ($p < 0.001$) for Npp as well as their interaction with trial ($p = 0.001$) (Table 10). At well-watered conditions, GH112 and VI060692 had the higher Npp of about 13 pods and 12 pods in the first trial and 13 pods and 14 pods each in the second trial (Figure 57C). Lower values for Npp were recorded for GH120 (4 pods), GH122 (5 pods) and VI060833 (6 pods) in the first trial, and GH122 (5 pods), GH120and GH150 (6 pods each) in the second trial (Figure 57C). These were largely reduced by drought stress. Similar to the observation among the well-watered crops, GH112 and VI060692 recorded the higher Npp under water stress across the two trials. In the first trial, GH112 yielded six pods, while VI060692 yielded five pods. In the second trial, both genotypes had

six pods each (Figure 57C). Fewer Npp were measured in GH120 and GH121 (1 pod each) in the first trial and GH122 and GH121 (2 pods each) in the second trial (Figure 57C). Overall, the difference in Npp between the well-watered crops and their respective water-stressed genotypes varied from 63 % to 128 % in the first trial and from 64 % to 119 % in the second trial (Figure 57C).

Genotype interaction with biochar was significant ($p < 0.001$) for Npp, as well as their interaction with trial ($p < 0.05$) (Table 10). Biochar improved Npp in all genotypes with an increasing application rate (Figure 58A). Without biochar amendment (0 t/ha), Npp varied from approximately two pods in GH122 (in each trial) to 6 pods in VI060692 in the first trial and 7 pods in GH112 in the second trial (Figure 58A). The Npp increased with 10 t/ha biochar application, varying from 3 pods in GH120 to 10 pods in GH112 in the first trial and from 4 pods in GH122 to 10 pods in VI060692 in the second trial (Figure 58A). The greatest Npp was obtained when 20 t/ha biochar was applied, ranging from 4 pods in GH120 to 13 pods in GH112 in the first trial and from 6 pods in GH122 to 14 pods in VI060692 in the second trial (Figure 58A). Overall, the highest Npp was obtained at 20 t/ha in all genotypes and differed from the 10 t/ha by a range of 1-fold- to 2-fold range in each trial (Figure 58A). Compared to the 0 t/ha, the 20 t/ha biochar application was superior in all genotypes by a range of 2-fold- to 3-fold range in each trial (Figure 58A). Irrespective of the biochar rate and trial, GH112 and VI060692 measured higher Npp, while GH120, GH121 and GH122 scored lower values.

The three interactions of Genotype, water and biochar were highly significant ($p < 0.001$), but their interaction with the trial was not significant ($p >$

0.05) for Npp (Table 8). Among the well-watered crops, Npp varied from 3 pods in GH122 to 8 pods in VI060692 in the first trial, and from 4 pods in GH122 to 9 pods in GH112 in the second trial (Figure 58B-C). These were substantially reduced at water deficit, with Npp varying from 1 pod (e.g., GH169, GH150, GH122 to 4 pods in VI060692 and GH112 in each trial). Biochar application at 10 t/ha marginally improved Npp in most genotypes, with Npp ranging from 1 pod (GH121 and GH120) to 6 pods in GH112 and VI060692 in the first trial and from 2 pods in GH122 to 6 pods in VI060692 and GH112 in the second trial (Figure 58B-C). The greatest improvement in Npp during the water deficit was observed with 20 t/ha biochar amendment. At 20 t/ha biochar rate, Npp varied from 1 pod in GH120 to 8 pods in GH112 in the first trial and from 4 pods (GH120, GH122, GH121, GH169) to 9 pods in VI060692 in the second trial.

Within the well-watered crops, there was also higher Npp in crops amended with biochar than their unamended counterparts. Among the well-watered crops unamended with biochar, Npp varied from 3 pods in GH122 to 8 pods in VI060692 in the first trial and from 4 pods in GH122 to 9 pods in GH112 in the second trial (Figure 58B-C). With biochar application at 10 t/ha, there was a general increase in most genotypes, with Npp ranging from 4 pods in GH120 to 13 pods in GH112 in the first trial and from 5 pods in GH122 to 13 pods in VI060692 in the second trial (Figure 58B-C). The highest increment was observed with 20 t/ha biochar amendment as Npp varied from 6 pods in GH120 to 18 pods in GH112 in the first trial and from 8 pods in GH122 to 19 pods in VI060692 in the second trial.

Thus, drought impact reduced the Npp in all genotypes by an amount ranging from about 2-fold to 6-fold compared to their various well-watered genotypes. However, the biochar amendment mitigated the impact of drought to a great extent, especially at higher rates. In the first trial, at a 20 t/ha rate, the Npp was comparable to the unamended well-watered crops in 80 % of the genotypes, except in two genotypes (Figure 58B).

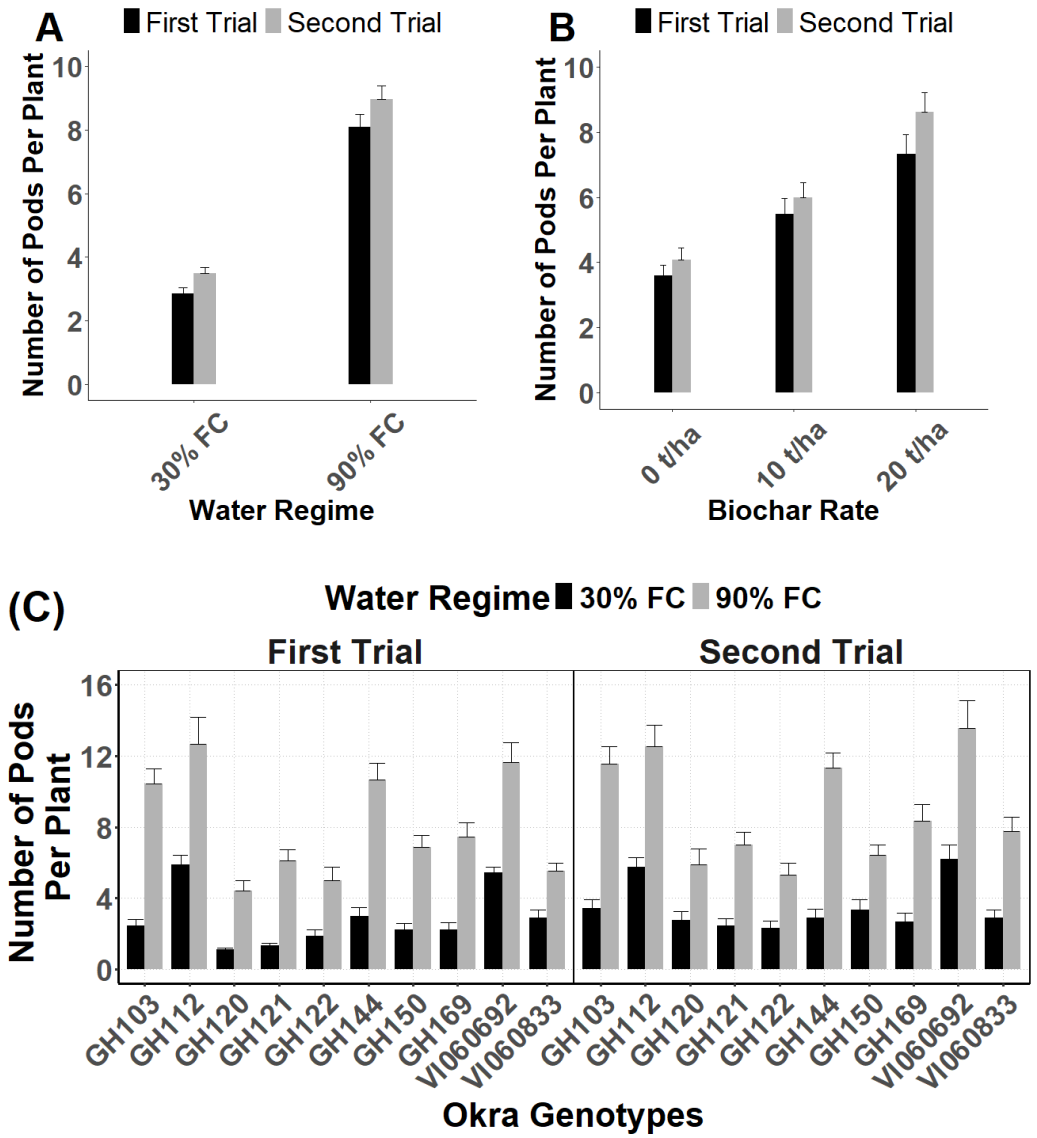

Figure 57: Variation in the number of pods per plant. (A) Single effect of water regime across trials; (B) Single effect of biochar rates across trials; (C) Interaction effect of genotype and water regimes across trials.

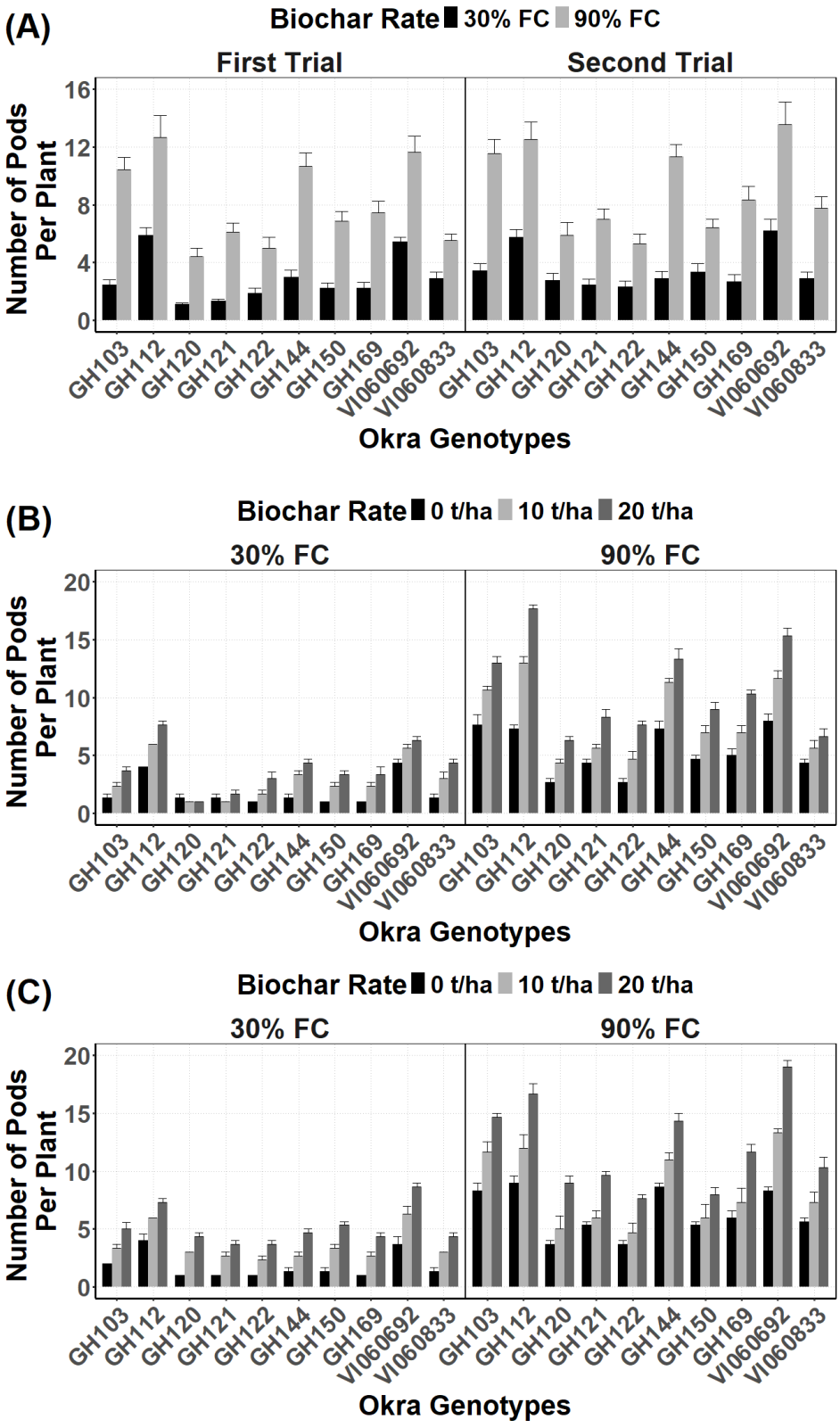

Figure 58: Variation in the number of pods per plant. (A) Interaction effect between genotype and biochar rates across trials. Panel B and C are interaction effect of genotype, water regime and biochar rates for: (B) First trial and (C) Second trial.

Total pod yield

The okra genotypes varied significantly ($p < 0.001$) in Tpy, but no significant ($p > 0.05$) trial effect was observed (Table 10). The water regime and its interaction with the trial were significant ($p < 0.001$) for Typ (Table 10). The Tpy at well-watered conditions was 1.66 t/ha and 1.72 t/ha in the first and second trials, respectively (Figure 59A). These were 66 % and 74 % more than the Tpy recorded at water deficit in the first trial (0.84 t/ha) and second trial (0.79 t/ha), respectively (Figure 48 B). The different biochar rates had a significant ($p < 0.001$) effect onTpy. However, no significant ($p > 0.05$) interaction effect was observed between biochar and the trial (Table 10). The first trial yielded 1.79 t/ha with 20 t/ha biochar amendment. This differed from the Tpy obtained when 10 t/ha (1.26 t/ha) biochar was applied by 35 % and from the 0 t/ha (0.71 t/ha) by 86 % (Figure 48B). This observation among the biochar rates did not differ significantly ($p > 0.05$) in the second trial (Figure 59B).

There was a significant ($p < 0.001$) interaction effect between genotype and water regime for Tpy, as well as their interaction with trials (Table 10). At well-watered conditions, GH112 and VI060692 maintained higher Tpy. Genotype GH112 measured 2.8 t/ha and 2.6 t/ha, while VI060692 recorded 2.5 t/ha and 2.7 t/ha in the first and second trials respectively (Figure 59C). Under drought conditions, Tpy remained higher for VI060692 and GH112. Genotype VI060692 yielded 1.8 t/ha and 1.6 t/ha in the first and second trials, while GH112 yielded 1.7 t/ha and 1.8 t/ha in the first and second trials, respectively. (Figure 59C). The remaining genotypes had Tpy less than 1.0 t/ha, including GH150, GH169, GH122,

VI060833, and GH121 in each trial (Figure 48C). Overall, compared to their various well-watered genotypes, Tpy declined in all genotypes under drought conditions, varying from 32 % to 103 % in the first trial and from 36 % to 105 % in the second trial (Figure 59C).

A significant ($p < 0.001$) interaction effect was observed between genotype and biochar rates, as well as the three interactions of genotype, biochar and trial (Table 10). Across all biochar rates, GH112 and VI060692 consistently recorded higher Tpy in each trial. At 0 t/ha, both genotypes had approximately 1.4 t/ha Tpy in each trial (Figure 60A). With 10 t/ha biochar rate, VI060692 measured 2.3 t/ha in the first trial and 2.1 t/ha in the second trial, while GH112 had 2.2 t/ha in the first trial and 2.3 t/ha in the second trial (Figure 60A). Genotype GH112 measured 3.1 t/ha and 2.9 t/ha in the first and second trials when a 20 t/ha biochar rate was used, while VI060692 had 2.7 t/ha and 3.0 t/ha Tpy in the first and second trials, respectively at 20 t/ha biochar (Figure 60A). On the other hand, GH122 measured the least Tpy at all biochar rates in each trial (0.3 t/ha, 0.7 t/ha, and 1.2 t/ha Tpy at 0 t/ha, 10 t/ha and 20 t/ha biochar rates each) (Figure 60A). Overall, soil amendment with biochar improved Tpy in every genotype with an increasing application rate. The 20 t/ha rate consistently gave the highest Tpy, higher than the 10 t/ha by a range of 20 % to 49 % in each trial (Figure 60A). Compared to the 0 t/ha biochar rate, the 20 t/ha rate was superior by a margin varying from 72 % to 111 % in both trials (Figure 60A).

There was a significant ($p < 0.001$) interaction effect between genotype, biochar and water regime, but no significant ($p > 0.05$) effect was observed in their

interaction with the trial (Table 10). The highest Tpy at water deficit was obtained for VI060692 (1.23 t/ha) in the first trial and GH112 (1.20 t/ha) in the second trial. These were less than the greatest Tpy obtained at ample water in GH112 (1.80 t/ha) in the first trial and VI060692 (1.85 t/ha) in the second trial by 1.5-fold and 1.6-fold, respectively. However, biochar application reduced drought impact in all genotypes with increasing application rate (Figure 60B-C).

When 20 t/ha biochar was applied under water deficit, the highest Tpy was 2.35 t/ha in GH112 and 2.30 t/ha in VI060692 in the first and second trials each. The remaining genotypes varied from 0.68 t/ha in GH150 to 2.30 in VI060692 in the first trial and 0.63 t/ha in GH169 to 2.25 t/ha in GH112 in the second trial. When compared to their respective unamended well-watered genotypes, biochar amendment at 20 t/ha at water deficit produced higher Tpy in 80 % of the genotypes by margins ranging from 1.1-fold to 2-fold in the first trial and 60 % of the genotypes by 1.1-fold to 1.7-fold in the second trial (Figure 60B-C)

With 10 t/ha biochar amendment during water deficit, Tpy remained higher for VI060692 and GH112 across trials. In the first trial, the highest Tpy was measured in VI060692 (1.95 t/ha), and this was marginally superior to the highest Tpy recorded in GH112 (1.80 t/ha) at ample water without biochar application by 1.1-fold. In the second trial, however, the highest Tpy was 1.90 t/ha in GH112, and it compared favourably to the highest Tpy measured in VI060692 (1.85 t/ha) at ample water without biochar. (Figure 60B-C)

Among crops treated with ample water, higher Tpy was obtained in groups treated with biochar than in the unamended group.

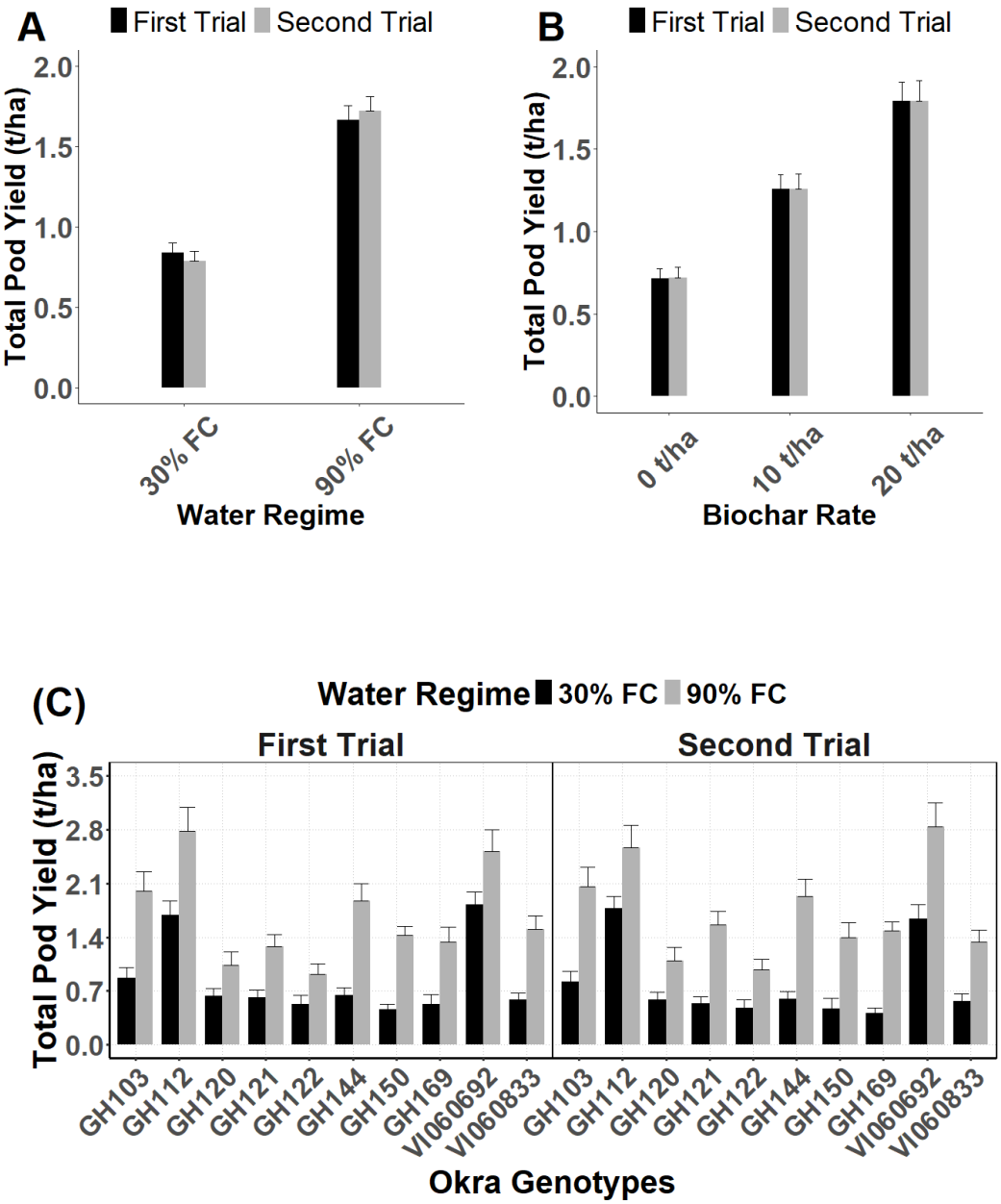

Figure 59: Variation in total pod yield. (A) Single effect of water regime across trials; (B) Single effect of biochar rates across trials; (C) Interaction effect of genotype and water regimes across trials.

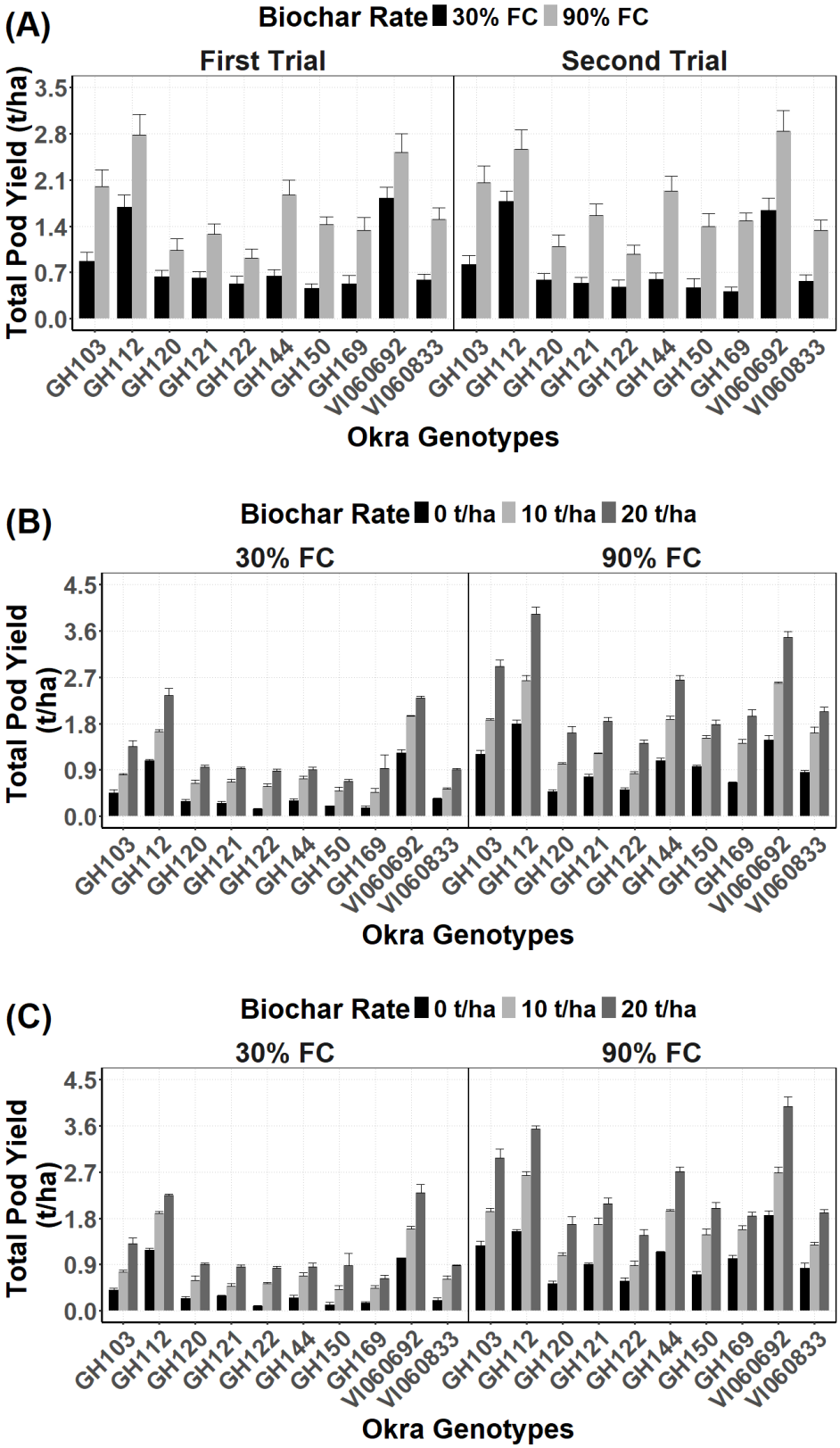

Figure 60: Variation in total pod yield. (A) Interaction effect between genotype and biochar rates across trials. Panel B and C are interaction effect of genotype, water regime and biochar rates for: (B) First trial and (C) Second trial.

Correlation between total pod yield and selected biochemical traits

Proline

A significant ($p < 0.001$) positive linear association was observed between leaf Pro content and Tpy at both water deficit ($R^2 = 0.33$) and well-watered ($R^2 = 0.63$) conditions (Figure 61A and Figure 61B).

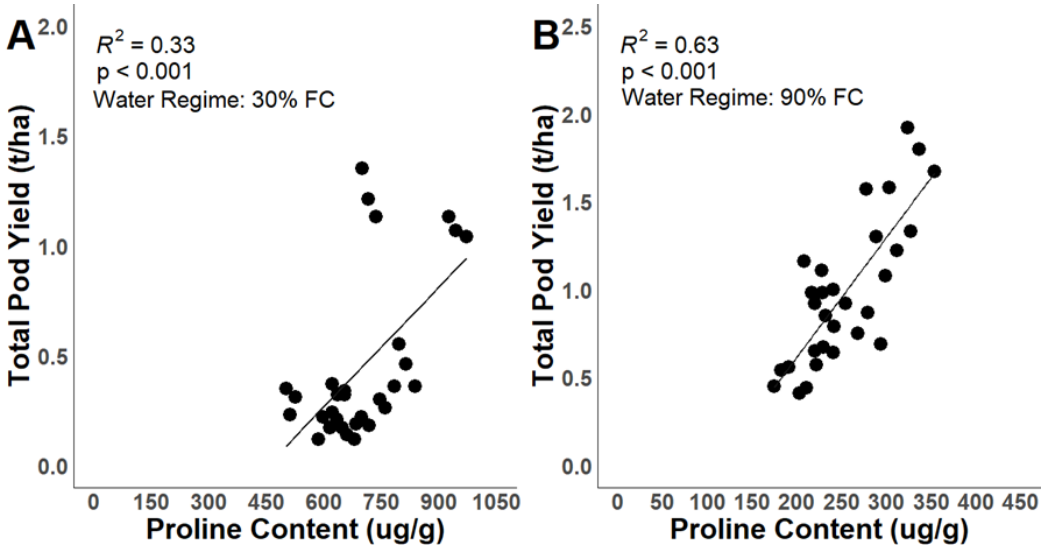

Figure 61: The relationship between leaf proline content and total pod yield. (A) Proline content per gram of fresh leaf and total pod yield at water deficit; (B) Proline content per fresh leaf and total pod yield at well-watered condition.

Salicylic Acid

There was a significant ($p < 0.001$) linear relationship ($R^2 = 0.41$) between Tpy and SA activity at water deficit (Figure 62A). At ample water, however, the relationship was observed to be moderate ($R^2 = 0.21$) but still significant ($p < 0.05$) (Figure 62B).

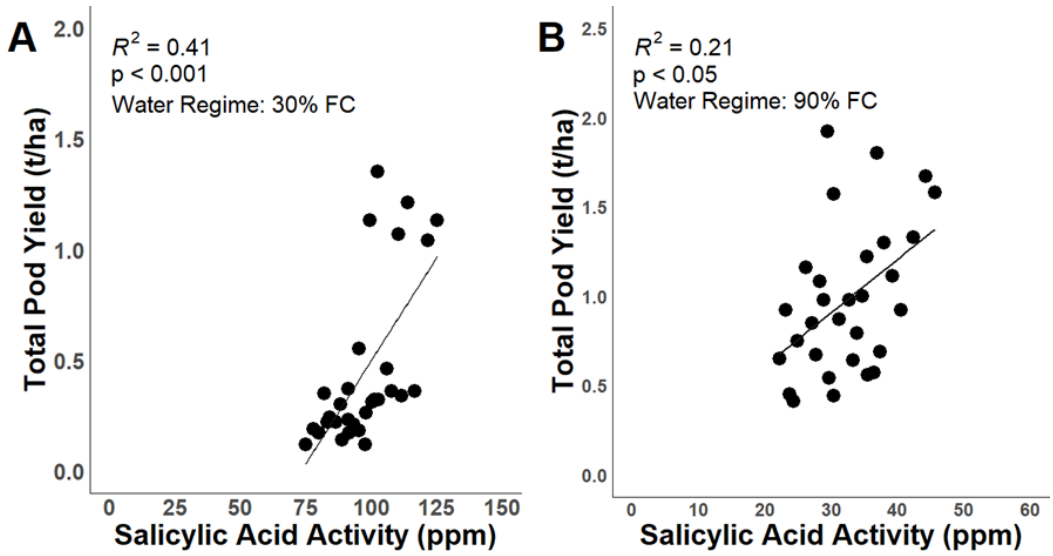

Figure 62: The relationship between leaf salicylic acid activity and total pod yield. (A) At water deficit; (B) At well-watered conditions.

Superoxide Dismutase

At water deficit, a linear positive association was observed between SOD and Typ ($R^2 = 0.76$; $p < 0.001$) (Figure 63A). Under well-watered conditions, SOD had a weak and insignificant ($R^2 = 0.12$, $p > 0.05$) relationship with Tpy (Figure 64B and 64D).

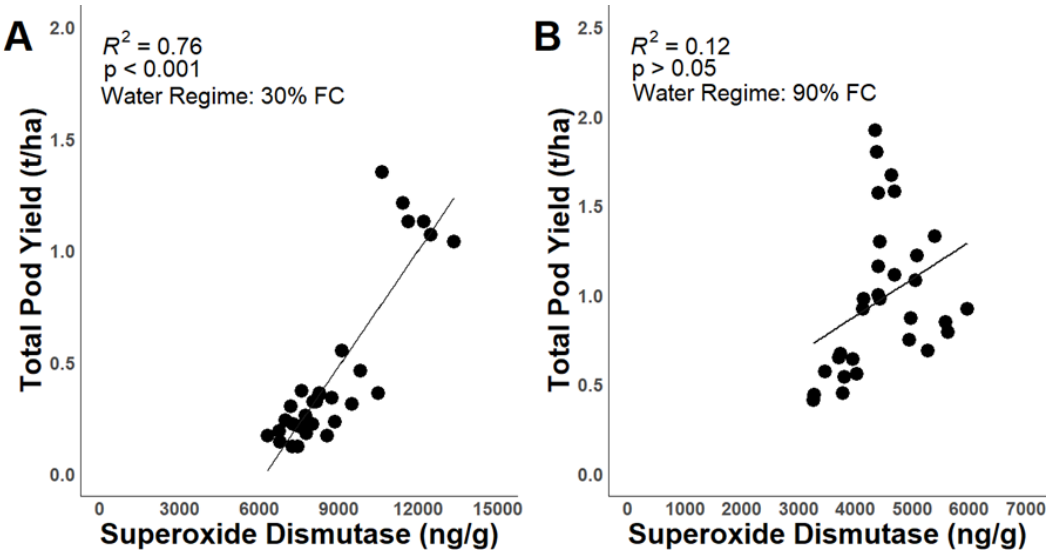

Figure 63: The relationship between total pod yield and leaf superoxide dismutase activity per gram of fresh leaf. (A) At water deficit; (B) At well-watered condition.

CHAPTER FIVE

DISCUSSION

First objective: assessing genotypic variation in the RSA of okra genotypes

Understanding the RSA traits and biomass production pattern of crops can be instrumental to selecting and breeding climate-resilient varieties. Yet, there is a shortage of root systems and shoot biomass genetic data on okra. The majority of the work has focused on yield and yield-related traits. Thus, in the present study, twenty-five RSA and three biomass traits were employed in assessing variations in 60 okra genotypes.

The majority of traits showed significant genetic variations.

Significant genotypic variations were observed in all three biomass traits. Similar result of significant genotypic differences in root dry biomass has been reported for five okra cultivars (Eltigani et al., 2021). Demelash et al. (2021) noted similarly for root dry biomass, shoot dry biomass and root-to-shoot ratio among 214 sorghum genotypes. Additionally, the CV, which measures the relative variation in quantitative traits (Zanklan et al., 2018), were also observed to be intermediate for Rdw and RS but marginally low for Sdw (Table 3). In conjunction with the ANOVA results, the CVs generally indicate moderate diversity among the okra genotypes in biomass production, which can potentially permit the selection of varietal improvement in these traits. The higher CV for Rdw than Sdw suggests more variability among the genotypes in Rdw than Sdw.

The presence of genetic variability forms the foundation of all selection strategies, as the extent of genetic diversity within a population directly correlates with the potential for enhancing specific traits through selection for varietal improvement (Hussain et al., 2021). The high GCV observed in all the biomass traits suggested a broad genetic base among the genotypes for these traits, which could be exploited in breeding works. The PCV were similarly high, indicating substantial environmental influence on these traits (Kulus, 2022). Nonetheless, the close similarity between the PCV and the GCV across all biomass traits implied that environmental factors have little impact on how these traits are expressed (Bello & Aminu, 2017). Overall, the high GCV and PCV obtained in this study for each biomass trait substantiated the presence of sufficient scope for phenotypic selection and genetic improvement in these characteristics. Albeit the scanty data on okra, studies have reported high GCV and PCV in the biomass traits of other important crop species. In line with the findings of this study, rice cultivars exhibited high GCV and PCV for both root dry weight and shoot dry weight (Ahmed et al., 2021). The PCV and GCV for root-to-shoot ratio was noted to be high by Duresso et al. (2023) among sorghum genotypes.

It was equally observed that some okra genotypes were inclined towards more root biomass than shoot biomass, such as VI063895, VI060692, GH154 and VI060691. This suggested that these genotypes will be more efficient foraging for limited soil resources, like water and mineral nutrients, than those with less root biomass. Given that parameters associated with biomass production could act as proxies for yield, the results from this study alluded to the possibility of these

genotypes having varying genetic potentials for yield, with genotypes having higher biomass expected to give higher yield. Thus, breeding programmes can exploit these variations to develop okra varieties with high-yield disposition. In this regard, genotypes with large shoots and root biomass should be given premium consideration (Opoku et al., 2022). However, when considering the carbon expenses for plants, it is notable that roots represent a significant carbon investment. Therefore, in conjunction with soil resource acquisition, an increase in root biomass, for instance, may not necessarily confer benefits (Adu et al., 2019).

Twenty-five root system traits were evaluated. These traits constitute essential descriptors of the RSA and its diversity, summarizing developmental processes like elongation and branching, as well as developmental features such as the distribution of root diameters, as demonstrated in prior studies such as Pagès (2014) and Salinier et al. (2021). This present study revealed significant genotypic variations for all 25 root system traits (Table 4). The scale of variability varied across the root traits, which corroborated the observation of Beroueg et al. (2021) for *Lactuca* genotypes. Adu et al. (2022b) evaluated two field-grown sorghum cultivars. Also, they reported significant genotypic variations in several RSA traits, such as volume, total root length, perimeter, root surface area, number of root tips, network area, median diameter, and angle-related traits, among others, all of which agreed with the result of the current study.

Juxtaposing the ANOVA result with the CVs, it can be said that, for traits with significant genotype effects and intermediate to high CVs (e.g., Bf, Trl, Nbp, Peri, Vol, Md, etc.), the okra genotypes exhibited high degree of diversity which

presents an opportunity for leveraging direct selection in breeding programmes to develop superior varieties for these traits. On the other hand, traits with significant genotype effect but low CVs (e.g., Lra, Prl, RLDR1, Ad, etc.) could mean that, although the genotypes differed significantly in these traits, the magnitude of these differences was not sufficiently large. Therefore, direct selection for these traits might yield little or no progress. Similar intermediate to high CVs have been reported by Adu et al. (2022b) for diverse RSA traits like volume, total root length, perimeter, root surface area, and network area among sorghum cultivars. An intermediate to high CVs were recorded among 6-day-old maize inbred lines for length-related RSA traits, including total root length, seminal root length and lateral root length (Kumar et al., 2012). Demelash et al. (2021) and Kumar et al. (2012) reported low CVs for root angle (5.9 %) and primary root length (18.8 %) for sorghum and maize inbred-lines respectively, which are consistent with the 7 % and 5 % observed for each of these traits in the present study. Low CVs have been reported for root diameter-related traits by Beroueg et al. (2021) for Lactuca genotypes (minimal diameter and maximal apical diameter), Ahmad et al. (2021) for 15 *Camellia sinensis* clones (root diameter) and Adu et al. (2022b) for sorghum cultivars (average diameter and maximum diameter). These observations corroborated the result of this study for two of the diameter traits (Mxd and Ad) but differed in Md, which recorded an intermediate CV.

Again, phenotypic variability estimates do not delineate the genotypic effect from the environmental effect. This underscores the critical role of genetic variability estimates in partitioning the real genetic differences (Reddy et al., 2012).

The estimates of GCV and PCV are, therefore, of greater use in determining the variability present in a set of germplasm (Reddy et al., 2012). The majority of RSA traits (e.g., Nbp, Bf, Trl, Na, Sa, SADR2, Mxd, etc.) recorded high GCV and PCV in the current study, and both were nearly equal in magnitude (Table 5). This suggested a large genetic diversity among the genotypes and little impact from the environment on these traits, with the possibility of making substantial improvement through direct selection. The least GCV and PCV observed in traits such as Lra and Prl implied higher environmental impacts on these traits (Bello & Aminu, 2017). Hence, reliance on phenotypic selection may not be effective for advancing the genetic improvement of the crop (Ibrahim et al., 2013; Shivaramegowda et al., 2016). For such traits, selective breeding may be achieved through quality molecular markers.

Several other studies on the RSA of various crops support the result of this study. Akshaya et al. (2020) observed high PCV and GCV for root length in 51 rice landraces, which agrees with most root length traits in this study (e.g., Trl, Peri, RLDR1, etc.). High GCV and PCV were reported among 54 rice varieties for root volume (Ahmed et al., 2021), similar to this study's observations for Vol, VDR1 and VDR2. Abtahi et al. (2017) reported high PCV and GCV among 30 orchardgrass genotypes for root area, which also corroborated the findings for the majority of the area traits in this study (e.g., Na, Sa, PADR1, etc.). In a study on *Camellia sinensis* by Ahmad et al. (2021), the number of lateral roots per plant measured high PCV and GCV, similar to all number traits in this study (e.g., Nfol, Nbp, etc.). However, contrary to the low GCV and PCV obtained in this study for

the root angle trait (Lra), Demelash et al. (2021) observed moderate PCV and GCV for root angle among sorghum genotypes. Soil type is among the myriad factors influencing root growth, and the differences in soil types between the two studies (Demelash et al., 2021) may have accounted for the differing results.

The significant trial and genotype-by-trial interaction effect observed for some traits (e.g., Sdw, Rdw, RS, etc.) could be attributed to vagaries in environmental factors (Table 4). Although the experiment was conducted in a greenhouse, climatic factors such as temperature and relative humidity were not under control, varying from 38 ^{0}C to 47 ^{0}C and 28 % to 50 %, respectively. Okra thrives best within the minimum and maximum temperatures of 18 °C and 35°C (Ezeakunne, 1984). The extremely high temperatures could have impeded vegetative growth, thereby introducing variability in biomass productivity between the two trials. Moreover, the root phenotyping procedures involved excavation, washing and imaging of the roots. Variations could be introduced if the protocol was not sufficiently robust, especially to minimise root losses during washing. Ensuring the least amount of root loss is key to ensuring the reproducibility of a root phenotyping result.

High broad-sense heritability existed in all traits.

The effectiveness of exploiting genotypic variability through selection hinges on the extent to which individual traits can be inherited (Bilgin et al., 2010). Heritability denotes how responsive a trait will be to selection. High H^2 was observed for all biomass traits (Table 5). This indicates minimal impact from the environment on these traits and the potential for improvement through phenotypic

selection (Mofokeng et al., 2019). Also, the high heritability posited that these traits will require fewer replications for screening, as such traits demand less replication to discern significant differences between genotypes, and vice versa (Adu et al., 2014). Similar H^2 heritability has been recorded for biomass traits of cleome (Houdegbe et al., 2022). Demelash et al. (2021) observed high heritability among sorghum genotypes in shoot dry biomass and root-to-shoot ratio. Ahmed et al. (2021) evaluated a group of 54 rice varieties. They reported high H^2 for root and shoot dry biomass as well as for root-to-shoot ratio and concluded that direct selection for these traits would be effective.

Similarly, all RSA traits recorded high H^2 in the current study (Table 5). For root traits in which high H^2 was observed, it indicated that phenotypic selection using these traits is likely to be effective, as the observed differences are primarily due to genetic factors that can be transmitted to the progenies, and vice versa. An ideotype breeding programme could, therefore, utilise these genotypes as breeding materials in developing varieties adapted to abiotic stresses, particularly drought stress (Demelash et al., 2021). For instance, steeper-angle roots are better suited for capturing mobile resources like water and nitrogen, which rapidly traverse the soil profile and accumulate at greater depths (Lynch & Wojciechowski, 2015). Thus, genotypes with steeper angles, such as VI060691, GH123, GH125, and GH156, could be useful for developing varieties adapted for soil resources foraging at depth.

A wider root angle, on the other hand, facilitates improved lateral access, potentially boosting water uptake in wide or skip-row agricultural practices (Ali et al., 2015). Thus, genotypes such as VI060686, VI060821, VI063912 and GH103,

having wider angles, could be exploited in breeding okra varieties suited for top-soil foraging. Similarly, more root volume (in GH111, GH121, and GH157), total root length (in GH108, VI063900 and GH125) and perimeter (in VI063895, GH106, and GH147) correspond to the ability to forage larger volume of soil for resources. Additionally, genotypes possessing larger root diameters have the mechanical strength to penetrate the hardpan associated with drought effects on soils, allowing them to access water and mineral nutrients at depth. Making large roots allows the root system to extend and forage in a large volume, because roots with a large meristem grow faster and longer (Pagès, 2014).

However, H^2 must be treated cautiously since it considers additive and non-additive (dominance and gene interactions) genetic effects. Only additive genetic effects are effectively heritable. Hence, despite the high broad-sense heritability observed in most RSA traits, additional studies are needed to delineate the additive genetic component from the non-additive effect. Similar results of high broad-sense heritability have been reported for diverse RSA traits in diverse crops, including volume, number of root tips, and root surface area for sorghum cultivars (Adu et al., 2022b); primary root length for *Brassica rapa* seedlings (Adu, et al., 2017b); total root length among soybean genotypes (Falk et al., 2020), and root diameter among *Camellia sinensis* (Ahmad et al., 2021).

Multivariate analysis – the relationship between traits and genotypes

The results of the current study demonstrated that RSA and biomass traits largely accounted for the observed variation within the dataset and could be essential in differentiating okra genotypes to advance breeding efforts for efficiency

in the acquisition and usage of soil resources. The percentage of explained variance was the higher for PC1 and PC2, suggesting that these are the most essential in accounting for the variability in the data (Table 6 and Figure 39A). However, traits with above-average contributions to at least one of the three significant PCs could be considered vital in accounting for the observed variations within the data.

The degree to which each variable contributes in explaining the variation in a particular PC is expressed in percentages. If each variable had an equal contribution, the expected value would be 1/total number of variables added in the PCA = 1/19 = 5.26 %. For a given PC, variables exceeding this threshold could be seen as significant contributors to the PC (Kassambara, 2017). The key variables in explaining the variation in a dataset are those that correlate with PC1 and PC2 (Adu et al., 2018; Kassambara, 2017). In the present study, 11 traits contributed above average (above the red dotted lines) to PC1. In comparison, eight traits contributed to PC2 (Figures 39C and 39D), as evidenced by their high loading scores (Figure 39B). These traits (e.g., Peri, Na, Sa, RLDR2, Nbp, Trl, Nrt, Vol, Bf, etc.) could be considered the most important in this study and merit consideration in efforts to develop okra varieties that are tolerant to drought stress and efficient in mineral nutrient acquisition. Some traits contributed above average to more than one of the significant PCs (e.g., Nfol, Nrt, RLDR2, PADR2, etc.) which further underscore their importance in this study (Figure 39C-39D). The diameter trait, Md, did not contribute above average to any of the significant PCs. Variables without major contribution to any of the significant PCs may be deemed redundant and thus

excluded to streamline the overall analysis (Kassambara, 2017). Thus, Md was eliminated from subsequent analysis.

The measure of how well variables are represented on the factor map is termed cos^2 (square cosine or squared coordinates). For any given variable, the sum of cos^2 across all principal components equals one (Kassambara, 2017). A high cos^2 value indicates that the variable is well represented on the principal component, typically positioning it closer to the circumference of the correlation circle (Kassambara, 2017). The closer a variable is to the circle of correlations, the better its representation on the factor map, and the more crucial it is for interpreting these components. Conversely, a low cos^2 suggests that the variable is perfectly represented by the PC, positioning it closer to the centre of the circle (Kassambara, 2017). The findings of this study indicated that nearly all traits analysed in the PCA were well represented on PC1 (see Figure 40A) and thus merit attention in breeding for improved okra varieties. One exception to this was root Md, which showed poor representation across all significant PCs.

The variable plot of PCA for PC1 and PC2 suggests an association between several traits (Figure 40B), which could be essential for okra breeding. The positive correlation between many traits (e.g., Nfol, Rdw, Vol, Trl, Peri, Nrt, Nbp, etc.) alluded to a potential simultaneous improvement of these traits. A breeder selecting for Nfol could be selected for all the other traits positively correlated with Nfol. Also, easily measurable counterparts of correlated traits that resolved on similar quadrant of the factor map (Figure 40B), such as Rdw, may serve as indirect trait of traits such as Nrt, Nfol, Nbp, etc., that are more difficult to measure.

Contrariwise, the negative correlation between Md and all other traits on the biplot is indicative that Md is inversely related to them (Figure 40B). Therefore, selecting for Md will mean selecting against every other trait negatively correlated with it. The correlation among key plant traits could indicate a functional strategy which, when described, could provide insights into why specific combination of traits are favored over others and the resulting implications (Wright et al., 2007).

Similar to the result of the PCA biplot, the Pearson correlation coefficients showed a significant positive association between many traits (Figure 41). For instance, Trl positively and significantly correlated with Sa. This was unsurprising given that it is a natural phenomenon where an increase in Prl automatically increases the number of lateral roots and total lateral root length, and therefore Trl and Sa (Adu et al., 2017b). Similarly, an increase in Bf will naturally increase the Nbp, Nrt, Rdw, etc., which could explain the positive association between these traits. The strong phenotypic correlations between traits indicated that selecting for one trait will not be detrimental to the other. harm the other. Additionally, in certain instances, a low correlation between traits can be advantageous as it allows for independent manipulation of those traits (Gifford et al., 2013). The result of this study corroborated Adu et al. (2017b) for *Brassica rapa* seedlings in which significant positive relationship between many RSA and biomass traits (e.g., root dry weight, total root length, root volume, root surface area, etc.) were observed.

The cluster dendrogram revealed a two-cluster solution (Figure 42A), with cluster 2 having a higher affinity for all traits that correlated with the positive quadrants of PC1 (Figure 42B and Figure 40B). It was unsurprising that Rdw was

associated with cluster 2 alongside RSA traits such as Trl, Nbp, Bf, Nrt, Nfol, and Peri, as an increase in these root system traits could lead to increased Rdw. This suggests that genotypes in cluster 2 could be more effective in producing biomass and yield (Adu et al., 2019). However, this is a general statement with a caveat. Roots impose a significant carbon expense on plants. Therefore, when the carbon expenses are weighed vis-à-vis soil resources acquisition, having increased root biomass may not always be advantageous (Adu et al., 2019).

Furthermore, since adaptations that confer drought tolerance in crop plants might involve higher ratios of root-to-shoot growth or mechanisms to avoid or escape drought conditions (Kooyers, 2015), the benefits of investing in greater root biomass would rely on the intrinsic drought tolerance strategies of plants (Adu et al., 2019). For a short-season genotype employing a drought-escape strategy, having greater root biomass might not be beneficial. Conversely, for long-season genotypes, a larger root system could offer a cost-effective advantage later in the cropping season (Adu et al., 2019).

Unlike cluster 2, cluster 1 (which correlated with the negative quadrant of PC1) will be more useful in selection for improved root diameter. The cluster dendrogram also implied that hybridising within each cluster might yield less genetic progress because of the close relationship among the genotypes within those clusters (Maranna et al., 2021). Conversely, crossbreeding between genotypes from different clusters would generate unique breeding materials. For instance, hybridising a member of cluster 1 with a member of cluster 2 and subsequent

backcrossing to the cluster 2 parent might yield a progeny with nearly all the traits of cluster 2 in addition to the pronounced root diameter of cluster 1.

Expectedly, the genotypes were not clustered according to geographical origin. The geographical origin of crops could impact their rooting traits, particularly if the locations have varying climatic and soil conditions, such as dry versus humid environments (Narayanan et al., 2014). Nonetheless, in the current investigation, although some genotypes were obtained from the World Vegetable Centre, all originated from Ghana or nearby West African countries with similar climatic conditions, hence the result.

Second objective: evaluating the effect of drought and the drought-mitigating potential of oil palm EFB biochar on the biochemical indices of selected okra genotypes.

The okra genotypes responded to water regimes and EFB biochar amendment in their biochemical production.

All five biochemical traits varied among the genotypes (Table 8). Similar significant genotypic differences have been reported for SOD in leaf samples of cabbage (Singh et al., 2010), Pro in common bean (Arteaga et al., 2020), Carb in peanut (Pereira et al., 2015), and AsA in cassava genotypes (Ibrahim & Opabode, 2019). The intermediate (Carb and SA) to high (Pro and SOD) CVs further substantiated a moderate to wide range of expression for these traits within the genotypes. This could be due to genetic factors, environmental factors, or a combination of both, and it could afford the opportunity for direct selection for improvement. However, the low CV recorded for AsA suggested limited diversity among the genotypes for this trait, which may significantly impede genetic progress through selection. Such traits will be easier to maintain and stabilize. However, if selection is so desired, molecular marker-assisted selection could be more rewarding. The high and low CVs measured for Pro and AsA respectively corroborated the findings of Špoljarević et al. (2011) and Ibrahim and Opabode (2019), who observed high and low CVs for leaf Pro content among three maize hybrids and leaf AsA content among cassava genotypes respectively. However, Pereira et al. (2015) and Fahimirad et al. (2013) reported low CVs for Carb and SOD among peanut and Canola cultivars, respectively, which contradicted the

result of the present study for these traits. This could be mainly attributed to differences in crop species assessed.

The single effect of the water regime and its interaction with genotype significantly influenced all the biochemical traits (Table 8). Drought elicited greater accumulation of all biochemicals relative to the well-watered control, both for the single effect (Figures 43A, 45A, 47A, 49A, and 51A) and interaction with genotype (Figure 43C, 45C, 47C, 49C and 51C).

The hyperaccumulation of Pro and Carb under water deficit could be attributed to their essential osmoregulatory function. As compatible osmolytes, also called osmoprotectants, the accumulated Pro and Carb trigger water potential reduction without decreasing crops' actual water content (Serraj & Sinclair, 2002). This, in turn, drives the influx of water into cells for turgor maintenance (Farooq et al., 2009a) during drought events. The osmolytes also shield the enzymes and macromolecules within cells from the damaging effects of drought-induced increases in ROS (Farooq et al., 2009b). Pro and Carb are also associated with recovery resistance by serving as a source of respiratory energy to plants under stress. The overproduction of Pro under drought in the present study agreed with Masheva et al. (2022), who reported a significant increase of 1.5-fold to 4.5-fold in leaf Pro concentration of mutant bean lines in response to water stress. A significant increase in Pro and Carb content has also been reported among peanut lines under drought stress relative to the unstressed crops (Pereira et al., 2015). Gupta et al. (2015) reported that drought-tolerant chickpea genotypes accumulate more Carbs than sensitive ones under drought. Thus, using osmoprotectant content as selection

criterion, it will not be out of place to consider genotypes with the greater Pro content (e.g., GH112, GH103, GH144 and VI060692) and Carb (e.g., GH103, VI060692, GH144 and VI060833) at water deficit as more tolerant than genotypes with the lower Pro (e.g., GH120 and GH122) and Carb (e.g., GH122 and GH150) content. A similar view was presented by Praxedes et al. (2006) and McKersie and Leshem (2010), who reported the role of Carb as a compatible solute under drought stress and emphasised its potential use as a marker for selecting more drought-tolerant genotypes.

The hyperactivity of various antioxidants under drought stress could be attributed to their role in neutralising harmful radicals. They function as scavengers of singlet and triplet oxygen, synergists, inhibitors of damaging enzymes, and peroxide decomposers (Manach et al., 1998). SOD, for instance, catalyses the dismutation of $O_2^{\bullet-}$ to H_2O_2 and O_2 (McCord & Fridovich, 1969; Monk et al., 1989). The various antioxidants increase at water deficit and work in concert to maintain cellular ROS levels at a minimum to prevent oxidative damage to plant cells. This study agrees with the results of other studies on the effect of drought stress on these antioxidants. Rahman et al. (2004) reported that SOD activity was increased by water stress in all four cultivars of tomato, and the increase was more rapid and pronounced in drought-tolerant cultivars than in the susceptible cultivars. Ibrahim and Opabode (2019) observed greater endogenous AsA in cassava genotypes at water deficit compared to the well-watered crops. They opined that this confirmed the physiological mechanism of reducing oxidative stress. Okuma et al. (2014) showed improved drought-stress tolerance through endogenous SA activity. If

antioxidant activity is employed as the sole selection criterion, VI060692, GH103, and GH112 could be considered more drought-tolerant due to their higher overall activities. Moreover, juxtaposing antioxidant activity with osmoprotectant content, VI060692, GH103 and GH112 still remained the most promising under drought-stress.

The single effect of biochar significantly influenced all the biochemical traits (Table 8). There was decreasing accumulation of osmoprotectants (Pro and Carb) and reduced antioxidant (SOD, AsA, and SA) activity with increasing biochar rates (Figure 43B, 45B, 47B, 49B and 51B). Similarly, biochar and genotype interaction had a significant effect on Pro, Carb, SA, and SOD, suggesting a differential genotypic response to biochar application (Figure 44A, 46A, 48A and 52A). However, no significant biochar and genotype interaction was observed for AsA. This indicated uniform genotypic response in AsA activity upon biochar application (Figure 50A). The interaction of biochar, genotype and water regime significantly influenced Pro, Carb, and SOD but not AsA and SA. Yet, there was a general trend: the production of each biochemical declined with the increasing rate of biochar application.

Most importantly, EFB biochar application elicited a decline in Pro, Carb, SA, AsA and SOD content under drought-stress relative to the unamended stressed crops (Figure 44B, 46B, 48B, 50B and 52B). This could be attributed to the fact that biochar application ensures the availability of water, proper soil porosity, and nutrients for plants under water deficiency. Torabian et al. (2018) reported that biochar amendment lowered the ROS production in leaf cells of mung beans under

salt stress. Thus, the observed decline in each biochemical in this study could be the case of reduced ROS production due to the improved soil-water availability associated with biochar amendment, making it unnecessary for the plant to activate its osmotic adjustment and antioxidant defence mechanisms. Yildirim et al. (2021) observed a decline in antioxidant (SOD, CAT and POD) activities among cabbage seedlings at water deficit after biochar amendment and opined that this could be due to biochar-mediated reduced negative impact of water stress on the crops. Hafeez et al. (2017) reported reduced Pro among *Glycine max* under drought after biochar amendment and attributed it to the absence of water stress due to biochar application. Biochar amendment significantly decreased Carb (soluble sugars, sucrose, and starch) contents in water-stressed barley relative to control plants and stressed untreated plants (Hafez et al., 2020).

On the whole, in this study, an increase in Pro and Carb content as well as SOD, AsA and SA activities were observed, and specific genotypic differences were established under water deficit. The genotypes with greater overall compatible osmolytes and antioxidant activities could be considered more drought tolerant than those with fewer biochemicals.

Third objective: evaluating the effect of drought and the drought-mitigating potential of oil palm EFB biochar on the yield of selected okra genotypes.

The okra genotypes responded to water regimes and EFB biochar amendment in pod yield.

All yield indices varied significantly among the ten selected okra genotypes (Table 10). An intermediate to high CV was also recorded for these yield indices, further substantiating appreciable variability among the genotypes and the potential for improvement through selective breeding. A similar result of highly significant genotypic difference was reported by Mohammed et al. (2022) for fruit length, number of fruits per plant, and fruit yield per hectare among 36 okra genotypes. Significant differences among okra genotypes have also been reported by Sood et al. (2018) for fruit per plant, fruit length and diameter and by Harris et al. (2019) for fresh pods per plant. However, contrary to the intermediate to high CVs recorded in the present study for various yield indices, other studies have reported low CVs for number of fruits per plant, green fruit length, green fruit width (Aminu et al., 2016; Kenaw et al., 2023) and fruit yield per hectare (Kenaw et al., 2023) among okra genotypes. These contrasting results could suggest greater diversity among the okra genotypes used in the present study and could offer more improvement opportunities through selection.

The single effect of the water regime and its interaction with genotype significantly influenced all the yield indices of okra (Table 10). Drought reduced each yield index compared to the well-watered control, both for the single effect

(Figures 53A, 55A, 57A, and 59A) and interaction with genotype (Figures 53C, 55C, 57C and 59C). This observation could be due to a myriad of factors. Water deficit impedes cell division and enlargement due to impaired enzyme activities, turgor loss, and reduced energy supply (Kiani et al., 2007; Taiz & Zeiger, 2010) and could have accounted for the reduced pod length and pod diameter. The reduction in Npp and Tpy could be explained by the stomatal closure and diminished photosynthesis associated with the drought effect. Stomatal closure during drought is primarily to limit transpirational water loss. However, this has the consequence of reduced CO2 and nutrient intake, thereby altering many physiological processes, such as those regulating photosynthetic reactions (Xiong & Zhu, 2002). ROS accumulate during water deficit, which can cause oxidative damage to lipids, proteins, and other macromolecules, disrupting normal plant metabolism (Rout & Shaw, 2001). This can significantly diminish the photosynthetic rate under drought conditions, primarily by interfering with the photosynthetic apparatus. The microbial activities of nutrient mineralisation could also be impeded by water deficit, resulting from reduced nutrient availability for crop growth, development, and productivity. These, either singly or in concert, could have resulted in the reduced yield observed.

The drought effect was more pronounced in some genotypes than others. For instance, GH120, GH121 and GH122 recorded less Npp under drought than VI060692, GH103 and GH112 (Figure 57C). Similarly, a greater reduction in Tpy was observed for VI060833, GH120, and GH122 than for VI060692, GH103 and GH112 (Figure 59C). This could indicate that the okra genotypes responded

differently to water deficit, with genotypes having the least yield reduction being more resistant to drought effect. Drought-resistant crops activate their defense mechanisms when faced with water scarcity (Chaves & Oliveira, 2004), allowing them to maintain appreciable yields compared to susceptible genotypes. In the present study, VI060692, GH103 and GH112 were the top individuals in overall biochemical contents. This could suggest that these genotypes effectively activated their biochemical defense mechanism during the drought events. It could further explain the higher Tpy and Npp obtained for them than the other genotypes. It is also worth mentioning that these genotypes were selected from cluster 2, which showed superiority in nearly all RSA traits. Hence, this alluded to the potential effectiveness of RSA traits as a selection criterion in breeding for higher yields under water deficit. This result corroborated the findings of Bahadur et al. (2013), in which pod yield reduction of 40.3 % and 45.6 % was reported in okra under moderate and severe drought stress relative to the well-watered control. Oluwasemire and Oladuji (2018) observed a substantial reduction in fruit number and fresh fruit weight among okra genotypes at 50 % potential evapotranspiration (ET_p) water regime than the 100 % and 125 % ET_p, and opined that the 50 % ET_p be avoided. Tiwari et al. (1998) and Bahadur et al. (2007) had earlier reported a significant reduction in okra yields due to the water-deficit.

The single effect of biochar, its interaction with genotype and the three-way interaction of biochar, genotype and water regime significantly influenced all the yield indices of okra (Table 10). More yield was recorded with increasing rate of EFB biochar application for the single effect (Figure 53B, 55B, 57B, and 59B),

interaction with genotypes (Figure 54A, 56A, 58A and 60A), and the three-way interaction with genotype and water regime (Figure 54B-C, 56B-C, 58B-C, and 60B-C). The drought-mitigation potential of the EFB biochar was ordered as 20 t/ha > 10 t/ha > 0 t/ha. A similar increasing trend of up to 30 t/ha and reaching 40 t/ha was observed by Li et al. (2018) in tomatoes under water deficit. The authors performed a cost-benefit analysis. They suggested that the net profit of biochar application compared to non-biochar treatment was positive at 10 t/ha, 20 t/ha, and 40 t/ha but negative with a 60 t/ha rate. Among the well-watered crops, too, biochar application enhanced crop performance, resulting in higher yields than their unamended counterparts.

A couple of reasons could have accounted for the improved yields observed with biochar amendment. First, biochar can improve the physical, chemical, and biological properties of soils (Jabborova et al., 2021). Singh et al. (2019) noted that biochar application increases soil organic matter content, reduces bulk density, improves aeration and cation exchange capacity, decreases leaching, enhances water-holding capacity, and promotes microbial activities (Singh et al. 2019). Second, in addition to its rich carbon content, biochar also contains essential plant nutrients such as N, P, and K and important cations such as Ca and Mg, which are key for plant growth (Major et al., 2010). Jabborova et al. (2021) noted that these nutrients significantly contribute to enhancing nutrient availability for crops. Hence, the improved okra yield could be due to improved soil physical and chemical properties, improved nutrient availability, or a synergy of both mechanisms. Studies abound in which biochar application has been shown to

improve crop yield under drought and well-watered conditions, which all agreed with the result of the present investigation.

Yakubu (2016) found that biochar improved okra fruit yield under deficit irrigation (DI) and recommended the practice of DI with biochar amendment in water-scarce areas. Applying 1.25 % biochar was observed to increase pod length and grain yield in soybeans under drought and well-watered conditions (Gavili et al., 2019). In a study by Akhtar et al. (2014) under full irrigation (FI), DI, and partial root-zone drying (PRD), biochar application at 5 % (w/w) increased the fresh fruit yield of tomato plants in all the water regimes. The authors observed that fruit yields obtained with biochar amendment at FI, DI and PRD were 20 %, 6 %, and 13 % higher than the non-biochar control. Ali et al. (2019) also observed applying biochar to cucumber plots increased the total yield of the crops under water-deficit conditions. Literature affirms that biochar application could minimize yield losses due to water deficits in vegetables. Hence, the result of the present study is unsurprising.

Relationships between traits

The regression studies uncovered a useful relationship between total pod yield and Pro, SA, and SOD under both drought stress and well-watered conditions (Figure 61A-63B). The positive significant relationship observed at both water deficit and ample water between total pod yield and Pro and SA suggested that an increase in Pro content and SA activity will result in a corresponding increase in total pod yield, irrespective of the water regime. Thus, Pro and SA could have contributed significantly to yields regarding both water deficit and ample water.

This is unsurprising, particularly under drought stress, given these biochemicals' osmoregulatory and antioxidant roles. The relationship between the biochemical traits and total pod yield was stronger at water deficit than ample water for SOD and SA. This suggested that SOD and SA had more activity and contributions to yield under water deficit than ample water. This was expected because, with ample water, plant cells do not need to produce high amounts of osmolytes and antioxidant enzymes. But at water deficit, these are needed for osmotic adjustment and maintenance of balance between production and removal of ROS. However, for Pro, the relationship with Tpy was stronger regarding ample water than in water deficit conditions, which is surprising and requires further investigation.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study employed custom-made rhizoboxes filled with unamended soil to rapidly screen for genetic variations in the RSA traits of 60 okra genotypes at the seedling stage (first experiment). From this, ten okra genotypes were selected to evaluate their biochemical and yield responses under drought stress and EFB biochar amendment (second experiment). Both experiments were carried out in a greenhouse. It was hypothesised that: (1) Genotypic variation does not exist in the RSA of okra; (2) Drought stress does not affect the biochemical traits of okra; (3) Drought stress does not affect the yield traits of okra; (4) Oil palm EFB biochar does not have modulating effect on okra's biochemical traits under drought stress; and (5) Oil palm EFB biochar does not have modulating effect on okra's yield traits under drought stress.

The result revealed significant genetic diversity among the okra genotypes in all the RSA and biomass traits. Also, while broad-sense heritability was high for all traits, GCV was high for the majority of traits, including all number, diameter, area and volume traits, and most length (e.g., Trl, Peri, RLDR1, etc.) traits. This suggested a broad genetic base among the okra genotypes and minimal environmental impact on these traits. These traits can be exploited among the genotypes through direct selection in a breeding programme to develop varieties with robust RSA for acquiring soil resources and large biomass for higher yield. However, a few traits recorded low GCV, such as Lra and Prl, suggesting limited

improvement opportunities through direct selection. For such traits, molecular marker-assisted selection could be more appropriate. The positive correlation between most RSA and biomass traits meant that selecting one would improve the other without major trade-offs.

Water deficit significantly affected the biochemical and yield traits of each okra genotype. There was an increased level of osmolytes and hyperactivity of antioxidants among the genotypes under water deficit relative to their well-watered counterparts. This suggested that the genotypes adopted the tolerance mechanisms of osmotic adjustment and antioxidant defense when challenged by drought. Interestingly, significant differences existed among the genotypes in their tolerance abilities, which could be exploited in a breeding programme to develop drought-tolerant okra varieties. The drought-stressed crops experienced significant yield penalties compared to their counterparts treated with ample water, indicating that, albeit okra is relatively drought-tolerant, major yield loses could be experienced. Remarkably, the okra genotypes varied significantly in their yield indices under drought, with some genotypes maintaining appreciable Npp and Tpy (particularly VI060692 and GH112) than the others. Thus, as the need to develop more drought-tolerant crop varieties in the face of changing climate continues, these genotypes could become valuable materials in breeding drought-tolerant okra varieties. The significant positive association between the biochemical traits and Tpy at both water regimes presented an opportunity for simultaneous improvement in these traits.

There was a substantial modulating effect of EFB biochar on the biochemical and yield indices of the okra genotypes under drought stress. EFB biochar application significantly mitigated the drought effect in each genotype, as evidenced by declined osmolyte accumulation, reduced antioxidant activity, and improved pod yield. The drought-mitigation potential of the EFB biochar increased with increasing rate of application. Hence, EFB biochar can be adopted to enhance okra productivity, especially in areas where production is majorly rainfed. This will not only improve okra yield but also contribute immensely to mitigating the environmental pollution associated with oil palm empty fruit bunch.

In all, the following specific conclusions can be made:

1. Genotypic variation exists in the RSA of okra.
2. Drought stress affects the biochemical traits of okra.
3. Drought stress affects the yield traits of okra.
4. Oil palm EFB biochar has modulating effect on okra's biochemical traits under drought stress.
5. Oil palm EFB biochar has modulating effect on okra's yield traits under drought stress.

Recommendations

Based on the results of this study, the following recommendations can be made:

1. Greenhouse conditions differ significantly from field conditions and can influence crops' RSA and biomass traits. Therefore, a similar experiment should be carried out under field conditions to validate these findings.

2. Further studies should investigate the narrow-sense heritability of the RSA traits and the specific and general combining ability of the selected genotypes for biochemical and yield traits.
3. The oil palm EFB biochar was effective in improving okra yields under drought. This, however, needs to be validated under field conditions before any large-scale adoption in mitigating drought impacts in okra production.
4. Genotypes VI060692 and GH112 were the most resilient under drought effect, evidenced by greater overall biochemical content and yield, and merit further investigation in view of breeding drought-tolerant varieties.

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APPENDICES

Appendix 1: Okra genotypes and their country of origin

Okra genotype	Originating country	Okra genotype	Originating country
VI059458	Malawi	CE118	Ghana
VI060686	Ghana	GH119	Ghana
VI060691	Benin	GH120	Ghana
VI060692	Benin	GH121	Ghana
VI060821	Mali	GH122	Ghana
VI060830	Sudan	GH123	Ghana
VI060831	Sudan	GH125	Ghana
VI060833	Cameroon	GH128	Ghana
VI060844	Cameroon	GH130	Ghana
VI060871	Niger	GH131	Ghana
VI060874	Senegal	GH132	Ghana
VI062547	Niger	GH133	Ghana
VI063894	Togo	GH135	Ghana
VI063895	Togo	GH136	Ghana
VI063900	Togo	GH144	Ghana
VI063912	Benin	GH145	Ghana
VI063926	Ghana	GH147	Ghana
VI063947	Nigeria	GH148	Ghana
GH102	Ghana	GH150	Ghana
GH103	Ghana	GH151	Ghana
GH104	Ghana	GH153	Ghana
GH106	Ghana	GH154	Ghana
GH108	Ghana	GH156	Ghana
GH111	Ghana	GH157	Ghana
GH112	Ghana	GH159	Ghana
GH113	Ghana	GH164	Ghana
GH114	Ghana	GH165	Ghana
GH115	Ghana	GH167	Ghana
GH116	Ghana	GH169	Ghana
GH117	Ghana	GH170	Ghana

Appendix 2: Root analysis meta-data from Rhizoviosion Explorer

RhizoVision Explorer Version	2.0.2	
Root type	Broken roots	
Image Thresholding Level	200	
Invert images	False	
Keep largest component	False	

Filter noisy components on background	True	
Maximum background noisy component size	1	
Filter noisy components on foreground	False	
Maximum foreground noisy component size	0	
Enable edge smoothing	False	
Edge smoothing threshold	2	
Enable root pruning	True	
Root pruning threshold	5	
Convert pixels to physical units	True	
Number of Pixels per mm	8.86	
Pixel to millimeter conversion factor	0.112867	
Diameter Range 1	0	1
Diameter Range 2	1	2

Appendix 3: Cos^2 of variables for the first five PCs, the first three of which had eigenvalues greater than one.

Trait	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Rdw	0.59	0.07	0.24	0.00	0.07
RS	0.31	0.00	0.38	0.01	0.18
Nfol	0.61	0.12	0.16	0.00	0.00
Nrt	0.49	0.23	0.13	0.00	0.00
Nbp	0.64	0.00	0.09	0.04	0.01
Trl	0.53	0.23	0.02	0.00	0.11
Bf	0.58	0.17	0.10	0.00	0.00
Na	0.84	0.12	0.02	0.00	0.00
Md	0.01	0.05	0.00	0.90	0.00
Peri	0.87	0.00	0.10	0.00	0.00
Vol	0.39	0.16	0.05	0.03	0.19
Sa	0.83	0.14	0.01	0.00	0.00
RLDR2	0.71	0.27	0.00	0.00	0.00
PADR1	0.63	0.10	0.23	0.00	0.02
PADR2	0.71	0.28	0.00	0.00	0.00
SADR1	0.59	0.08	0.29	0.01	0.01
SADR2	0.71	0.28	0.00	0.00	0.00
VDR1	0.68	0.03	0.23	0.01	0.01
VDR2	0.70	0.29	0.00	0.00	0.00

Appendix 4: Biochar application rate estimation

The mass of soil to be sampled from one-hectare space was determined as:

- Soil bulk density = 1.3 gcm^{-3}
    - Converting this into kgm^{-3} = 1.3 x 1000 = 1300 kgm^{-3}
- Depth at which the soil was sampled = 20 cm
    - Converting this into m = 20/100 = 0.2 m
- Land area = I hectare = 100 m x100 m = 10,000 m^2
- Mass of sampled soil = bulk density x depth at which soil was sampled x land area
    - 1300 kg~~m^{-3}~~ x 0.2~~m~~ x 10,000 ~~m^2~~ = 2,600,000 kg soil
    - Note: This will be the amount of soil sampled from 1ha land at 20 cm depth and 1.3 gcm^{-3} bulk density.

Biochar rates: three biochar rates were used: 0, 10 and 20 t/ha.

0 t/ha: This served as the control without biochar.

10 t/ha

- 10 tons of biochar is to be applied to the 2,600,000 kg soil.
- Converting 10 t/ha to kg/ha = 10 x 1000 = 10,000 kg/ha
    - Therefore, 10,000 kg of biochar is to be applied to the 2,600,000 kg soil.
- Mass of soil per PVC = 37.6kg
- If 2,600,000 kg soil = 10,000 kg of biochar

  37.6kg soil = y?

  y = 37.6/2,600,000 x 10,000 = 0.16538kg = **144.6 g per PVC**

20 t/ha

- 20 tons of biochar is to be applied to the 2,600,000 kg soil.
- If 10 t/ha = 144.6 g

  20 t/ha = y?

y = 20/10 x 144.6 = **289.2 g biochar**.

Appendix 5: Estimation for various water-regimes.

The 100% field capacity (FC) of UCC soil is 30% of soil mass.

- 30% soil mass = 100% FC

  y% soil mass = 90% FC; y = 27%.
- Find the 27% of 37.6kg
  - 27/100 x 37.6 kg = 10.152kg = 10, 152 g per PVC
- Convert 10,150 g to volume
  - density of water = 1g/cm^3
  - density (P) = mass (m)/volume (v); v = m/P = 10152g/1gcm^{-3} = 10,152 cm^3
- Convert cm^3 to Liters
  - 1 cm^3 = 0.001 liter

    10152 cm^3 = y liter; **y = 10.152 liters**

To find the amount of water needed to maintain the soil at 30% FC

- 100% FC = 11.28 L

  30% FC = y L = 0.3 x 11.28 L = **3.384 liters**