Harnessing genetic variability for root and shoot morpho-physiological traits contributing to drought stress tolerance in African Spider Plant *Gynandropsis gynandra* (L) Briq accessions.

By

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Thesis Abstract

African spider plant (*Gynandropsis gyanandra* (L.) Briq.) is a promising leafy vegetable, which is an important source of zinc, iron, calcium, magnesium, and vitamins. It forms one of the strategic crops that are being used to improve the resilience of local communities to drought. These crops, often referred to as underutilized, forgotten, neglected or orphan crops are adapted to the local environment and have potential to address climate change induced abiotic stresses such as drought. Drought is major biotic stress threatening the sustainability of agriculture and food security, especially in SSA and leads to shortened cropping seasons with significant yield reductions. This study was conducted to i) assess the variation of morpho-physiological and biochemical traits of *Gynandropsis gynandra* in response to drought stress, ii) estimate the heritability and variance components for morphological and physiological traits in *Gynandropsis gynandra* under drought stress and iii) assess drought indices together with root traits to identify tolerant African spider plant (*Gynandropsis gynandra* (L.) Briq.) genotypes.

In the first experiment, 18 African spider plant accessions were evaluated in the tunnels at the University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa under drought-stressed and non-stressed conditions using a split-split plot design with four replications across three water regimes: severe drought (30% field capacity), intermediate drought (50% field capacity), and well-watered (100% field capacity). The following morpho-physiological and biochemical traits were recorded; days to 50% flowering (Fl), plant height (Ph), leaf length (Ll), leaf width (Lw), stem diameter (Sd), chlorophyll content (Spad), relative water content (Rwc), net photosynthesis (Photo), transpiration rate (Trans), stomatal conductance (Cond), proline content (Pro), number of leaves per plant (Nl) and leaf yield (Ly). Data were subjected to the following statistical analysis: analysis of variance (ANOVA), Pearson's correlation coefficient, principal component analysis (PCA), and cluster analysis. Proline content rose significantly under stress but was inversely associated with agronomic traits under both optimal and water-limited conditions. Two accessions with high leaf yield under drought stress and beneficial adaptive traits were identified for further use in breeding for drought tolerance in the crop species.

The second experiment investigated variance components, heritability and path coefficient analysis of yield and yield-related traits in the 18 spider plant accessions. A randomized complete block design (RCBD) experiment was carried out with four replications across three water regimes: severe drought (30% field capacity), intermediate drought (50% field capacity), and well-watered (100% field capacity). The genotypic coefficient of variation (GCV) ranged from 0.10 % (relative water content) to 46.86 % (leaf yield) under well-watered conditions, 0.11% (relative water content) to 54.42 % (leaf yield) under mild stress conditions, and from 0.00% (stomatal conductance) to 74.22% (leaf yield) under severe stress. The phenotypic coefficient of variation (PCV) ranged from 0.10% (relative water content) to 46.91% (leaf yield) under well-watered conditions, 0.11 from 0.10 % (relative water content) to 46.86 % (leaf yield) for mild stress and 0.00% (stomatal conductance) to 74.34% (leaf yield) severe stress. Under drought stress conditions, the experiment showed a high to moderate heritability estimate along with a high to moderate genetic advance for the number of leaves, leaf width, plant height, and stem diameter, indicating that these attributes are governed by additive gene action. The number of leaves per plant, plant height, days to 50% flowering, relative water content, net photosynthesis and leaf length were identified as target traits that could be used to improve spider plant leaf yield under drought-stressed conditions.

In the third experiment, the same African spider plant accessions were evaluated to compare changes in root traits under intense drought stress conditions and identify drought-tolerance indices that can be utilized in selecting African spider plant genotypes. The following six drought tolerance indices were evaluated: stress susceptibility index (SSI), yield index (YI), stress tolerance index (STI), geometric mean of productivity (GMP), stress tolerance (TOL), and mean productivity (MP). Six root traits including: total root length (TRL), total root volume (TRV), root dry weight (RDW), root: shoot ratio (RR), total root area (TRA), and average root diameter (ARD) were recorded as well as leaf yield (Ly). The experiments were laid out in a randomized complete block design with three replications and two water regimes treatments namely non-stressed and drought stressed conditions. The ANOVA showed that genotypes varied in leaf yield, but non-significant values were observed for root traits. Five drought tolerance indices namely TOL, GMP, STI, MP, and YI, were discovered to be effective in selecting stress-tolerant genotypes. Root: shoot ratio was identified as a useful trait in the selection of tolerant genotypes. Overall, the study managed to capture and identify genotypes that can be used for future breeding programs for drought stress. Lastly, root traits together with stress indices that can be utilized in selecting drought-tolerant African spider plant genotypes were identified.

Declaration 1: Plagiarism

I, Tinashe Chatara, declare the following.

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As the candidate's supervisors, we agree to the submission of this thesis:

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Prof. Julia Sibiya (Supervisor)

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Dr. Cousin Musvosvi (Co-supervisor)

Declaration 2: Publications

Chatara, T.; Musvosvi, C.; Houdegbe, A.C.; Sibiya, J. Variance Components, Correlation and Path Coefficient Analysis of Morpho-Physiological and Yield Related Traits in Spider Plant (*Gynandropsis gynandra* (L.) Briq.) under Water-Stress Conditions. Agronomy 2023, 13, 752. **https://doi.org/10.3390/agronomy13030752**

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Assessment of drought tolerance indices to identify tolerant African Spider plant (*Gynandropsis gynandra* (L.) Briq.) genotypes. **Under review in Heliyon.**

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Dedication

This work is dedicated to the almighty God, the giver of life.

My mother Sheillagh Zimonte for the sacrifices and unconditional love for us your children. I hope I will be a son you can be proud of.

My father Mr. Vicus Chatara for the encouragement and teachings you have given me up to this day.

My brothers and sisters

For all the small community farmers around the continent, this work is for you

Abbreviations

ALV	African leafy vegetables
ANOVA	Analysis of variance
ABA	Abscisic acid
ARD	Average root diameter
Cond	Stomatal conductance
CV	Coefficient of variation
Df	Degrees of freedom
Fl	Days to 50% flowering
GA	Genetic advance
GAM	Genetic advance as a percentage of the mean,
GCV	Genotypic coefficient of variation
GMP	Geometric mean of productivity
H2	Broad-sense heritability
LWP	Leaf water potential
LSD	Least Significant Difference
ROS	Reactive oxygen species
Rubisco	Ribulose 1,5 bisphosphate carboxylase/oxygenase
RuBP	Ribulose 1,5 bisphosphate
SSA	sub-Saharan Africa
WUE	Water use efficiency
RWC	Relative water content
Ly	Leaf yield
Ph	Plant height

Photo	Net-Photosynthesis rate
Pro	Proline content
Ll	Leaf length
Lw	Leaf width
Nl	Number of leaves
Sd	Stem diameter
Spad	Chlorophyll content
Trans	Transpiration rate
VG	Genotypic variance
VG×E	Genotype × environment interaction variance
VE	error variance
VP	Phenotypic variance
PCV	Phenotypic coefficient of variation,
RDW	Root dry weight
RR	Root to shoot ratio
TR	Total root length
TRV	Total root volume
TRA	Total root area

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Chapter 1. Introduction

Background of the study

African spider plant *Gynandropsis gynandra* (L.) Briq also known as *Cleome gynandra* L. is an African leafy vegetable that belongs to the *Cleomaceae* family. The crop is important in contributing to the healthy nourishment of a great number of rural Africans (Chweya, 1997; Mishra *et al.,* 2011; Wenyika *et al.*, 2015). The species is a diploid that has a recurrent chromosome number of twenty (2n = 2x = 20) (Chweya, 1997). African spider plant can also be identified by various names such as African cabbage, cat's whiskers, and spider wisp (Mishra et al., 2011). It has become one of the principal African leafy vegetables (ALV) behind amaranth (*Amaranthus spp.)*, African nightshade (*Solanum spp),* and cowpea (*Vigna unguiculata*). Spider plant grows as a weed in most countries of (SSA). Traditionally, the spider plant is not grown as a commercial crop; however, for several years, it has been a semi-domesticated volunteer crop in fields as well as on fertile soils close by and at homesteads (Mnzava and Chigumira Ngwerume, 2004).

Although the spider plant grows as a weed in most countries of SSA, this leafy vegetable is rich in vitamins B together with micronutrients such as zinc, iron and iodine that could play a significant role in allaying hunger and malnutrition(Sogbohossou et al., 2020; Houdegbe et al., 2022a). In addition, the spider plant is nutritionally superior in comparison to exotic vegetables such as cabbage, cauliflower, and broccoli. Spider plant is also a rich source of proteins, in addition to having high levels of vitamins A and C, magnesium, iron, calcium, and antioxidant enzymes such as peroxidases, dismutase, and catalase (K'Opondo, 2011a; Sogbohossou et al., 2020; Chataika et al., 2021; Houdegbe et al., 2022b). For this reason, it has been identified as a vegetable that will play an essential role in food security, nourishment, and generation of income among the poor in rural as well as urban communities (Abukutsa-Onyango, 2007b; Onyango et al., 2013c).

Importance and utilization of spider plant

The spider plant has been shown to have several uses, which highlight its importance. Nearly every organ of the plant is essential. The tender leaves, shoot and flowers can be eaten as vegetables in regions where the plant grows (Chataika et al., 2020c). This ALV can be eaten as a tasty relish, stew, or a side dish consumed with staple foods (Sogbohossou et al., 2018a).

Another essential attribute of the spider plant is its antioxidant properties in particular radical scavenging activity. A free radical scavenger, as defined by (Prakash et al., 2011) is an antioxidant that aids in defending cells from damage triggered by free radicals. These free radicals are a culmination of unsteady molecules formed during regular cell metabolism. Free radicals multiply in cells damaging other molecules in the process. There is a high risk of getting cancer and other diseases as a result of the damage caused by these free radicals (Calabrese et al., 2008; Perrone et al., 2010, 2018). The radical scavenging properties which help to suppress harmful effects of free radicals in various diseases such as cancer, underline the economic importance of the African spider plant (Rahman et al., 2015).

Spider plant has also been found to have medicinal properties. In Africa, this leafy vegetable has been applied traditionally to treat various diseases such as stomach aches, headaches, diabetes, and cardiovascular diseases (Chataika et al., 2021). The spider plant has pharmacological characteristics. Its anti-inflammatory activity has been documented by Narendhirakannan et al. (2005). This was also confirmed by the results of the study conducted by Mibei et al. (2012), who observed that *Gynandropsis gynandra* has active compounds which include alkaloids, flavonoids, saponins, phenols, terpenoids, and steroids, which are responsible for the anti-inflammatory activity. A study conducted by Dzomba et al. (2014) also reviewed how leaf extracts of this leafy vegetable can be possibly used in preserving beef. The leaf extracts were discovered to enhance shelf life, improve sensorial features and nutrition of the meat.

Constrains to production

Despite its significance, the African spider plant is underutilized in many sub-Saharan African countries. Poor germination and a shortage of good seed , erratic rainfall , poor soil fertility, insect pests, competing producer preferences, and insufficient agronomic skills are all production challenges (Liang et al., 2011; Mbugua et al., 2011; Onyango et al., 2013b; Katengeza et al., 2019; Blalogoe et al., 2020; Chataika et al., 2022). The latter emphasizes the shun spider plant has received from extension and research services, resulting in low yields. One of the constraints in spider plant production is the scarcity of seed and the low fertility of the soil. African spider plant has been neglected for a long time. Because of the lack of research on the crop there has been a lack of quality seed that can be used by farmers. Farmers use recycled seeds that might harbour pests and diseases. African spider plant is mainly grown in the marginal areas characterized by poor soils hence low yields are achieved (Mbugua et al., 2011; Katengeza et al., 2019; Chataika et al., 2022) .

Another issue that is more prevalent in African spider plant is poor germination. The findings are in line with previous research that revealed poor germination and seed dormancy as major constraints to spider plant production (Blalogoe et al., 2020). Drought is one of the major challenges hampering spider plant production in African countries. This is in line with the findings of several authors who have identified frequent droughts in many parts of Sub-Saharan Africa (SSA) as having disastrous effects on agriculture and food security. This study was, therefore, undertaken to explore and identify key traits that can be used in screening African spider plant genotypes for drought.

Drought and selection for drought tolerance in spider plant

Due to the complexities of drought, conventional breeding methods must be utilized to enhance selection efficiency. The accessions must be thoroughly and effectively screened under drought stress environments to identify suitable genotypes. Drought stress triggers a series of physiological and biochemical responses in plants. Characterizing these responses provides insights into the mechanisms underlying drought tolerance. It also helps identify key traits associated with drought adaptation, such as root architecture, osmotic adjustment, stomatal regulation, antioxidant defence, and water-use efficiency. Understanding these mechanisms can guide the development of targeted strategies to enhance plant resilience to drought.

Estimating variance components, heritability, and conducting path coefficient analysis is essential in plant breeding under drought stress conditions. These estimates provide insights into the genetic basis of drought tolerance, guide breeding strategies, identify drought-tolerant genotypes, understand trait relationships, and enhance breeding efficiency. Furthermore, these estimates contribute to the development of improved African spider plant crop varieties that can withstand and thrive under drought conditions, ensuring food security and agricultural sustainability (Bernardo, 2020).

Root traits and drought tolerance indices are vital in plant breeding for drought tolerance. Roots contribute to enhanced water uptake, drought avoidance, and nutrient acquisition. More importantly, root traits are closely related to above-ground traits and can be used as indirect selection criteria. Drought tolerance indices provide a comprehensive assessment of plant performance under drought conditions. By focusing on root traits and using drought tolerance indices, breeders can develop crop varieties with improved drought tolerance, ensuring sustainable agricultural productivity in water-limited environments (Dayoub et al., 2021).

Rationale of the study

The universal population is projected to come up to 8.3 billion by the year 2030, then expected to reach 9.1 billion by 2050 (Yaduvanshi et al., 2022). Furthermore, an estimated 2.4 billion people are projected to be residing in developing nations centralized in South Asia and SSA. Traditionally, agriculture is pivotal to the economy in these regions and the main creator of employment (Lipper et al., 2014). Additionally, studies conducted show that Africa's population is experiencing the fastest growth globally with 50% of the growth expected in the next 18 years. Because of this rise in the population, a food crisis is imminent (Dubey et al., 2019).

This has also been exacerbated by an insubstantial number of recognized and edible crops in world agriculture at present, thus signifying a serious threat to food security especially in the wake of climate change which is likely to give rise to a series of extreme events (Lipper et al., 2014). Climate change continues to be one of the most predominant conundrums faced by humankind presently as temperatures keep on escalating. The escalations in temperature in turn have triggered a myriad of extreme weather events such as floods, heatwaves, and droughts (Feulner, 2017; Mabhaudhi et al., 2019a). Akpinar et al. (2013) reported that drought stress could potentially lower the average crop yields by an excess of 50% and would hamper agricultural productivity.

With the advent of droughts, ALVs play a crucial role in the livelihood of people in the developing world (Mayes et al., 2012a; Baldermann et al., 2016). One such crop with enormous potential is *G. gynandra.* This leafy vegetable is capable of producing yield under stressed conditions due to its ability to adapt to prolonged periods of drought (Chivenge et al., 2015a). However, the spider plant is characterized by a lack of improvement relative to its potential. It is not conventionally grown as a commercial crop, for that reason it is still viewed as a weedy and volunteer crop in most farming systems. This ALV has been overlooked by agricultural researchers and governments' agricultural enhancement policies (Ebert, 2014), hence classified as neglected, forgotten, underutilized or orphan crop. Because it is an underutilized and orphaned crop, there are no established varieties of *G. gynandra*, the majority of accessions exist as landraces, that is, an assortment of genotypes often adapted in their locations (Adhikari et al., 2017).

An organized collection system of *G. gynandra* has not been realized in all the geographical areas of several countries in which the diversity of the species exists. Furthermore, traits closely associated with leaf yield in *G. gynandra* have been discovered, but little is known on morphological and physiological traits associated with the leaf yield especially under drought stress (Sogbohossou et al., 2018c; Dubey et al., 2019). A great deal of crop breeding effort has been concentrated on the expression of aboveground traits with the goals of boosting yield and drought tolerance attributes. The importance of roots in achieving these goals has greatly been ignored. Phenotyping continues to be the major criterion for selecting breeding materials. The utilization of morphological and physiological parameters in the evaluation of genotypes is vital for exploring the association between morpho-physiological processes and drought tolerance (Sharif et al., 2018). In this respect, several morphological and physiological parameters can potentially be used in the identification of drought-tolerant genotypes (Chu et al., 2014; Hussain et al., 2018).

Overall Objective

Overally, the study aimed to establish the genetic basis for breeding drought-tolerant cultivars in *Gynandropsis gynandra* to improve the resilience of local communities to climate change.

1.5.1 Specific objectives

The specific objectives were to:

i) Determine the variation of morphological, physiological and biochemical traits among *G. gynandra* accessions in response to drought stress.

ii) Estimate heritability, variance components and path coefficient analysis to identify secondary traits for aiding the selection of drought-tolerant *G. gynandra* accessions .

iii) Assess leaf yield-based drought tolerance indices and root characteristics under drought stress and optimal conditions in *G. gynandra.*

1.5.2 Research hypotheses

The study tested the following hypotheses:

i) There is variation among *G. gynandra* accessions for morphological physiological and biochemical traits in response to drought stress.

ii) Heritability estimates of leaf yield and related morpho-physiological traits differ under drought and non-drought conditions; and some secondary traits can be used to aid selection for leaf yield among *G. gynandra* accessions under drought.

iii) There are differences in stress tolerance indices and root characteristics in *G. gynandra* under varied soil moisture deficit regimes.

1.6 Overview of the dissertation

This dissertation contains five written chapters that are presented in the form of research papers closely linked to the objectives (Table 1.2), a predominant format endorsed by the University of KwaZulu-Natal. Each objective follows a stand-alone format of a research paper. Consequently, introductory details between chapters and repetition of references were unpreventable, The Crop Science journal referencing style was used in the chapters of this dissertation.

Table 1. 1 Structure of the thesis

Chapter	**Chapter title**
1	Introduction of the thesis
2	Literature review
3	Morpho-physiological and biochemical characterization of African spider plant (*Gynandropsis gynandra* (*L.*) Briq.) genotypes under drought and non-drought conditions
4	Variance components, heritability, correlation, and path-coefficient analysis for morpho-physiological traits of African spider plant (*Gynandropsis gynandra* (L.) Briq.) under drought and non-drought conditions
5.	Assessment of drought indices and root traits to identify tolerant African spider plant (*Gynandropsis gynandra* (L.) Briq.) genotypes.
6	Overview and implications of study

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Chapter 2. Breeding for drought tolerance in *Gynandropsis gynandra:* A review of literature

Abstract

Despite increased knowledge of the potential of *Gynandropsis gynandra* in contributing to food security and improvement of farmer livelihoods, coordinated investigation agendas to facilitate African spider plant production and use by local communities are oftentimes lacking. However, throughout the world, multiple efforts are being made to mitigate the effects of drought by breeding drought-resistant varieties. Nevertheless, progress is hindered because drought tolerance is a complex trait regulated by multiple genes, and its full expression is influenced by the environment. As a result, breeding for drought tolerance necessitates the integration of various knowledge systems and methodologies from various plant sciences disciplines. This review discusses the factors involved in the development of African spider plant cultivars with high leaf yield and drought tolerance. It discusses the impact of drought stress on spider plant, crop adaptation, mechanisms to drought stress, plant development and synthesis of high biomass, and methods for screening African spider plant accessions for drought tolerance and high leaf yield. Moreover, the review highlights African spider plant breeding approaches, genotype x environment interaction, and yield stability. The information provided in this review could be used as a guideline in African spider plant breeding to increase leaf yield and drought tolerance.

Keywords: Breeding, phenotyping, drought tolerance, African spider plant, leaf yield

2.1 Introduction

African spider plant *Gynandropsis gynandra* (L.) Briq is an underutilized African leafy vegetable, also referred to as an orphan crop, that has been cultivated in Africa, Asia, and the Americas. Although information to describe its exact origin is not clear, it is thought to have originated in Africa and Asia (Gahukar, 2014). *Gynandropsis gynandra* is a fast-developing leafy vegetable that is becoming more popular as a commercial crop in West and East Africa (Gahukar, 2014). It is grown at elevations ranging from 0 to 2400 meters above sea level and prefers well-drained clay loam soils with a pH of 5.5–7.0 and temperatures ranging from 18 to 25°C (Sogbohossou et al., 2019). The African spider plant belongs to the C4 photosynthetic pathway, which is well adapted to high temperatures and variable agroclimatic conditions (Figure 2.1).

Figure 2. 1 The morphology of African spider plant from A-B flower types, C-D leaf types and E-F seed size

Along with amaranthus, cowpea, and African nightshades, the African spider plant is an important leafy vegetable (Omondi et al., 2017a). According to Onyango et al. (2013a), the African spider plant is planted 57% of the time for household consumption and 43% of the time for money creation. Furthermore, it has medicinal value to the people, where it is used to boost milk production in mothers, treat anaemia, rubefacient, vesicant, rheumatism, diarrhoea, and chest pain (Mnzava and Ngwerume, 2004).

Additionally, the African spider plant has been used in the biological control of insect pests such as diamondback moth larvae, and flower thrips (Rensburg et al., 2018). The production of the African spider plant among communities has increased in various African countries. Omondi et al. (2017b) identified African countries with high spider plant production to include: Kenya, Uganda, Zimbabwe, Zambia, Burkina Faso, Ghana, South Africa, Malawi, Botswana, Cameroon, Namibia, Tanzania, and Benin As a result, it contributes to regional diets, and the capacity to cope with climate change, and may already be incorporated into existing production systems, despite little investment to improve productivity or quality (Sogbohossou et al., 2019).

More than 295 African spider plant accessions have been collected and ex-situ conserved at the World Vegetable Center, various African countries, and the National Plant Germplasm Center of the United States (Mayes et al., 2012b). Several investigations on its characterization have been conducted to improve the species, revealing the existence of significant genetic diversity (Wasonga, 2014). Significant differences were observed in seedling emergence date, leaf formation, and days to flowering among the African spider plant accessions (Onyango et al., 2013a), demonstrating possibility of genetic differences and variation among farmer varieties. There have been phenotypic variations among African spider plant accession in traits such as petiole length, plant height, and days to flowering (Onyango et al., 2013e; Houdegbe et al., 2022a). Therefore, the following questions are addressed throughout this review: Why should we improve the African spider plant? What research gaps are impeding the production and promotion of this African leafy vegetable? What are the essential elements of a successful breeding program for this underutilized leafy vegetable?

2.2 Taxonomy, origin, and geographical distribution

The spider plant is a member of the family *Cleomaceae* which was previously known as Capparidaceae and of the subfamily *Cleomoideae*. In addition, the family consists of approximately 700-800 species that are categorized into 45 genera (Lock and Kokwaro, 1995; Oshingi et al., 2019). The genus has over 200 species that consist of greatly polymorphic herbaceous plants (Iltis, 1967; Bruinsma, 1985). Furthermore, indigenous African Cleome is made up of about 50 spineless species (Iltis, 1967). Evidence suggests that spider plant is related to the species *Cleome Spinosa*, *Cleome viscosa, Cleome speciosa Raf, Cleome monophylla, Cleome rutidosperma DC* and *Cleome hirta Oliv* (Chweya and Mnzava, 1997). Other related synonym terms to *Gynandropsis gynandra* (L.) Briq include *Gynandropsis pentaphylla* (L.) DC, *Cleome pentaphylla* (L.) Schrank, and *Gynandropsis gynandra* (Kwarteng et al., 2018). Moreover, the spider plant is an annual herbaceous plant that grows to a height of approximately 0.5 m – 1.0 m and can extend up to 1.5 m if environmental conditions are favourable. The plant has an erect stem with sticky and in many cases vastly branched thin branches. In addition to the above, the stem is also densely glandular as well as hairy with a small number of varieties being hairless (Chweya and Mnzava, 1997; Holm *et al.*, 1977).

Evidence suggests that the spider plant might have originated in tropical Africa as well as Southeast Asia (Oshingi et al., 2019; Chataika et al., 2020a). It is from these locations that it began to spread further to other tropical and subtropical nations in the northern and southern hemisphere (Lock and Kokwaro, 1995). Kwarteng et al. (2018) indicate that spider plant is spread by birds as well as seed dispersal, which is a result of capsule dehiscence. The genus *Cleome* is extensively scattered in the dry parts of the tropics and subtropics. Despite all that, the genus Cleome predominantly occurs in Africa (Figure 2.2). Furthermore, the spider plant is generally disseminated to different tropical and subtropical countries such as Mexico, Brazil, Colombia, and India where it relatively grows as a weedy plant (Holm *et al.*, 1979; Kwarteng, *et al.*, 2018). Van Den Heever and Venter (2007b) identified Ethiopia, Nigeria, Angola, Egypt, Tanzania, Ghana, Kenya, South Africa, Zimbabwe, and Zambia as countries where the Spider plant commonly grows (Figure 2.2). In South Africa, the Spider plant is a very common herb that extends from Limpopo, the North-West, Mpumalanga, Gauteng, Free State, KwaZulu-Natal, and the Northern Cape (Mishra et al., 2011).

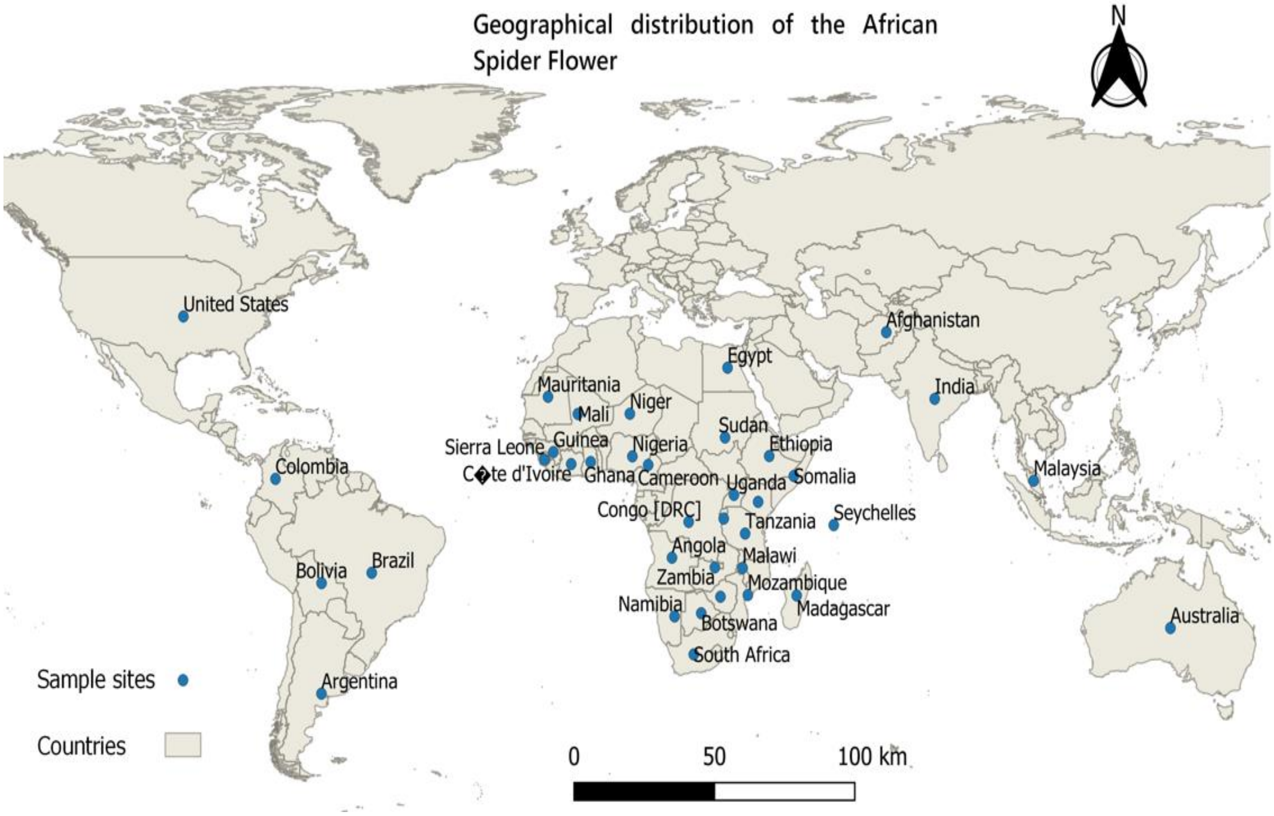

Figure 2. 2 The geographical distribution of the African spider plant across the world

Sources (Oshingi et al., 2019; Chataika et al., 2020a).

2.3 Constraints limiting African spider plant production.

African spider plant is mainly grown in small-holder farming set up in SSA. Because of this, several constraints limit its production and reduce its potential. These can be divided into the following broad categories; socio-economic, and biotic and abiotic environmental stresses. Each of these stresses is discussed below and how they impact spider plant production.

2.3.1 Socio-economic constraints

Amongst the socio-economic constrains is the lack of technical advice to the smallholder farmers regarding production of this leafy vegetable. This is because agricultural extension officers lack the indigenous knowledge of this vegetable, which underscores adopted agronomic practices (Weinberger, 2007). There is barely any knowledge of indigenous vegetables like the African spider plant that exists in both local and global literature. Secondly, improved seed or planting material is lacking in the African spider plant. The majority of smallholder farmers grow their home-saved seeds of landraces (Modi, 2003). The consequence of utilizing landraces for planting is that the plants are not uniform, and viability tests are generally not conducted before sowing leading to poor seed germination (Weltzien and Fischbeck, 1990; Mazvimbakupa et al., 2015; Ghaleb et al., 2021).

The third socio-economic constraint to the production of the African Spider plant is a poor market framework. A systematic and effective marketing channel for this leafy vegetable is lacking (Shackleton et al., 2009a). This is because most of the attention has been given to exotic vegetables, such as cabbage, cauliflower, and broccoli. Furthermore, traders have to travel long distances and spend a lot of money just to buy this vegetable in the market (Okwoche, 2012). On account of the expertise and time needed to prepare African spider plant for cooking, the majority of the younger generation often shun it in the market (Chataika et al., 2022).

Another major constraint limiting African spider plant production is the shortage of farming inputs, for example seeds for planting. Because there are no improved varieties, there are no hybrid seeds that are being produced for commercial sale, thus seeds can only be obtained from other smallholder farmers in the form of landraces (Zakari et al., 2014). Commercial fertilizers are also expensive making them inaccessible to most smallholder farmers, while those that can afford to buy are unable to obtain adequate quantities (Denning et al., 2009). In addition, organic fertilizers such as cow manure are of low quality and usually inadequate. The shortage of fertilizer is exacerbated by nutrient-poor soil conditions of the cropland area utilized by the farmers. Draft power and shortage of manual labor are other constraints to African spider plant production as the majority of young and able-bodied men and women are migrating to urban areas in search of a better life (Alwang and Siegel, 1999) and some smallholder farmers do not have the required livestock for draft power (Antwi and Seahlodi, 2017).

2.3.2 Biotic stresses

Living organisms, such as viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds, constitute the biotic stress factors in plants (Singla and Krattinger, 2016). The African Spider plant is not spared from the effects of these biotic factors in its production. This African leafy vegetable is prone to several insect pests and diseases. Studies have been conducted to identify these insect pests affecting its growth and yield. The pests include the African bollworm (*Helicoverpa* *armigera),* cucurbit ladybird beetle (*Epilachna* spp), leaf roller (*Sylepta derogate*), and cotton aphids (*Aphis gossypi),* which have been reported to be more prevalent in East Africa (Hutchinson et al., 2011). Diamondback moth (*Plutella xylostella*), black bean aphids (*Aphis fabae*), cotton aphids (*Aphis gossypi)* flea beetles (*Chrysomelidae spp*),) and cucurbit ladybird beetle (*Epilachna* spp), Lugari and cutworms (*Agrotis spp*), were reported to cause more economic and devastating damage to African spider plant (Goldman, 1996; Kunjwal & Srivastava, 2018; Wu & Miyata, 2005). Attacks by these beetles were commonly reported during dry months in Zambia, and they can be efficiently suppressed by spraying the plants with an insecticide such as Ambush (Permethrin), and Ripcord (200 g/L Cypermethrin). Weaver birds (*Quelea quelea*) are also known to eat the young seeds of African spider plant. Studies conducted by Atheya and Marthur (1966), Raghava and Purnachandra (1980), and Singh (1983b) showed that African spider plant is a host to several diseases including powdery mildew fungi namely, *Cercospora uramensis, Sphaerotheca fuliginea* and *Oidiopsis taurica.* All these diseases have a negative impact on Spider plant as they cause a sharp decline in economic yields.

2.3.3 Abiotic stresses

Light, carbon dioxide, water, temperature, nutrients, and salinity are the most significant abiotic variables for plants. They are linked to the functional network that includes all important plant physiological processes in plant ecological performance (Lüttge, 2019). Abiotic stresses are a major constraint to crop production and food security around the world. The situation has worsened as a result of the extreme and rapid changes in the global climate. In simple terms, environmental factors induced by climate change have resulted in a slew of abiotic stresses, including changes in rainfall patterns (high rainfall, low rainfall, shortened or prolonged rainy seasons), salt accumulation in soils (salinity), temperature extremes (heat shocks and chilling), increased carbon dioxide, and ozone layer depletion (Ashraf et al., 2008; Ashraf, 2010; Fahad et al., 2017).

Drought and heat are undeniably the two most influential stresses affecting crop growth and productivity. Drought disrupts growth, nutrient and water interactions, photosynthetic activity, assimilate partitioning, and ultimately reduces crop yields in African spider plant (Abbate et al., 2004; Ashmore et al., 2006; Bain et al., 2013). Heat stress can cause severe protein damage, disrupt protein synthesis, deactivates important enzymes, and impair membranes. Heat stress may also have a significant impact on the mode of cell division (Berti et al., 2004). All these damages can severely restrict plant growth while also promoting oxidative damage. Furthermore, in crops where seed is the desired harvestable product, brief exposure to high temperatures during seed filling can lead to accelerated filling, resulting in poor quality and a decrease in yield (Araus et al., 2008; Abid et al., 2018).

2.4 Effects of drought on the morphology of plants

2.4.1 Morphological traits

Proper crop plant growth and development are critical for the initiation of normal plant structures that conduct all physiological and metabolic processes and provide potential yield. Drought stress severely hampers African spider plant growth and development (Mosenda et al., 2020). Plant height, leaf area, structural and functional characteristics of the root, plant biomass, plant fresh weight, plant dry weight, and stem diameter are some of the morphological traits used to estimate growth and development. Drought reduces plant height, stem diameter, plant biomass, and leaf area (Khan et al., 2001; Zhao et al., 2006).

African spider plant leaves range from 8 to 140, and they appear alternately on nodes. A leaf is made up of structural and functional elements. Leaf size and the number of leaves are structural components of leaf growth. Leaf functional traits include photosynthesis, transpiration, and light interception. Drought stress reduces the size and number of leaves in the African spider plant (Farooq et al., 2009) and drought stress reduces cell division and elongation by impairing enzyme activities, decreasing turgor, and decreasing energy supply (Kiani et al., 2007; Farooq et al., 2009). Drought-induced turgor loss reduces plant growth and productivity by lowering leaf water potential (LWP), cell division rate, and elongation (Hussain et al., 2009). Reduced dry matter accumulation occurs in all plant organs during drought, with varying degrees of reduction in different organs.

Drought affects different crops in different ways, for example, drought, reduced the fresh and dry weights of marigold (*Tagetes erecta L.*) shoots and flowers (Asrar and Elhindi, 2011). It also reduced shoot and root dry weights, significantly in Asian red sage (*Salvia miltiorrhiza L.*), though roots are less affected than shoots (Liu et al., 2011). Furthermore, drought decreases leaf area and leaf number due to turgor loss (Farooq et al., 2010). With a few exceptions, drought has a significant impact on crop phenology by shortening the crop growth cycle. Water scarcity initiates a signal that causes an early switch in plant improvement from the vegetative to the reproductive stage (Desclaux and Roumet, 1996). Drought reduced the total growth duration of both bread wheat (*Triticum aestivum L.*) and barley (*Hordeum vulgare L.),* resulting in significant yield reductions (McMaster and Wilhelm, 2003). Furthermore, drought delayed flowering in maize (*Zea mays* L.) (Abrecht and Carberry, 1993), quinoa (Geerts et al., 2008), and rice (Fukai et al., 1999), whereas it hastened flowering and physiological maturity in soybean (Desclaux and Roumet, 1996), wheat, and barley (McMaster and Wilhelm, 2003).

While drought occurs during the vegetative phase of crop growth, it has the potential to significantly reduce economic yield. Drought during flowering is critical because it increases pollen sterility, resulting in a reduced yield set. Drought causes pollen sterility during the mitotic stage of flowering and only influences female fertility when stress is severe (Saini and Westgate, 1999; Alqudah et al., 2011). Plants enhance their roots and generate an extensive root system to improve water uptake under water stress conditions. Under drought conditions, increased biomass distribution to roots and root system expansion generally result in increased water uptake capacity (Rahdari and Hoseini, 2012; Salehi-Lisar and Bakhshayeshan-Agdam, 2016a). As a result, under drought conditions, plant root-to-shoot ratios typically increase, and plant total biomasses decrease significantly (Shao et al., 2008; Zare et al., 2011). Although a study by Mosenda et al., (2020) has tried to look at the effects of drought on morphological traits, more traits need to be explored. There is still little to no information on how drought affects plant morphology in African Spider plant. Exploring these traits will give an insight as to how drought stress affects spider plant morphologically and to identify traits that can aid in the selection of drought tolerant genotypes.

2.4.2 Physiological and biochemical traits

Plants' stomata close early in response to drought stress to prevent transpiration of water loss and leaf dehydration influencing all plant water relations (Alscher and Cumming; Chaves, 1991; Chaves et al., 2003). Stomatal closure under drought stress can be caused by a decrease in leaf turgor and water potential (Ludlow and Muchow, 1990) or by low atmospheric relative humidity (Maroco et al., 1997). Stomata closure is considered the first step in drought adaptation by means of maintaining cell turgor to continue plant metabolism and to avoid potential loss of its water transport capacity (Jones and Sutherland, 1991; Lipiec et al., 2013).

Drought stress reduces relative leaf water content, leaf water potential (LWP), and transpiration rate while increasing leaf temperature in plants (Siddique et al., 2000). In-plant–water relationships, the relative water content (RWC), LWP, stomatal resistance, transpiration rate, leaf temperature, and canopy temperature are all important factors. Under drought stress, stomatal closure and a decrease in stomatal conductance and transpiration rate have been linked to higher water-use efficiency (the ratio of dry matter produced to water consumed) in crops such as wheat, *Trifolium alexandrium*, and alfalfa (*Medicago sativa*) (Lazaridou and Noitsakis, 2003).

The plant hormone abscisic acid (ABA) regulates stomatal closure. A decrease in soil water content and plant turgor stimulates ABA biosynthesis (Morgan, 1990; Zhang et al., 2003). ABA is synthesized in the roots and transferred by the xylem to the shoot during soil drying to prevent leaf expansion and trigger stomatal closure prior to a change in leaf water status (Speirs et al., 2013). The abundance of ABA in the xylem sap of leafy vegetables has been found to be related to stomatal conductance (Stoll et al., 2000). Furthermore, the exposition of genes involved in ABA synthesis (the 9-cis-epoxycarotenoid dioxygenase), *NCED1*, and *NCED2*, was higher in the roots than in the leaves, particularly when soil moisture decreased, and vapor pressure deficit increased. Diverse hormones, apart from ABA, play a role in regulating stomatal closure. Increased cytokinin concentrations in the xylem foster stomatal opening by decreasing ABA sensitivity (Wilkinson and Davies, 2002) whereas, a reduction in root cytokinin corresponds with an increase in xylem ABA and a decrease in stomatal conductance (Speirs et al., 2013). Plant hormones such as ABA, auxin, cytokinin, ethylene, and gibberellins have been implicated in plant response to various environmental stresses (Dodd, 2005; Schachtman and Goodger, 2008).

One of the primary effects of drought in higher plants is a reduction and/or inhibition of photosynthesis (Keyvan, 2010). Stomatal and nonstomatal factors can both inhibit photosynthesis in water-stressed conditions. Carbon dioxide limitations caused by an extended period of stomatal closure, particularly during light saturation result in the amassment of reduced photosynthetic electron transport components (Snape and Pánková, 2013; Bhargava and Sawant, 2013). The build-up of these compounds can lower molecular oxygen and lead to the formation of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals, as well as H_2O_2, causing oxidative damage in chloroplasts (Xoconostle-Cázares et al., 2010). Furthermore, low CO_2 uptake caused by stomatal closure is the main stomatal-dependent factor that reduces photosynthesis rate due to decreased activity of CO_2 reduction enzymes (Lisar et al., 2012). ROS can harm photosynthetic apparatus such as thylakoid membranes, photosynthetic pigments, and enzymes (Osakabe et al., 2014).

Another factor influencing photosynthesis rate reduction is a decrease in the chlorophyll content of leaves under water stress (Sapeta et al., 2013; da Silva et al., 2013). A reduction in chlorophyll content during drought stress is dependent on the length and severity of the drought and indicates a reduced ability for light-harvesting (Salehi-Lisar and Bakhshayeshan-Agdam, 2016b). The reduction in chlorophyll content under water stress has been identified as a common sign of oxidative stress and may be caused by pigment photo-oxidation and chlorophyll degradation (Figure 2.2).

Plants require photosynthetic pigments primarily for light-harvesting and the production of reducing powers. Each of the two chlorophyll a and b are susceptible to soil dehydration (Bijanzadeh and Emam, 2010). Furthermore, drought stressed-induced chlorophyll content depletion has been attributed to chloroplast membrane loss, uncontrolled swelling, lamellae vesiculation distortion, and the presence of lipid droplets (LI et al., 2006). From a physiological standpoint, leaf chlorophyll content is an important parameter in and of itself. According to studies, the majority of chlorophyll loss in plants in response to water deficit occurs in mesophyll cells, with a lesser amount lost in bundle sheath cells (Gujjar et al., 2020).

Plants accumulate various organic and inorganic solutes in the cytosol to reduce osmotic potential and maintain cell turgor (Schultz and Matthews, 1993). Under drought conditions, leaf turgor can be maintained through osmotic adjustment in response to the accumulation of proline, sucrose, soluble carbohydrates, glycine betaine, and other solutes in the cytoplasm, which improves water uptake from drying soil (Xiong et al., 2012). The process of such solute accumulation under drought stress is referred to as osmotic adjustment, and it is highly dependent on the rate of plant water stress (Hare et al., 1998). Because of its significance in stress tolerance, proline is the most widely studied of these solutes. Proline accumulation is the initial response of plants to water deficiency stress to reduce cell injury.

Proline can function as a signalling molecule to regulate mitochondrial functions, control cell proliferation or cell death, and precipitate specific gene expression, all of which are important for plant stress recovery (Sofo et al., 2004; Parida et al., 2008). Proline accumulation under stress has been linked to stress tolerance in many plant species, and its concentration has been proved to be higher in stress-tolerant plants than in stress-sensitive plants. It affects protein solvation and conserves the quaternary structure of intricate proteins, continues to preserve membrane integrity under drought stress, and decreases lipid membrane oxidation or photoinhibition (Yamada et al., 2005; Anjum et al., 2012). Furthermore, it aids in the stabilization of subcellular structures, the scavenging of free radicals, and the buffering of cellular redox potential under drought-stress conditions (Ábrahám et al., 2003; Verbruggen and Hermans, 2008). However, the function of proline in African spider plant is unknown as currently we are not aware of any studies pertaining to its action. This study aims to establish the effect drought stress on proline and to determine if it can be used as a reliable biochemical marker to screen for drought tolerant African spider plant genotypes.

A study conducted by Mosenda et al., (2020) explored physiological traits like chlorophyll content and relative water content. In his study a sharp decrease in chlorophyll content and relative water content were observed in African spider plant under drought stress conditions. More physiological traits need to be incorporated in future research programs to have more information on the physiological processes involved in African Spider plant under stress conditions. This study will therefore aid to the existing study and will explore more physiological traits such as net photosynthesis, stomatal conductance and transpiration rate. Incorporating more traits is beneficial as it gives more insights on the crop's response to drought stress. To date there is no progress that has been made to improve physiological and biochemical traits in African Spider plant. Therefore, future breeding programs should look at this aspect.

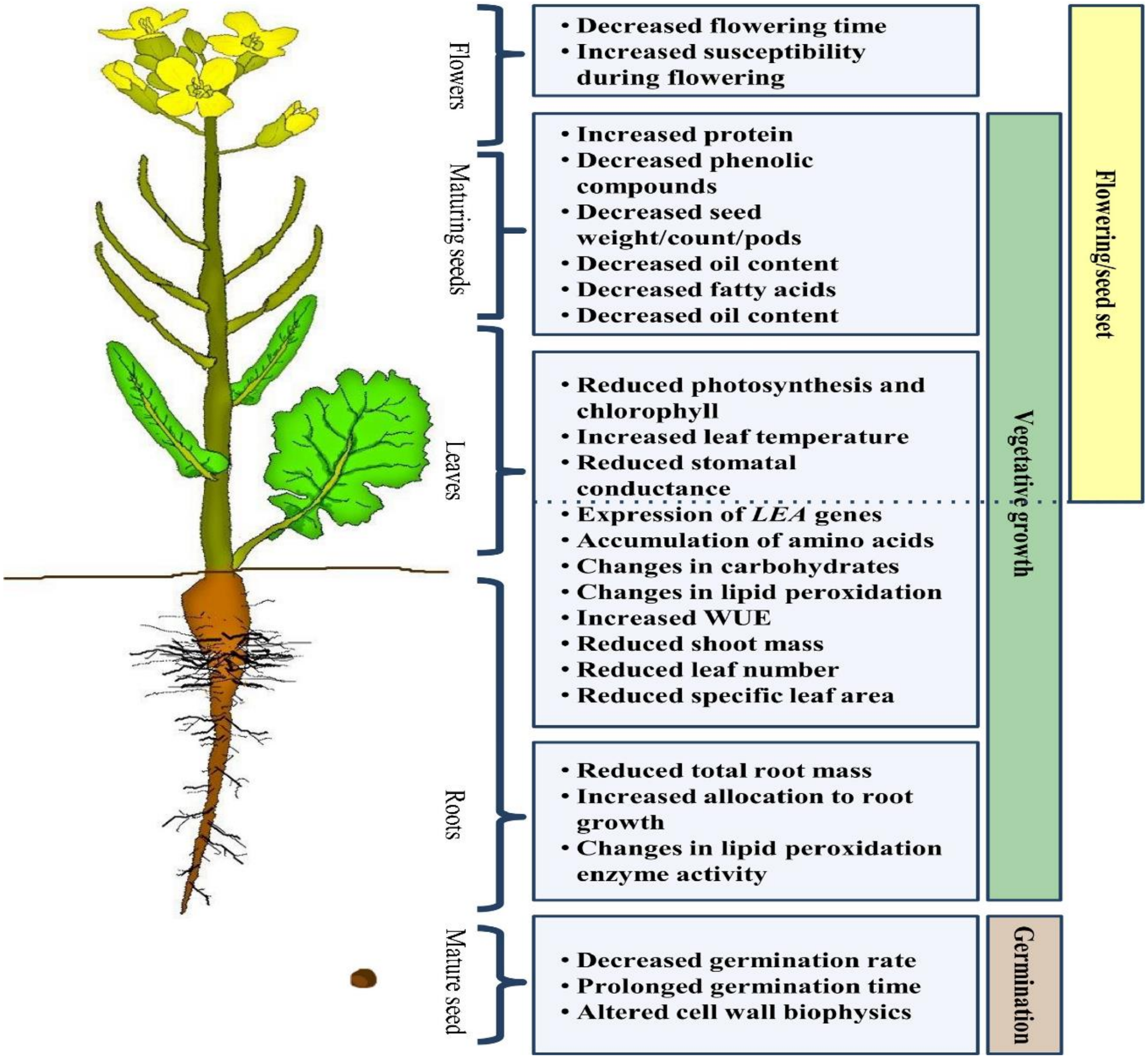

Figure 2. 3 Crop physiological and morphological trait responses to water deficit stress

Source: (Zhu et al., 2016)

2.5 Drought Resistance Mechanisms in plants

Drought stress heterogeneity, variable effects in space and time, and degree and severity of stress are all contributing to stress's erratic and inconsistent behaviour. Nature has endowed plants with the capacity to adapt for survival and production efficiency under extreme stress (YIN et al., 2017). Plants have various morphological, physiological, and biochemical characteristics that allow them to adapt or resist stress. Resistance is defined as the lowest yield reduction under drought stress conditions compared to normal water availability (Jafari et al., 2019). Resistance can come in the form of drought escape, drought avoidance, or drought tolerance. Drought resistance is defined in evolutionary terms as the capacity of varieties or species to withstand and successfully reproduce in environments with limited water availability. In agriculture, drought resistance is defined as the plant's ability to achieve an economic yield despite limited water availability (Vincent et al., 2005). Drought resistance refers to plant mechanisms that lead to lower yield losses under drought conditions compared to higher yields under normal water availability.

2.5.1 Drought escape

The goal of breeding for earliness is to synchronize phenology with water availability (Bidgoli, 2018). Drought escape is defined as the shortening of a crop's growing season or life cycle prior to the onset of drought stress. Drought escape is critical against terminal drought stress, so the reproductive growth phase must be involved in drought escape (Zhang et al., 2017). Days to sowing, days to flowering, and days to maturity are all genetically heritable traits that can be modulated by water availability. The interaction between genotype and environment (GE) determines crop duration and induces plants to complete the life cycle before drought onset. The effective synchronization of phenological development with soil moisture availability and the predominance of terminal drought stress are the factors that necessitate drought escape (Du et al., 2020). The development of short-duration and early maturing cultivars aids in the crop's resistance and its ability to escape terminal drought stress (Benjamin and Nielsen, 2006). Yield is directly related to crop duration, and any decrease in crop duration decreases yield (Chartzoulakis et al., 2002). Early maturing genotypes have relatively low evapotranspiration, lower leaf area index, and lower yield potential, whereas late maturing genotypes have higher evapotranspiration, lower leaf area index, and higher yield potential. It is not known whether African Spider plant uses drought escape as a mechanism. This study therefore seeks to identify African spider plant drought tolerance mechanism. This information will be valuable for future research and breeding programs.

2.5.2 Drought avoidance

Various physiological and metabolic processes are not affected by water stress and continue to function normally as regards drought avoidance (Picotte et al., 2009). Drought avoidance is calculated as an assessment of the tissue water status expressed as turgor water ability under moisture stress. Crop water status is preserved by either decreasing transpiration or maximizing water uptake. Stomata closure, leaf firing, leaf rolling, canopy condition, leaf characteristics, and root attributes are all essential drought avoidance selection criteria in plants. Water absorption can be maintained with the assistance of a large root system (Gowda et al., 2011; Cao et al., 2013). Functional or phenotypic root characteristics, as well as operational or hydraulic root characteristics, are critical for efficient drought avoidance in plants.

A thick, as well as deep root system, allows for further water extraction from the soil. The key determinants of drought avoidance are root characteristics such as root length, root density, and root biomass (Gowda et al., 2011; Cao et al., 2013). Distinctive root thickness, greater root depth, root length over weight ratio, seminal root weight, and root length density are root characteristics that guarantee increased water uptake under water stress (Rucker et al., 1995; Picotte et al., 2009; Khaleghi et al., 2019; Bhusal et al., 2020).

Pubescence, also known as hairiness, is an essential attribute of xeromorphic plants. Leaf hairs have the ability to reduce leaf temperature and transpiration (Misra et al., 2020). The glaucousness or waxiness of the leaf surface contributes to their water potential (Anjum et al., 2017). In the African spider plant, there is significant genetic variability for glaucous relative to none and hairy comparison to none. As a result, there is broad agreement that stomatal conductance, hairiness, and glaucousness/waxiness can minimize water losses, whereas structural and operational root attributes can enhance water uptake, allowing maize plants to avoid drought stress (Li et al., 2020).

2.5.3 Drought tolerance

Drought tolerance refers to the ability of crop plants to sustain their development and growth in the face of drought stress (Yang et al., 2021). Under existing drought conditions, yield stability is related to drought tolerance. Drought tolerance is remarkably a complex system, and plants have developed various physiological and molecular adaptations to confer drought tolerance. Drought-tolerant accessions have a higher economic yield when subjected to drought stress. Plant development and growth, plant phenology, and photo assimilate reserve translocation are all essential characteristics to improve drought tolerance in crops (Yang et al., 2021). Drought tolerance in crop species is conferred by osmotic adjustment and antioxidant scavenging defence mechanism, growth regulators, water channel proteins, stress-responsive proteins, transcription factors, and signalling pathways (Escalona et al., 1999). It is not clear which drought resistance mechanism African spider plant uses to cope with stress. This study will therefore identify the mechanisms employed by African spider plant to cope with stress. The mechanisms can be drought escape, drought avoidance, drought tolerance or a combination of the three mechanisms.

2.6 Breeding African spider plant for drought tolerance

2.6.1 Creating an African spider plant breeding program

Creating breeding programs for the African spider plant starts with cultivar improvement on the basis of user preferences and adequate acclimatization to various environmental conditions, with specific product targets dictated by particular market regions (Shackleton et al., 2009b). Smallholder farmers generally prefer full-season varieties with a high leaf yield, disease and pest resistance, and abiotic stress (Afari-Sefa et al., 2016). Retailers and consumers are looking for products that have a good appearance, a long shelf life, superior aroma and taste, a high nutritional value, and are affordable. Growers' and consumers' preferences are crucial in shaping breeding goals and product programs, they must be explored early in the breeding program to steer germplasm collection and characterization approaches and afterward given preference later once genetic diversity and breeding constraints are known (Neven and Reardon, 2004; Blandon et al., 2009; Colen et al., 2012).

Breeders can develop varieties with desirable traits, and wide acceptability through cultivar improvement with various stakeholder engagement including consumers, retailers, and farmers (Reardon et al., 2003; Hernández et al., 2007). An interdisciplinary breeding framework that takes advantage of the new advancements in breeding to steer coordinated, inclusive initiatives by researchers to ensure better nutritional benefits for consumers is suggested.

The following steps (Fig 2.3) have been established by (Sogbohossou et al., 2018b) as key stages for an effective African spider plant improvement program: (2) germplasm collection, characterization, and regulation (3) product target definition (4) description of genetic control of essential traits (5) cultivar development process design; (6) incorporation of genomics data to optimize that process; (7) multi-environment collaborative trials and end-user evaluation; and (8) crop value chain development (Neven and Reardon, 2004).

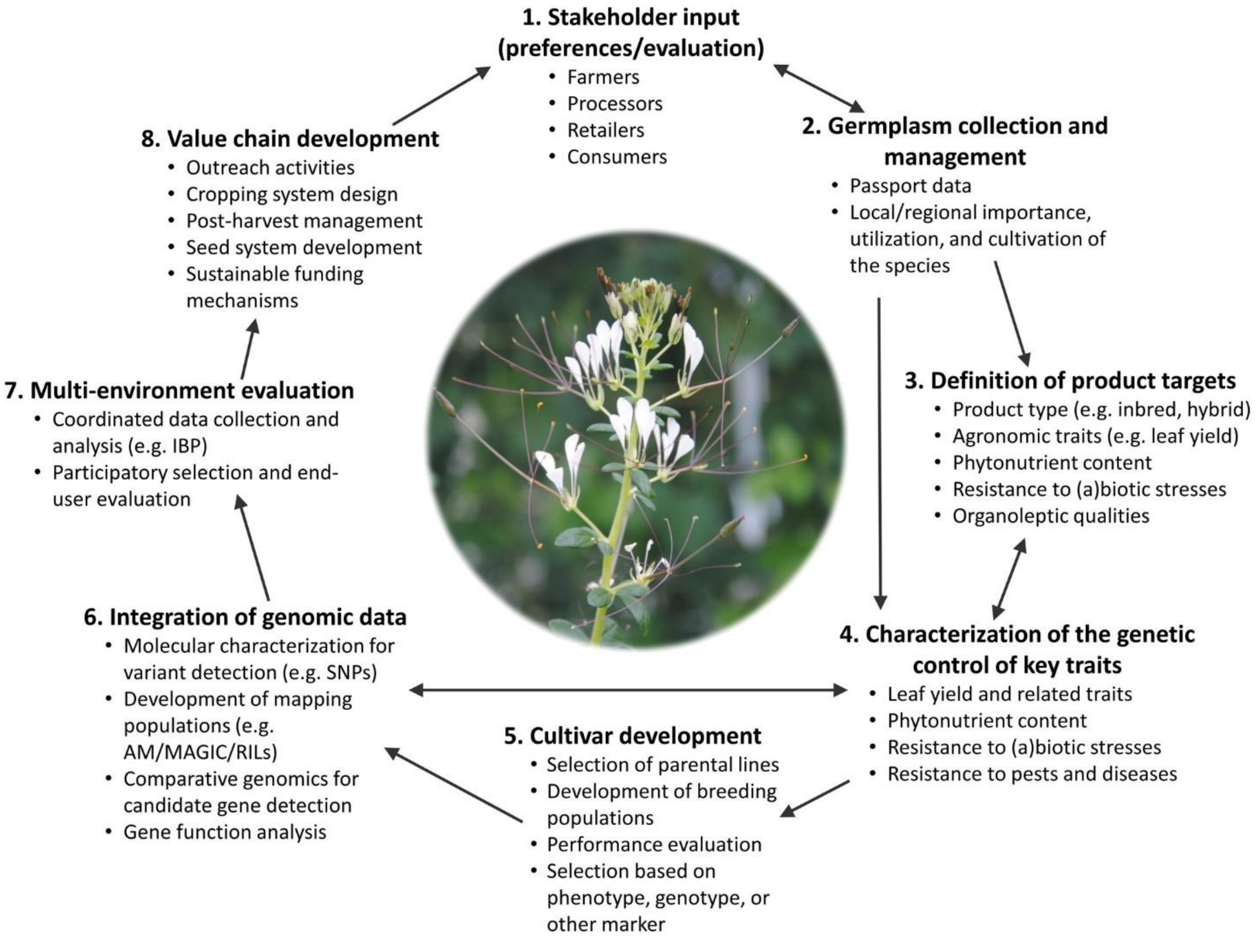

Figure 2. 4 An integrated breeding program for the African spider plant

Source: (Sogbohossou et al., 2018b)

2.6.2 Germplasm assembly, characterization, and management

Information on a crop species' taxonomy, distribution, and ecology is required for effective germplasm assembling. The African spider plant has little to no information and this information must be sought after in this species as there are such information gaps (Odong et al., 2013). Regions can be identified and germplasm collection plans devised using the information on the taxonomy and distribution of the species. To assist downstream analysis of genetic data from genebank accessions, all collections must be supported by complete passport data, including reliable geo-referencing, habitat descriptions, and sampling techniques. Sampling procedures that provide adequate coverage of habitats related to key features of interest must be defined. Ex-situ conservation is frequently used for African spider plants (Volk and Richards, 2011).

Some of these African leafy vegetable ex situ germplasm collections already exist in local, regional, and global gene banks, but they need to be extended. The World Vegetable Center, for example, presently maintains 295 accessions of *G. gynandra*, including 112 from Eastern and Southern Africa and 183 from Asia *(http://seed.worldveg.org/)*. Future collection efforts in South and Central America, as well as Australia, could contribute to the global repository of *G. gynandra* variety. Pure lines, cultivars generated by research institutes, and farmer collections must be added to the existing germplasm of African spider plant regularly as well as plant material gathered in the wild (Glaszmann et al., 2010). When it comes to pure breeding lines, self-pollinated accessions are kept as individual plants, but in the case of landraces and heterogeneous materials, they are kept as populations. Cross-pollinated plants are generally kept as populations, with an emphasis on inbreeding depression, and genetic drift (Rafalski, 2010).

2.6.3 Characterization of the genetic control of key traits

While there is some knowledge on farmer and consumer preferences, little is documented about the gene regulation of important characteristics of interest yet, such information is essential for establishing a breeding program (Tang et al., 2010). The number of genes that control trait expression, the type of gene action involved, for example, additive, dominant, and epistatic, the extent of genetic and phenotypic variances, potential environmental influences, and the heritability of key characteristics can all affect the design of the "process" by which advanced cultivars will be produced (Varshney et al., 2012). Furthermore, other features associated with essential characteristics may be used to develop more efficient and cost-effective breeding strategies. Other traits that are connected to essential characteristics may be used to develop more efficient and cost-effective breeding procedures (Kumar et al., 2012). In order to promote meaningful data comparison across settings, the phenotypic characterization of specified features in the African spider plant should be accomplished utilizing a set of standardized methods developed and shared throughout research institutes. Some species provide morphological characterization data, including *Amaranthus spp,* and *Gynandropsis gynandra* (Hall et al., 2005; Nikooei et al., 2015).

Researchers at the World Vegetable Center have produced and amended a standard list of morphological descriptors for *Gynandropsis gynandra*, which was then refined by the Cleome Consortium. Gene banks as well as other *Gynandropsis gynandra* -related institutions are advised to use and contribute to the ongoing refining of this listing of phenotypic descriptors. The first and most important aim feature in leafy vegetable breeding is leaf yield (Bala et al., 2014). Leafy vegetable breeding necessitates accurate characterization and substantial relationships between phenotypic variables and leaf yield. Some species, notably *Gynandropsis gynandra* have undergone such research (Sogbohossou et al., 2018b).

Omondi, (1989) reported low heritability (< 60%) for leaf yield and yield-related variables such as plant height, number of leaves, leaf length, and leaf width, but a high heritability (> 60%) for days to flowering, a maturity indicator. (Kangai Munene et al., 2018) also found high heritability for a number of traits, including days to flowering (91%), number of leaves per plant (99%), plant height (99%), number of primary branches (94%), chlorophyll content (94%), and single leaf area (87%). High heritability indicates that a large proportion of the phenotypic variation in a trait is due to genetic factors. Breeders can target such traits for selection, as there is a higher probability of achieving desired improvements through selective breeding. This can lead to more efficient and targeted selection of superior plant individuals.

Chweya and Mnzava, (1997) discovered a negative association between days to flowering as well as leaf dry weight in the African spider plant, implying that full-season variants of *Gynandropsis gynandra* may enhance leaf production. The number of leaves per plant were found to be correlated positively and significantly with plant height and number of primary branches implying that these traits can be considered as target traits for selection. This is promising for improving yield in the crop through various plant breeding methods. Further research involving several genotypes, environments, and sites is needed to validate these findings and explore other main traits.

Plant geneticists and breeders are well-positioned to develop plants that increase yield under drought stress. To enhance crop yield under varying drought conditions, a comprehensive knowledge of root functional traits and how these traits are strongly associated with whole plant strategies is required. Root diameters, root length, and root density are root traits related with plant yield productivity under drought, particularly at depths in soil with available water (Comas et al., 2013). Current studies have only focused on above ground traits. There us need to explore these root traits in African spider plant and how these traits can aid in the selection of drought tolerant genotypes.

Drought tolerance phenotyping under controlled environments can be utilized to pre-screen genotypes for field validation. Despite the fact that field trials are prone to variation, specific strategies can be used to closely monitor environmental parameters or establish semi-controlled environments for example rain shelters, irrigation, and enclosures (Hall et al., 2005; Lopes et al., 2014). The growth periods under which these stresses occur, as well as their length and severity, are important factors in determining tolerance/ resistance. Aside from yield assessment under drought challenges, a comprehension of the physiological mechanisms behind this drought stress tolerance is required to identify which physiological, morphological and biochemical features should be included in the screening process (Fukai et al., 1999). Water uptake, photosynthetic efficiency, and water usage efficiency parameters such as stomatal conductance, photosynthetic assimilation rate, chlorophyll content, leaf thickness, and leaf stable carbon isotope ratio, for example, might be studied across genotypes (Sannemann et al., 2015).

Yield based selection should be accompanied by the proper estimation, application, and interpretation of multiple drought stress indices that evaluate genotypic yield response to water deficit (Rosielle and Hamblin, 1981a). To distinguish drought-tolerant genotypes, the tolerance index (TOL), harmonic mean (HARM), mean productivity (MP), geometric mean productivity (GMP), and stress-tolerance index (STI), and stress-sensitivity index (SSI) are the most commonly used drought-tolerance indices. To date no indices have been adopted to select for drought tolerance in African Spider plant. These can be employed in the selection of African spider plant genotypes for drought tolerance (Shojaei et al., 2022) .

In lettuce screening for drought tolerance, leaf relative water content, and different plant growth during drought stress have been considered as markers of water stress such metrics might be examined to construct screening methods suited to each vegetable species. When compared to C3 species, C4 species such as *G. gynandra*, would have alternative drought escape strategies (Varshney et al., 2012).

2.6.4 Cultivar development process design

A thorough understanding of the reproductive biology of the selected species will be required for cultivar development. Possible sources of beneficial alleles for all essential traits of interest in existing germplasm, for example, leaf yield, time to maturity, pest, disease resistance, and drought tolerance should be established, and a strategy for successfully integrating them should be developed (Odong et al., 2013). To start, a testing system for evaluating the progeny of breeding crosses must be developed in order to successfully and accurately estimate the heritability of and relationships among the characteristics in the refined product target, and recognize any agronomic traits that can be used as a basis for selection toward the product target (Volk and Richards, 2011). The parents will be chosen depending on the outcomes of germplasm characterization, and the offspring chosen based on performance for key features will be evaluated at different locations indicative of the range of agroclimatic conditions in which the final product would be cultivated.

Since product targets are not about a single feature, a multi-trait selection strategy must be developed. This strategy must consider the relative significance of the features for end-users, the scope of such features (qualitative vs. quantitative), the depth of their genetic control, for example, additive, dominant, epistatic, additive x dominant, and their degree of heritability (low vs. high), their relationships to each other, and the breeder's selection intensity (Hall et al., 2005). Another effective way of breeding species of interest for various features for example maturity, leaf yield, and vitamin content could be to use selection indices, especially if substantial negative correlations between target traits are discovered.

A key asset for effective breeding is the use of molecular markers related to characteristics or as a mechanism for whole genome selection. A provisional reference genome and a transcriptome map of the species are among the key genetic resources available in *G. gynandra* (Cannarozzi et al., 2014). Building on such scientific research will allow for a more precise examination of genetic diversity within species, and rapid trait improvement in breeding programs (Mwadzingeni et al., 2021). Throughout the breeding process, multi-location studies must be performed, with a concentration on the end-user approval of the emerging advanced lines of the crops of interest (Yan et al., 2007). During the cultivar development phase, choices on the number and location of testing sites for evaluating populations and developed lines should consider the variety of agroclimatic conditions under which the species is cultivated, the existing breeding stations or experimental farms, and the available resources to allocate to such experiments.

Conclusion

Because of the African spider plant's highly nutritious qualities and economic potential to achieve stable nutritional food security in developing nations, large-scale production and increased commoditization of this leafy vegetable are desirable. Large-scale production, on the other hand, will require coordinated efforts from various stakeholder groups across the value chain, from research to production to advertising to the end-use. This review established the effects of drought stress on plants' morpho-physiological and biochemical traits. There is little to no literature on how African spider plant copes with drought stress. Furthermore, the effect of leaf gas exchange parameters on drought stress in spider plant has not been explored. It is not known if proline content is a reliable marker for selecting drought tolerant African spider plant genotypes. This study therefore seeks to address those gaps mentioned above to have a better understanding on how this leafy vegetable can adapt under different stress conditions. Identifying key morpho-physiological and biochemical traits that confer drought tolerance is also of importance in African spider plant crop improvement. The review also established an integrated breeding program that can be adopted for the African spider plant.

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Chapter 3 Morpho-physiological and biochemical characterization of African spider plant (*Gynandropsis gynandra* (L.) Briq.) genotypes under drought and non-drought conditions

Abstract

The African spider plant (*Gynandropsis gynandra* (L.) Briq.) is a nutrient-dense, climate-resilient indigenous vegetable with a C4 carbon fixation pathway. Understanding African spider plant drought tolerance mechanisms is essential for improving its performance in water-stressed areas. Numerous hypotheses have been suggested to explain how the African spider plant can withstand prolonged drought. The objective of this study was to evaluate the stress tolerance potential of 18 African spider plant accessions based on 13 morphological, physiological, and biochemical character traits under three different water treatment regimes, namely, optimum, mild drought and severe drought. Two experiments were carried out in 2020 and 2021 in the tunnels at the University of KwaZulu-Natal, Pietermaritzburg, South Africa using a split-split plot design with four replications and three treatment-regimes namely optimum (100% field capacity), intermediate drought (50% field capacity) and, severe drought (30% field capacity). The results revealed that water regime had a significant effect ($P < 0.01$) on the accessions for the traits studied. A significant reduction across all studied traits was observed under drought conditions, except for proline content, which showed higher levels under drought conditions. Several morphological and physiological parameters, including days to 50% flowering ($r = 0.80$), leaf length ($r = 0.72$), net photosynthesis ($r = 0.76$) and number of leaves per plant ($r = 0.79$), were positively associated with leaf yield under drought conditions. Cluster analysis categorized the 18 accessions and 13 measured parameters into 4 clusters, with cluster-1 exhibiting greater drought tolerance for most of the studied traits, followed by those in cluster-2. Among the accessions tested, accessions L3 and L5 demonstrated excellent drought tolerance and yield performance under both conditions. As a result, these accessions were selected as candidates for African spider plant drought tolerance breeding programs.

Keywords: Drought tolerance, water regime, African spider plant, Phenotyping

3.1 Introduction

African spider plant (*Gynandropsis gynandra* (L.) Briq.) is a member of the Cleomaceae family which is native to SSA. It is also widely referred to as spider flower, spider plant, cats' whiskers, as well as African cabbage. The plant has multiple uses, which include human food and medicine, animal feed, and has crop protectant abilities. *Gynandropsis gynandra* (L.) Briq is an essential leafy vegetable for achieving food security for households in remote regions of several African countries including South Africa, Zimbabwe, Zambia, Kenya, Namibia and Botswana (Mbugua et al., 2011; Keatinge et al., 2015). African spider plant has been found naturally thriving in seven of South Africa's nine provinces ; KwaZulu-Natal, Free State, Gauteng, Mpumalanga and Northern Cape Limpopo, Northwest (Moyo and Aremu, 2022; Chataika et al., 2022).

Vitamins C, A, E, B1, B2, and B9 as well as minerals including iron, zinc, calcium, copper, potassium, magnesium, manganese, phosphorus, and sodium are abundant in the species' leaves and surpass those in most exotic vegetables (Abukutsa-Onyango, 2005; Odhav et al., 2007; Uusiku et al., 2010; Singh et al., 2013; Houdegbe et al., 2022a). The leaves of *Gynandropsis gynandra* (L.) Briq are also high in proteins and fatty acids (Mnzava, 1990), as well as essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine). Furthermore, spider plant has a variety of health-promoting secondary metabolites, including flavonoids, terpenoids, tannins, glucosinolates, aldehydes, ketones, sesquiterpenes, and several other phenolic compounds (Neugart et al., 2017; Somers et al., 2020; Sogbohossou et al., 2020; Chataika et al., 2022) with various therapeutic applications (plant extracts, drugs, etc.). The species is a valuable resource for the pharmaceutical industry because its extracts have antimicrobial (fungi and bacteria), anthelmintic (Ajaiyeoba et al., 2001), antimalarial (Igoli et al., 2016), hepatoprotective (Lakshmi Narsimhulu et al., 2019), antiarthritic (Narendhirakannan et al., 2005b), antioxidant, anti-inflammatory (Chandradevan et al., 2020), immunomodulatory (Kori et al., 2009), antinociceptive (Ghogare et al., 2009), anticancer (Bala et al., 2010a), antidiabetic (Ravichandra et al., 2014), and vasodilatory (Runnie et al., 2004) properties. Improvement of this vegetable will thus help to combat malnutrition, promote health, and generate income for stakeholders such as pharmaceutical companies and local communities.

Drought stress has continuously posed a threat and has become a serious problem to agricultural production. Recent studies have revealed that the majority of rural families in SSA rely on agriculture for their livelihood and food, thus extended periods of droughts in those areas can have a devastating effect on the families (Meze-Hausken, 2000; Mupangwa et al., 2011; Rusinamhodzi et al., 2012). Drought is an extreme climatic phenomenon that is a prevalent natural hazard that leads to water scarcity and eventually famines (Abaje., et al., 2014). In plants, drought stress has been found to have an adverse effect on the morphological, physiological, and biochemical characteristics which leads to low yields (Baroowa et al., 2016).

Due to its efficient drought avoidance, tolerance, and escape mechanisms (Cernansky, 2015; Mabhaudhi et al., 2019b), *G. gynandra* (L.) Briq thrives under insufficient and untimely precipitation. However, the leafy vegetable has received insufficient research in the past, although it is fast gaining attention of researchers and policymakers (Gido et al., 2017). As an underutilized horticultural crop, there are no established varieties of the African spider plant, and the majority of accessions exist in the form of landraces, that is mixtures of genotypes with common local adaptation (Houdegbe et al., 2022a). Genetic diversity of the African Spider plant has been the focus of research as breeders aim to establish knowledge on the existing genetic diversity. Genetic diversity can be identified by morphological, physiological, and biochemical markers (Chakhchar et al., 2015).

Phenotyping continues to be a fundamental criterion for evaluating germplasm on the basis of drought adaptability and essential morpho-physiological and biochemical traits, together with yield and its components (Monneveux et al., 2012; Passioura, 2012). Conventional breeding methods have enhanced crop productivity considerably due to the use of such traits in both ideal and low rainfall conditions. Thus, studying drought-associated traits is extremely important in enhancing crops for drought tolerance. Earlier studies have been centered on identifying a few traits in *G. gynandra* (L.) Briq (Masuka et al., 2012; Wasonga et al., 2015; Omondi et al., 2017; Kangai Munene et al., 2018; Adeka et al., 2019) However, there is no comprehensive information on the characterization of *G. gynandra* (L.) Briq genotypes for morphological, physiological, and biochemical traits under drought stress. The objective of this study was to evaluate the morphological, physiological, and biochemical traits as selection markers for drought tolerance in African Spider plant genotypes. Two key aspects were addressed. Firstly, identifying and selecting genotypes that are tolerant and sensitive to drought based on morpho-physiological and biochemical characteristics. Secondly to identify traits that can be used as markers in identifying tolerant genotypes under drought stress conditions.

3.2 Materials and methods

3.2.1 Plant material

Eighteen African spider plant accessions originating from East Africa (5), West Africa (5), Southern Africa (4) and Asia (4) were evaluated in this study. The accessions were obtained from the gene bank of the University of Abomey-Laboratory Calavi, Genetics, Biotechnology, and Seed Science (Benin); the World Vegetable Center; the Kenya Resource Center for Indigenous Knowledge (Kenya); and the University of Ouagadougou (Burkina Faso) (Table 3.1). The chosen accessions are grown mainly under rain-fed conditions by smallholder farmers and are frequently exposed to prolonged drought stress. These accessions were also selected based on germination percentage and ability to produce high leaf yield under optimum conditions.

3.2.2 Experimental design, growth conditions and agronomic practices

Two experiments were carried out in 2020 and 2021 seasons at the University of KwaZulu-Natal, School of Agricultural, Earth, and Environmental Sciences in Pietermaritzburg, South Africa (29°37'34.1"S and 30°24'14.3"E), under controlled conditions in the Controlled Environment Research Unit (CERU). The 18 African Spider plant accessions were evaluated for drought tolerance using morphological, physiological and biochemical traits under three moisture regimes i.e. severe stress (30% field capacity), moderate stress (50% field capacity), and well-watered (100% field capacity), defined based on the findings of Masinde et al., (2005).

The seeds were sown in October and February of 2020 and 2021 seasons, respectively. The experiment was performed in a split-split plot design with four replicates, with the two seasons comprising the main plot, water stress (normal, mild-drought stress and severe-drought stress) making-up the sub-plots, and the 18 genotypes constituting the sub-sub plots. Under each water regime, the seeds of each accession were sown in dedicated seedling trays and nurtured into seedlings before transplanting into individual pots two weeks after sowing. During the first seven days after transplanting, the seedlings were irrigated to keep the soil moisture in the pots at 100% field capacity. Drought stress was applied from the 8th day after transplanting by ceasing irrigation until the harvest maturity stage, which occurred 21 days after transplanting. Using the method outlined by Kesiime et al. (2016), the amount of water applied in the pots was calculated based on the field capacity (FC) of the potting mixture. Soil moisture was constantly checked using a HS2 and HS2P Hydro sense II soil moisture measurement system and a Campbell scientific CR1000 series data loggers (Campbell scientific, Logan, Utah, USA).

The pots used for the study were medium-sized plastic pots with a pot height of 28cm and a diameter of 30 cm. Each plot consisted of three plants, with a plant in each pot which had a capacity of 4.5 kg. The potting medium used was composted pine bark. Fertilizer containing (N:P: K) (2:3:2) was applied to seedlings in the pots during the transplanting process through the basal application technique at 40g pot^{-1}.

Table 3. 1 List of genotypes used for the study.

Genotype	**Genebank of Origin**	**Country of origin**	**Region**
L1	KENRIK*	Kenya	East Africa
L2	Universityof Ouagadougou	Burkina-Faso	West Africa
L3	GBioS/UAC	Benin	West Africa
L4	GBioS/UAC	Benin	West Africa
L5	GBioS/UAC	Togo	West Africa
L6	Universityof Ouagadougou	Burkina-Faso	West Africa
L7	World Vegetable Center	Thailand	Asia
L8	World Vegetable Center	Zambia	Southern Africa
L9	World Vegetable Center	South Africa	Southern Africa
L10	World Vegetable Center	Malaysia	Asia
L11	World Vegetable Center	Uganda	East Africa
L12	World Vegetable Center	Malaysia	Asia
L13	KENRIK	Kenya	East Africa
L14	World Vegetable Center	Uganda	East Africa
L15	LUANAR	Malawi	Southern Africa
L16	Otjiwarongo	Namibia	Southern Africa
L17	World Vegetable Center	Laos	Asia
L18	KENRIK	Kenya	East Africa

KENRIK, Kenya Resource Centre for Indigenous Knowledge; LUANAR, Lilongwe University of Agriculture and Natural Resources; GBioS/UAC, Laboratory of Genetics, Biotechnology and Seed Science, University of Abomey-Calavi*.**

3.3 Data collection

Data for the following traits were collected from samples taken from 3 plants from each plot.

3.3.1 Morphological and phenological characteristics

1. Days to 50% flowering (Fl) - Recorded from planting date to the date when 50% of the plants initiate flowering.
2. Plant height (Ph, cm) measured from the surface of the soil to the tip of the flower for the three plants in each plot.
3. Leaf length (Ll, cm) determined on fully expanded leaves by measuring the length per each leaf from the pointy part at one end to the point at which the leaf joins the stalk at the other end with a meter ruler. Four leaves per plant were used.
4. Leaf width (Lw, cm) measured on fully expanded leaves, was achieved by recording the longest extension of any two points on the blade edge perpendicular to the leaf length axis using a meter ruler.
5. Stem diameter (Sd, mm) was recorded in mm on the thickest part of the stem using a digital Vernier caliper.

3.3.2 Physiological characteristics

The following physiological traits were assessed:

Relative water content

Fully expanded relatively young leaves from each treatment were collected to get a precise estim ation of relative water content (Rwc). After meticulously drying the surface of the leaf with tissue paper, the samples were covered in polythene bags and taken to the laboratory. The leaf samples were weighed to establish the fresh weight of the leaf (FW). Following that, the samples were placed in petri dishes containing distilled water and left in the dark for an entire night. The excess water from the leaves was wiped with blotting paper before measuring the turgid weight (TW). The leaves were then dried in an oven at 80°C for 24 h, and their dry weight (DW) was measured.

The following formula was used to calculate the relative water content (Rwc):

$$(\%)\ \boldsymbol{RWC} = \frac{\boldsymbol{FW-DW}}{\boldsymbol{TW-DW}} \boldsymbol{X} \quad \mathbf{100} \qquad \text{Equation 3.1}$$

Where FW = Sample of fresh leaf weight TW = Sample of turgid leaf weight and DW = Sample of dry leaf weight.

Leaf gas exchange parameters

During the growing season, the following parameters were recorded three times: Net photosynthesis (Photo), transpiration rate (Trans, mmol m^{-2} s^{-1}), and stomatal conductance (Cond, mol m^{-2} s^{-1}) using a LI-6400XT Portable Photosynthesis System (Licor Bioscience, Inc. Lincoln, Nebraska, USA) equipped with an infrared gas analyser (IRGA) interconnected to a leaf chamber fluorometer (LCF). The outward leaf CO_2concentration (C_a) and the constructed saturating photosynthetic active radiation (PAR) were set to $400\ \mu mol^{-1}$ and $1000\ \mu mol^{-2}\ \ m^{-2}s^{\ -1}$, respectively. The temperature of the leaves was kept constant at 25 °C. The water flow rate and relative humidity were both held constant at $500\ \mu mol$ and 43 %, respectively. To avoid stomatal closure due to low air humidity, the cuvette's leaf-to-air vapour pressure deficit was kept constant at at 1.7 kPa. Parameters were measured on the third half-formed leaf from the plant's tip between 08.30 and 12.00 a.m. by attaching the leaf inside the sensor head. Measurements were taken from three plants in both non-stressed and drought-stressed conditions for each accession.

Chlorophyll content

Chlorophyll content (Spad) was measured on three flag leaves of all plants using a Biobase portable chlorophyll meter (Biobase, Jinan, China).

3.3.3 Proline content

Proline content was determined in the Plant Physiology Laboratory at the University of KwaZulu-Natal, Pietermaritzburg, South Africa. During harvesting, ten fully expanded flag leaf samples were randomly selected from non-stressed and drought-stressed treatments for proline analysis. The samples were freeze-dried and kept at 74 °C. To obtain 0.1 g, the dried leaf tissue was ground and weighed. Upon obtaining the 0.1 g, 10 ml of 3% aqueous sulfosalicylic acid were used to homogenize the 0.1 g of leaf tissue. Proline was extracted using the acid-ninhydrin method described by (Bates et al., 1973). After heating the samples for 1 hour at 100°C, 5 ml of toluene was added. The absorbance of the proline extract in toluene at 520nm was measured using a UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

The concentration of proline was then calculated using the formula proposed by (Bates et al., 1973).

$$\mu g\ proline/ml\ x\ toluene)/115.5\mu g/\mu mole]/[(g\ sample)/5] =\ \mu moles\ proline/g\ of\ fresh\ weight\ mater$$

Equation 3. 2

Where 115.5 μg is the molecular weight of proline

Yield and yield components.

1. Leaf yield (Ly, g) determined by weighing and summing-up all leaf harvests per plot.
2. Number of leaves per plant (Nl) was determined by counting the total number of leaves per plant in each plot. All three plants were counted, and the mean was determined per plot.

The above mentioned traits measured are summarized in Table 3.2

Table 3. 2 Agronomic, physiological, and biochemical traits evaluated in this study.

Acronym	**Name of trait**	**Method of measurement**
Ly	Leaf yield	Weighed leaf yield per plot.
Ph	Plant height	The PH was measured from the surface of the soil to the tip of the flower
Fl	Days to flowering	Recorded from planting date to the date when 50% of the plants initiate flowering.
Ll	Leaf length	Measured the length per each leaf from the pointy part at one end to the point at which the leaf joins the stalk at the other end with a rule. Three leaves per plan were used
Lw	Leaf width	Measured the longest extension of any two points on the blade edge perpendicular to the leaf length axis measured using a meter rule.
Sd	Stem diameter	the thickest part of the stem recorded at the base of the plant stem was measured using a digital Vernier caliper.
Nl	Number of leaves	Counted the total number of leaves per plant.
Spad	Chlorophyll content	evaluated from the adaxial surface of the second top fully expanded leaf of all plants per plot using measured using a Biobase portable chlorophyll meter (Biobase, Jinan, China).
Photo	Net-Photosynthesis rate	were recorded on the upper third flag leaf from 10:00 a.m. to 12:00 p.m.an using an LI-6400XTPortable Photosynthesis System.
Cond	Stomatal conductance	were recorded on the upper third flag leaf from 10:00 a.m. to 12:00 p.m.an using an LI-6400XT Portable Photosynthesis System.
Rwc	Relative water content	Was estimated using the formula (%) $\boldsymbol{RWC} = \frac{\boldsymbol{FW-DW}}{\boldsymbol{TW-DW}}\boldsymbol{X} \quad \boldsymbol{100}$
Trans	Transpiration rate	were recorded on the upper third flag leaf from 10:00 a.m. to 12:00 p.m.an using an LI-6400XT Portable Photosynthesis System.
Pro	Proline content	Evaluated according to the procedure of (Bates et al., 1973).

3.4 Data analysis

R-software version-4.1.1 was used to perform all statistical analysis for the study (R Core Team, 2021). The package *agricolae* version 1.3-5 was used to perform the analysis of variance (ANOVA). Fisher's Least Significant Difference (LSD) was used to compare the significance of the three water treatments at a probability of *P < 0.05*, and the results were presented in a boxplot created in R version 4.1.1 (R Core Team, 2021) using the *ggplot2* package. A hierarchical clustering heatmap showing the studied genotypes and traits were constructed using the R package *ComplexHeatmap.* To analyze the correlation matrix plot, the R package *corrplot* was used, whilst two R packages *FactoMineR* and *factoextra* were utilized to produce the principal component analysis (PCA) and the PCA biplot. Data for split-split plot design was analyzed using a linear mixed model based on the following statistical model:

$$\boldsymbol{Y_{ijkl} = \mu + block_i + A_j + BlockA_{ij} + B_k' + AB_{jk} + BlockB_{ik} + c_i + AC_{jl} + BC_{kl} + ABC_{jkl} + e_{ijkl}} \qquad \text{Equation 3. 3}$$

Where $Y_{ijkl} =$ measurement of outcome variable $\mu =$ overall mean $block_i =$ Random effect of block or replication $A_{j=}$ Fixed effect of factor A (main plot) $BlockA_{ij}$= Random interaction between the Block or Rep and Factor A (Main plot factor)- this is the error term for Factor A - Main plot error B_k = Fixed effect of Factor B - Sub plot AB_{jk}= Fixed interaction between Factor A and Factor B $BlockB_{ik}$= Random interaction between the Block or Rep and Factor B (Sub plot factor) - this is the error term for Factor B and the interaction between Factor A and B -Sub plot error. c_i= Fixed effect of your Factor C-Sub plot. AC_{jl}= Fixed interaction between Factor A and Factor C. BC_{kl} = Fixed interaction between Factor B and Factor C. ABC_{jkl}= Fixed interaction between Factor A, Factor B, and Factor C. e_{ijkl}= Residual error — correct error term for Sub -Sub plot Factor C, AC, BC, and ABC.

Table 3. 3 Skeletal ANOVA for the split-split plot design used in the study.

Source of variation	df	Expected mean square
Replication	r-1	$\sigma^2 + c\sigma_\theta^2 + bc\sigma_y^2 + abcr\sigma_R^2$
A	a-1	$\sigma^2 + C\sigma_\theta^2 + bc\sigma_y^2 + r\sigma_{ABC}^2 + rb\sigma_{AC}^2 + rc\sigma_{AB}^2 + rbc\sigma_A^2$
Error (a) = Rep × A	(r-1) (a-1)	$\sigma^2 + c\sigma_\theta^2 + bc\sigma_y^2 2$
B	b-1	$\sigma^2 + c\sigma_\theta^2 + r\sigma_{ABC}^2 + ra\sigma_{BC}^2 + rc\sigma_{AB}^2 rac\sigma_B^2$
A × B	(a-1) (b-1)	$\sigma^2 + c\sigma_\theta^2 + r\sigma_{AB}^2 + rc\sigma_{AB}^2$
Error (b) = Rep × B(A)	a(r-1) (b-1)	$\sigma^2 + c\sigma_\theta^2$
C	c-1	$\sigma^2 + r\sigma_{ABC}^2 + ra\sigma_{BC}^2 + rb\sigma_{AC}^2 + rab\sigma_C^2$
A × C	(a-1) (c-1)	$\sigma^2 + r\sigma_{ABC}^2 + rb\sigma_{AC}^2$
B × C	(b-1) (c-1)	$\sigma^2 + r\sigma_{ABC}^2 + ra\sigma_{BC}^2$
A ×B × C	(a-1) (b-1) (c-1)	$\sigma^2 + r\sigma_{ABC}^2 + ra\sigma_{BC}^2$
Error(c) = Rep × C (A × B)	ab(r-1) (c-1)	$\sigma^2 + r\sigma_{ABC}^2$
Total	rabc-1	σ^2

3.5 Results

3.5.1 Effect of genotypes, environments, and water regimes on morpho-physiological and biochemical traits

The ANOVA results provided in Table 3.4 show the effects of growing season, water regime, and genotype factors, along with their interaction effects, and coefficients of variation (CVs) on the studied morpho-physiological and biochemical traits. The effect of planting season was not significant for most traits ,except for days to 50% flowering, chlorophyll content, and stomatal conductance *($P < 0.05$)*. Highly significant differences ($P < 0.001$) were recorded for the different water regimes for all traits (Table 3.4). The interaction between season and water regime was not significant for most traits except for Ph, Cond, Photo, Trans and Pro which were significantly affected *($P < 0.001$)* (Table 3.4).

The accessions were significantly different for most studied traits ($P < 0.001$), except for Pro (Table 3.4). Season by genotype interaction was not significant for most traits, except for plant height, leaf length, leaf width and stem diameter ($P < 0.001$) (Table 3.4). Except for transpiration rate and proline content where the interaction was not significant, the interaction between genotype and water regime was highly significant ($P < 0.001$) on all other traits studied. Most of the traits had non-significant interaction effects of season, water regime and genotype, except for plant height, leaf length, leaf width, and net photosynthesis which were significant.

Table 3. 4 F-values of the 13 traits from a combined analysis of variance of 18 African spider plant accessions under 3 water regimes and over two seasons

Sources of variation	**Df**	**Fl**	**Ph**	**Ll**	**Lw**	**Sd**	**Spad**	**Rwc**	**Cond**	**Photo**	**Trans**	**Nl**	**Ly**	**Pro**
Season	1	9.01*	0.70^{ns}	5.64^{ns}	4.76^{ns}	2.24^{ns}	9.76*	0.31^{ns}	6.17*	0.17^{ns}	0.03^{ns}	0.02^{ns}	0.12^{ns}	1.72^{ns}
Rep (Season) = Error a	6	9.10***	8.29***	2.12^{ns}	2.26*	5.25***	1.43^{ns}	1.99^{ns}	2.97**	16.75***	25.45***	3.56**	16.62***	7.68***
WR	2	198.71***	312.25***	166.28***	147.49***	232.06***	210.85***	12761.3***	61.06***	1170.40***	45.64***	2326.75***	1858.77***	611.23***
Season * WR	2	0.37^{ns}	2.27^{ns}	0.23^{ns}	0.13^{ns}	0.39^{ns}	0.09^{ns}	0.44^{ns}	2.07^{ns}	6.42*	2.35***	0.08^{ns}	0.02^{ns}	2.11^{ns}
Rep (Season*WR) = Error b	12	5.91***	2.52**	3.01**	3.79***	2.70**	2.46**	1.17ns	2.85*	1.99*	7.31***	1.59ns	5.59***	4.67***
Gen	17	10.50***	14.56***	12.83***	9.21***	13.44***	2.31**	212.71***	4.02***	22.27***	3.49***	150.40***	467.16***	1.23^{ns}
Season * Gen	17	0.84^{ns}	2.63**	4.43***	2.37**	3.21***	0.75^{ns}	0.44^{ns}	2.32**	1.48^{ns}	1.25^{ns}	0.27^{ns}	0.22^{ns}	0.52^{ns}
WR * Gen	34	2.11**	2.97***	3.64***	4.21***	2.07**	1.80**	45.73***	3.00***	4.48***	1.10ns	40.88***	164.33***	1.00^{ns}
Season * WR * Gen	34	0.64^{ns}	1.69*	2.99***	2.34***	1.37^{ns}	1.17^{ns}	0.36^{ns}	1.96**	2.37***	1.10ns	0.49^{ns}	0.24^{ns}	0.74^{ns}
CV		9.51	17.54	15.60	15.60	18.11	7.79	3.13	30.90	6.38	21.16	12.26	10.38	17.37

WR= Water regime, Gen = Genotypes, CV = Coefficient of variation , Df = Degrees of freedom, Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content, Rwc = Relative water content, Photo = Net photosynthesis rate, Cond = Stomatal conductance, Trans = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield, Pro = Proline content, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.5.2 Performance of the accessions under different water regimes

Figures 3.1, 3.2 and Table 3.5 show the mean values and LSD values comparing the accessions for morpho-physiological and biochemical traits under non-stressed and drought-stressed conditions. When compared to optimum conditions, drought stress reduced morpho-physiological traits. Days to 50% flowering (Fl) under all three conditions showed significant genotypic differences *($P < 0.05$)* (Figure 3.1). A phenological trait, days to 50% flowering was reduced as the stress intensified. Drought stress considerably reduced days to flowering by 11 days under mild stress and 20 days under severe stress conditions. Accession L3 and L5 showed the greatest number of days to flowering under optimum and severe stress conditions, while accessions L8 and L5 recorded the most days to flowering under mild stress conditions.

Plant height (Ph) across all three water regimes showed significant genotypic differences ($P< 0.05$). [Figure 3.1 (b)]. Plant height ranged from 44.26 cm to 61.64 cm under optimum conditions, 18.41- 44.46 cm under mild stress conditions and 14.25-33.71 cm under severe stress. Drought stress significantly reduced the plant height by 34.7% under mild stress and 56.63% under severe stressed conditions. Accessions L5 and L8 recorded the tallest plants whilst accessions L16 and L18 recorded the lowest plant height under optimum conditions. The highest plant height was observed in accessions L8 and L3, whereas accessions L12 and L18 recorded the lowest plant height under mild stress. Under severe stressed conditions, Accessions L12, L16, and L13 recorded the lowest plant height whereas accessions L6 and L8 had the tallest plants.

Leaf length significantly differed among the spider plant accessions *($P < 0.05$)* across all three water regimes [Figure 3.1 (c)]. Drought stress reduced leaf length by 43.6% under severe stress and 28.3% under mild stress conditions. Leaf length ranged from 4.74-8.11 cm under optimum conditions, 3.58-5.61 cm under mild stress and 2.64-4.01 cm under severe stressed conditions. Accessions L3 and L5 recorded the highest leaf length under optimum whist accessions L18 and L10 recorded the lowest leaf length. Under mild stress conditions, accessions L3 and L17 had the highest observed leaf length measurements whereas accessions L16 and L11 recorded the lowest leaf length measurements. Accessions L3 and L14 had the highest leaf lengths under severe stress whereas accessions L2, L12 and L16 recorded the lowest leaf lengths under severe stress.

There were significant *($P < 0.05$)* genotypic differences in leaf width [Figure 3.1 (d)]. Drought stress reduced leaf width by 29.1% in mild stress conditions and 45.54% in severe stress conditions. The range for leaf width was 6.39-10.20 cm under optimum conditions, 3.96-7.16 cm under mild stress and 3.31-4.76 cm under severe stress. Under optimum conditions, accessions L3 and L5 recorded the highest leaf width whereas accessions L1 and L18 recorded the lowest leaf width. Accessions L3 and L8 had the highest leaf widths, whilst L11, L12 and L4 recorded the lowest leaf widths under mild stress. Accessions L15 and L9 recorded the highest leaf width under severe stress whereas accessions L11 and L12 had the lowest leaf widths.

Genotypic differences were significant *($P < 0.05$)* for stem diameter [Figure 3.1 (e)]. Water stress reduced stem diameter by 35% in mild stress conditions and by 52.6% in severe stress conditions. Stem diameter ranged from 4.86-9.00 mm under optimum conditions, 3.13- 6.24 mm under mild stress 2.24-4.05 mm under severe stress conditions. Accessions L3 and L5 recorded the highest stem diameter whereas the lowest were observed for accessions L1 and L18 under optimum conditions. Under mild stress conditions, L18 and L1 were the lowest whist accessions L3 and L8 recorded the highest stem diameters. The lowest recorded stem diameters under severe stress were for accessions L16 and L18 and the highest was observed for accessions L3 and L11.

Chlorophyll content was significantly different under drought stress with a reduction of 14.4% under mild stress and 15.5% under severe stress conditions. The genotypic differences were significant across all three water regimes *($P < 0.05$)* [Figure 3.1 (f)]. Chlorophyll content ranged from 37.78- 43.33 under optimum condition, 32.59- 36.88 units under mild stress and 27.78-32.51 units under severe stress conditions. The highest chlorophyll content readings were observed in accessions L14 and L17 under optimum, L14 and L8 under mild stress and accessions L14 and L11 under severe stress conditions. The lowest readings for chlorophyll content were observed for accessions L10 and L8 under optimum conditions, L16 and L1 under mild stress and accessions L12 and L2 under severe stress conditions.

With regards to relative water content, significant genotypic differences were observed across all water regimes *($P < 0.05$)* [Figure 3.1 (g)]. A decrease in available water caused a significant reduction in the relative water content of the leaves by 28.5% under mild stress and 47.3% under severe stress conditions. Relative water content ranged from 56.75- 88.00% under optimum, 42.31-62.93% under mild stress and 31.84- 49.16% under severe stress conditions. Accessions L3 and L5 recorded the highest relative water content across all three water regimes. The lowest readings in relative water content were noted for accessions L7 and L18 under optimum, L2 and L13 under mild stress and L1 and L12 under severe stress conditions.

Net photosynthesis varied significantly *($P < 0.05$)* among the African spider plant accessions studied [Figure 3.1 (h)] , with L3, and L5 having significantly higher net photosynthesis across all three water regimes. Accessions L10 and L16, accessions L6 and L18 and accessions L18 and L13 recorded the lowest net photosynthesis under optimum, mild stress and severe stress respectively. Drought stress reduced net photosynthesis by 20.6% under mild stress and 47.3% under severe stress conditions. The ranges for net photosynthesis varied from 69.74- 89.08% under optimum, 54.82-65.15% under mild stress and 37.77-56.74 under severe stress conditions.

For stomatal conductance, accessions L3 and L14, had significantly higher conductance under optimum conditions [Figure 3.1 (j)], L16 and L14 under mild stress and L3 and L17 under severe stress conditions. The genotypic differences were significant across all three water regimes *($P < 0.05$)* . Drought reduced stomatal conductance by 35% under mild stress and 47.5% under severe stress. In terms of range, stomatal conductance ranged from 0.1625-0.2931 mol m^{-2} s^{-1} under optimum, 0.0866-0.2050 mol m^{-2} s^{-1} under mild stress and 0.1008-0.1703 mol m^{-2} s^{-1} under severe stress.

Across all water treatments, significant *($P < 0.05$)* genotypic transpiration rates were observed [Figure 3.1 (i)]. Drought stress reduced the transpiration rate by 47.1% under severe stress conditions and 30.3% under mild stress conditions. The transpiration rate ranged from 0.0082-0.0114 mmol m^{-2} s^{-1} under severe stress, 0.0099-0.0138 mmol m^{-2} s^{-1} under mild stress and 0.0146-0.0211 under optimum conditions. Accessions L18 and L15 had the lowest transpiration rates under severe stress whereas the highest rates were observed on L5 and L9. Under mild stress the lowest transpiration rates were found in accessions L15 and L12 whilst the highest rates were found in accessions L1 and L10. Accessions L3 and L5 had the highest transpiration rates whilst L15 and L18 had the lowest rates under optimum conditions.

Water stress decreased the number of leaves by 53.1% and 72.8.4% under mild and severe stress respectively (Table 3. 5). Significant *($P < 0.05$)*) genotypic differences were observed across all three water regimes [Figure 3.1 (k)] . The number of leaves ranged from 11- 44 under severe stress, 26-76 under mild stress and 52-131 under optimum conditions. Accessions L3 and L5 had the highest number of leaves under optimum and severe stress conditions. Accessions L3 and L14 had the highest number of leaves under mild stress conditions. Accessions L2 and L12, L18 and L12 together with accessions L10, L12 and L13 had the least number of leaves under severe stress, mild stress, and optimum conditions respectively.

There were no significant genotypic differences observed among the genotypes for proline content [Figure 3.1 (l)], . However, Pro varied significantly across water regimes and its concentration increased significantly as the stress intensified.

Water stress decreased leaf yield by 63.5% and 85.4% under mild and severe stress respectively (Table 3.5). There were significant ($P < 0.05$) genotypic differences observed across all water regimes (Figure 3.2). Leaf yield ranged from 3.33-26.99 g per plot under severe stress, 11.98-59.27 g per plot under mild stress and 26.57-131.19 g per plot under optimum conditions. Accessions L3 and L5 produced the highest leaf yields in all three water regimes. Accessions L18 and L2 had the lowest yield under severe stress conditions. Accessions L2 and L13 under mild stress and accessions L10 and L18 under optimum produced the lowest yields.

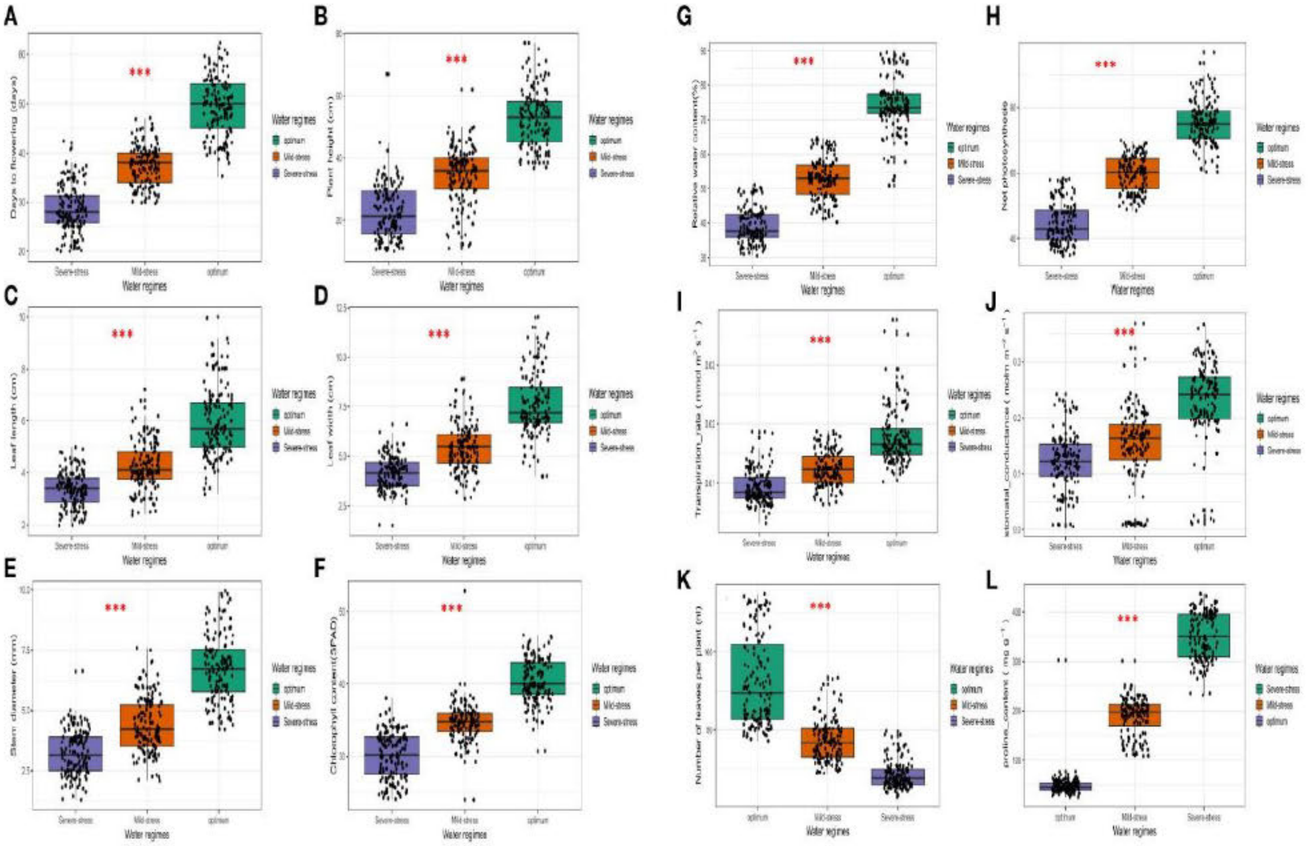


Figure 3. 1 Boxplots showing variation in all 13 morpho-physiological and biochemical traits recorded in 18 African spider plant accessions grown under optimum, mild-stress and severe stress conditions. a) = Days to 50% flowering, b) = Plant height, c) = Leaf length

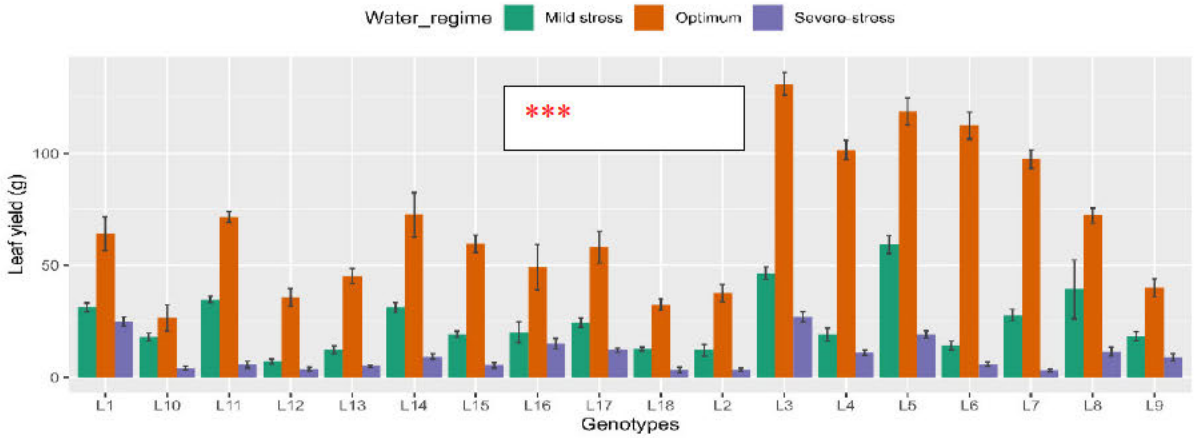


Figure 3. 2 Bar plots showing variation in leaf yield recorded in 18 African spider plant accessions grown under three water regimes

Table 3. 5 Mean values for 13 morpho-physiological and biochemical traits of 18 African Spider plant accessions across three water regimes over two seasons.

	Fl				**Ph**				**Ll**			
Gen	**SS**	**MS**	**WW**	**Mean**	**SS**	**MS**	**WW**	**Mean**	**SS**	**MS**	**WW**	**Mean**
L1	31	37	49	**39**	22.13	32.64	55.09	**36.62**	3.74	3.95	5.50	**4.40**
L2	27	38	47	**37**	16.93	33.34	46.56	**32.28**	2.64	4.11	5.48	**4.08**
L3	35	39	58	**44**	25.53	41.61	54.58	**40.57**	4.01	5.61	8.11	**5.91**
L4	31	39	52	**41**	26.18	27.75	47.86	**33.93**	3.14	3.90	6.90	**4.65**
L5	32	44	55	**44**	26.43	38.29	61.64	**42.12**	3.65	4.38	7.20	**5.08**
L6	29	37	55	**40**	33.71	33.66	58.28	**41.88**	3.05	4.13	5.35	**4.18**
L7	26	37	53	**39**	28.24	37.11	54.06	**39.80**	3.44	4.56	5.41	**4.47**
L8	31	40	50	**40**	30.86	44.46	61.35	**45.56**	3.49	5.34	7.05	**5.29**
L9	29	36	48	**38**	19.26	37.58	53.00	**36.61**	3.56	4.06	6.25	**4.62**
L10	27	36	45	**36**	19.73	35.38	52.11	**35.74**	3.30	4.16	5.10	**4.19**
L11	27	38	49	**38**	18.66	31.88	50.44	**33.66**	3.09	3.79	6.25	**4.38**
L12	25	34	45	**35**	14.49	21.89	52.08	**29.49**	2.90	3.58	5.31	**3.93**
L13	28	37	48	**38**	14.25	32.34	47.35	**31.31**	3.25	4.26	5.59	**4.37**
L14	27	37	50	**38**	26.79	41.30	53.30	**40.46**	3.93	4.59	6.61	**5.04**
L15	26	37	49	**37**	27.18	37.46	60.59	**41.74**	3.43	3.93	5.74	**4.37**
L16	29	37	47	**38**	14.29	37.95	45.84	**32.69**	2.90	3.34	5.38	**3.87**
L17	30	37	46	**38**	29.01	37.26	51.65	**39.31**	3.60	4.63	5.26	**4.50**
L18	27	37	46	**37**	18.36	18.41	44.26	**27.01**	3.38	4.50	4.74	**4.21**
Mean	**29**	**38**	**49**	**39**	**22.89**	**34.46**	**52.78**	**36.71**	**3.36**	**4.27**	**5.96**	**4.53**
Minimum	**27**	**36**	**45**	**35**	**14.25**	**21.89**	**44.26**	**27.01**	**2.64**	**3.34**	**4.74**	**3.87**
Maximum	**32**	**44**	**56**	**44**	**33.71**	**44.46**	**61.64**	**45.56**	**4.01**	**5.61**	**8.11**	**5.91**
LSD p=0.05 water regime			**2.29**				**2.63**				**0.31**	
LSD p=0.05 genotype			**2.08**				**3.66**				**0.40**	
LSD p=0.05 water regime x genotype			**3.40**				**5.46**				**0.60**	
CV			**9.51**				**17.54**				**15.60**	

Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length b Means for leaf width, stem diameter, and chlorophyll content of 18 African Spider plant accessions across three water regimes over two seasons.

	Lw				**Sd**				**Spad**			
Gen	**SS**	**MS**	**WW**	**Mean**	**SS**	**MS**	**WW**	**Mean**	**SS**	**MS**	**WW**	**Mean**
L1	4.56	5.06	6.73	**5.45**	3.08	3.78	6.36	**4.41**	30.23	33.10	41.34	**34.89**
L2	3.56	5.45	6.80	**5.27**	2.68	4.54	7.01	**4.74**	28.46	34.70	40.40	**34.52**
L3	4.56	7.16	10.20	**7.31**	4.05	6.24	9.00	**6.43**	30.65	34.76	41.98	**35.80**
L4	4.00	4.78	8.64	**5.81**	3.44	4.35	7.20	**5.00**	31.55	33.71	40.88	**35.38**
L5	4.18	5.11	8.98	**6.09**	3.54	4.99	7.85	**5.46**	28.53	34.49	41.89	**34.97**
L6	4.08	5.14	6.95	**5.39**	3.96	4.04	6.75	**4.92**	28.93	34.84	40.09	**34.62**
L7	3.98	5.61	7.11	**5.57**	3.83	4.26	6.60	**4.90**	29.38	36.50	39.05	**34.98**
L8	4.20	6.10	8.93	**6.41**	3.76	5.14	7.50	**5.47**	29.71	35.13	42.45	**35.76**
L9	4.75	5.18	8.30	**6.08**	2.79	4.33	6.66	**4.59**	30.86	33.50	40.60	**34.99**
L10	4.53	5.86	6.80	**5.73**	2.84	4.15	5.95	**4.31**	29.94	33.79	37.78	**33.84**
L11	3.36	3.96	8.21	**5.18**	3.99	3.80	7.25	**5.01**	32.14	34.73	39.86	**35.58**
L12	3.31	4.78	7.11	**5.07**	2.84	3.94	5.60	**4.13**	27.78	34.05	40.03	**33.95**
L13	4.16	5.89	7.14	**5.73**	3.00	4.34	7.14	**4.83**	30.48	34.83	38.50	**34.60**
L14	5.15	5.84	7.71	**6.23**	3.15	4.43	6.78	**4.79**	32.51	36.88	43.33	**37.57**
L15	4.76	5.26	7.46	**5.83**	2.73	4.40	6.71	**4.61**	31.49	35.46	40.21	**35.72**
L16	3.70	4.88	6.81	**5.13**	2.24	4.05	5.35	**3.88**	31.14	32.59	39.44	**34.39**
L17	3.90	5.88	7.16	**5.65**	3.21	5.08	6.91	**5.07**	28.91	34.38	42.73	**35.34**
L18	4.05	5.38	6.39	**5.27**	2.55	3.13	4.86	**3.51**	29.94	36.08	38.26	**34.76**
Mean	**4.16**	**5.41**	**7.64**	**5.73**	**3.20**	**4.39**	**6.75**	**4.78**	**30.15**	**34.64**	**40.49**	**35.09**
Minimum	3.31	3.96	6.39	5.07	2.24	3.13	4.86	3.51	27.78	32.59	37.78	33.84
Maximum	5.15	7.16	10.20	7.31	4.05	6.24	9.00	6.43	32.51	36.88	43.33	37.57
LSD p=0.05 water regime			**0.45**				**0.36**				**1.10**	
LSD p=0.05 genotype			**0.51**				**0.49**				**1.55**	
LSD p=0.05 water regime x genotype			**0.78**				**0.71**				**2.30**	
CV			**15.60**				**18.11**				**7.79**	

Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content

3.5 cont. ….

		Rwc				**Photo**				**Cond**		
Gen	**SS**	**MS**	**WW**	**Mean**	**SS**	**MS**	**WW**	**Mean**	**SS**	**MS**	**WW**	**Mean**
L1	33.46	53.34	73.03	**53.28**	47.50	60.18	71.76	**59.81**	0.1008	0.1265	0.1796	**0.1356**
L2	36.95	42.31	66.98	**48.75**	42.48	58.94	70.89	**57.44**	0.1345	0.0942	0.2148	**0.1478**
L3	48.83	62.93	88.00	**66.59**	52.57	64.64	89.08	**68.76**	0.1703	0.1187	0.2931	**0.1940**
L4	35.22	47.85	73.18	**52.08**	44.98	61.86	78.91	**61.92**	0.1182	0.1334	0.2547	**0.1688**
L5	49.16	61.70	87.43	**66.10**	56.74	65.15	84.97	**68.95**	0.1212	0.1629	0.2847	**0.1896**
L6	43.14	63.42	77.00	**61.19**	44.35	54.82	82.74	**60.64**	0.1252	0.1724	0.2787	**0.1921**
L7	40.04	45.80	56.75	**47.53**	41.86	59.85	74.83	**58.85**	0.1111	0.1727	0.2507	**0.1782**
L8	36.42	48.05	73.95	**52.81**	46.41	64.13	73.80	**61.45**	0.1117	0.1434	0.2184	**0.1578**
L9	42.33	52.80	75.60	**56.91**	42.60	57.80	73.00	**57.80**	0.1594	0.1268	0.2495	**0.1786**
L10	37.43	51.29	70.58	**53.10**	41.11	61.07	69.93	**57.37**	0.1180	0.1544	0.1625	**0.1450**
L11	38.43	53.27	77.62	**56.44**	45.27	61.81	74.06	**60.38**	0.1143	0.1784	0.2451	**0.1793**
L12	31.84	52.21	77.06	**53.70**	41.76	59.58	72.97	**58.10**	0.1031	0.1679	0.2274	**0.1661**
L13	34.45	44.31	71.71	**50.16**	41.11	56.94	73.41	**57.15**	0.1078	0.1838	0.2422	**0.1779**
L14	41.32	53.10	81.96	**58.79**	46.11	59.37	77.15	**60.88**	0.1157	0.1885	0.2908	**0.1983**
L15	36.68	54.11	72.79	**54.53**	43.80	56.54	74.85	**58.40**	0.1131	0.1058	0.2058	**0.1416**
L16	41.50	57.45	72.27	**57.07**	45.95	60.95	69.74	**58.88**	0.1184	0.2050	0.1504	**0.1579**
L17	34.70	57.03	76.97	**56.23**	41.23	58.45	74.00	**57.89**	0.1387	0.1794	0.2120	**0.1767**
L18	43.78	55.53	65.91	**55.07**	37.77	54.92	71.72	**54.80**	0.1017	0.0866	0.1971	**0.1285**
Mean	**39.20**	**53.14**	**74.38**	**55.57**	**44.64**	**59.83**	**75.43**	**59.97**	**0.1213**	**0.1500**	**0.2310**	**0.1674**
Minimum	31.84	42.31	56.75	48.75	37.77	54.92	69.74	54.80	0.1008	0.0866	0.1504	0.1285
Maximum	49.16	63.42	88.00	66.59	56.74	65.15	89.08	68.95	0.1703	0.2050	0.2931	0.1983
LSD p=0.05 water regime			**0**				**1.39**				**0.02**	
LSD p=0.05 genotype			**0**				**2.17**				**0**	
LSD p=0.05 water regime x genotype			**0**				**3.16**				**0.01**	
CV			**3.13**				**6.38**				**30.90**	

Rwc = Relative water content, Photo= Net photosynthesis rate, Cond = Stomatal conductance

3.5 cont. ….

	Trans				Nl				Ly			
Gen	**SS**	**MS**	**WW**	**Mean**	**SS**	**MS**	**WW**	**Mean**	**SS**	**MS**	**WW**	**Mean**
L1	0.0093	0.0138	0.0184	**0.0138**	32	46	79	**52**	24.89	31.34	64.19	**40.14**
L2	0.0092	0.0115	0.0162	**0.0123**	11	33	53	**32**	3.43	11.98	37.60	**17.67**
L3	0.0094	0.0131	0.0211	**0.0145**	44	56	126	**75**	26.99	46.49	131.19	**68.22**
L4	0.0110	0.0126	0.0178	**0.0138**	16	39	76	**44**	10.97	19.04	101.67	**43.89**
L5	0.0114	0.0134	0.0206	**0.0151**	37	49	131	**72**	19.06	59.27	118.78	**65.70**
L6	0.0091	0.0122	0.0187	**0.0133**	20	44	125	**63**	5.81	14.15	112.42	**44.13**
L7	0.0087	0.0135	0.0197	**0.0140**	22	51	112	**62**	3.20	27.80	97.50	**42.83**
L8	0.0085	0.0141	0.0175	**0.0134**	25	55	94	**58**	11.45	39.31	72.26	**41.01**
L9	0.0116	0.0121	0.0172	**0.0136**	14	33	62	**36**	8.80	18.33	40.00	**22.38**
L10	0.0091	0.0140	0.0170	**0.0134**	17	44	53	**38**	4.05	17.95	26.57	**16.19**
L11	0.0092	0.0111	0.0174	**0.0126**	18	36	58	**37**	5.67	34.87	71.69	**37.41**
L12	0.0088	0.0107	0.0163	**0.0119**	13	26	52	**30**	3.74	6.96	35.66	**15.45**
L13	0.0083	0.0131	0.0173	**0.0129**	16	31	53	**33**	4.98	12.21	45.17	**20.79**
L14	0.0097	0.0134	0.0200	**0.0144**	24	76	113	**71**	9.26	31.25	72.49	**37.67**
L15	0.0082	0.0099	0.0146	**0.0109**	15	53	79	**49**	5.33	19.19	59.71	**28.08**
L16	0.0099	0.0129	0.0171	**0.0133**	26	38	65	**43**	15.07	20.10	49.21	**28.13**
L17	0.0095	0.0112	0.0174	**0.0127**	25	44	80	**50**	12.02	24.43	58.18	**31.54**
L18	0.0082	0.0110	0.0158	**0.0117**	14	26	54	**31**	3.33	12.68	32.54	**16.18**
Mean	**0.0094**	**0.0124**	**0.0178**	0.0132	**22**	**43**	**81**	49	**9.89**	**24.85**	**68.16**	**34.30**
Minimum	0.0082	0.0099	0.0146	0.0109	11	26	52	30	3.20	6.96	26.57	15.45
Maximum	0.0116	0.0141	0.0211	0.0151	44	76	131	75	26.99	59.27	131.19	68.22
LSD p=0.05 water regime			**0**				**1.93**				**2.16**	
LSD p=0.05 genotype			**0**				**3.30**				**2.04**	
LSD p=0.05 water regime x genotype			**0**				**4.77**				**3.27**	
CV			**21.16**				**12.26**				**10.38**	

Trans = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield

3.5 cont. ….

		PRO		
Gen	**SS**	**MS**	**WW**	**Mean**
L1	355.34	176.44	46.25	192.68
L2	323.38	185.28	42.88	183.85
L3	356.10	182.21	50.65	196.32
L4	349.11	202.31	41.27	197.56
L5	362.28	210.48	51.45	208.07
L6	345.63	169.39	44.51	186.51
L7	367.03	175.29	75.85	206.06
L8	325.80	182.33	49.65	185.93
L9	362.23	196.43	48.25	202.30
L10	345.72	189.73	47.03	194.16
L11	338.60	191.12	40.06	189.93
L12	345.27	184.25	44.55	191.36
L13	356.44	189.18	45.28	196.97
L14	369.44	184.54	46.01	200.00
L15	354.20	183.83	49.14	195.72
L16	312.53	200.02	44.57	185.71
L17	369.52	180.58	53.60	201.23
L18	373.38	203.37	48.70	208.48
Mean	**350.67**	**188.15**	**48.32**	**195.71**
Minimum	312.53	169.39	40.06	183.85
Maximum	373.38	210.48	75.85	208.48
LSD p=0.05 water regime		**18.86**		
LSD p=0.05 genotype		**19.31**		
LSD p=0.05 water regime x genotype		**30.59**		
CV		**17.37**		

Pro = Proline content

3.5.3 Principal component analysis

Principal component analysis (PCA) showing the proportion of total variance explained, and cumulative variance of studied morpho-physiological and biochemical traits among African spider plant accessions under non-stressed and drought-stressed conditions are presented in Table 3.4. Three principal components (PCs) with a cumulative variance of 82% were identified under optimum conditions. PC1 positively related to leaf yield, days to 50% flowering, net photosynthesis, and the number of leaves per plant, accounting for 61% of total variation. Proline content and plant height were negatively correlated with PC2. In contrast, relative water content was positively correlated with PC2, which was responsible for 14 % of total variation. Plant height and chlorophyll content positively correlated with PC3, which explained 7% of total variation.

Similarly, three PCs with a cumulative variance of 84% were identified under drought-stress conditions. PC1 negatively correlated with all the thirteen studied and accounted for 69% of total variation. PC2 positively correlated with leaf width, chlorophyll content, leaf length, number of leaves per plant and negatively correlated with net photosynthesis and stomatal conductance accounting for 9% of total variation. PC3 positively correlated with number of leaves per plant, and relative water content accounting for 6% of total variation.

The association between African spider plant accessions and investigated traits is represented using principal component bi-plots under optimum and drought-stressed conditions (Figure 3.3). In terms of discriminating accessions, relatively small angles between dimension vectors indicated high trait correlation. Accessions that exceled at a specific trait were plotted closer and further away from the vector line. Under optimum conditions, the biplot was created using PC1 (61.5%) and PC2 (13.7%) (Figure 3.3a). The biplot results revealed that most traits clustered together in the biplot's rightmost part region except for proline. However, most the accessions were scattered at the leftmost part region of the biplot.

Under drought stressed conditions, the biplot was created using PC1 (69.2%) and PC2 (8.9%) (Figure 3.3b). The biplot findings confirmed that traits such as leaf width and proline content were clustered together in the biplot's leftmost region. The accessions under severe stress were clustered at the left most region whilst mild-stress accessions were clustered at the right. Traits such as leaf yield, number of leaves per plant, stem diameter, days to 50% flowering, net photosynthesis, transpiration rate, chlorophyll content, stomatal conductance, relative water content, leaf length and plant height were clustered to the right side.

Table 3. 6 Rotated component matrix of 13 morpho-physiological and biochemical traits of 18 African spider plant accessions under drought-stressed and optimum conditions.

	Optimum			**Drought-Stressed**		
Traits	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Fl	**0.32043**	-0.19588	-0.21092	-0.33774	-0.1781	0.232332
Ph	0.211481	-0.11642	**0.686428**	-0.31664	0.07903	-0.38559
Ll	**0.30578**	0.242105	-0.04807	-0.31816	**0.364343**	0.004674
Lw	0.294836	0.231222	-0.05752	-0.2421	**0.428673**	-0.12701
Sd	0.299911	0.126289	-0.06238	-0.33257	-0.04518	-0.23727
Spad	0.229639	0.222319	**0.499979**	-0.07015	**0.528818**	-0.09657
Rwc	0.239282	**0.440847**	0.120608	-0.23018	-0.03766	**0.321672**
Photo	**0.327278**	-0.01867	-0.2026	-0.32746	-0.31684	0.138791
Cond	0.286774	-0.01922	-0.25965	-0.13662	-0.2583	-0.3314
Trans	0.286964	-0.19249	-0.15048	-0.24614	-0.25001	0.113058
Nl	**0.306739**	-0.28105	0.167221	-0.0536	**0.319616**	**0.64286**
Pro	0.057781	**-0.653**	0.169809	-0.3664	0.102217	-0.13569
Ly	**0.321105**	-0.18197	-0.14963	-0.36568	-0.14215	0.196314
Explained variance (eigenvalue)	2.8278	1.3367	0.96179	8.990	1.154	0.812
Proportion of total variance (%)	61.512	13.744	7.11566	69.154	8.879	6.248
Cumulative variance (%)	61.512	75.25	82.37254	69.154	78.033	84.280

Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content, Rwc = Relative water content, Photo= Net photosynthesis rate, Cond = Stomatal conductance, Trans = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield, Pro = Proline content

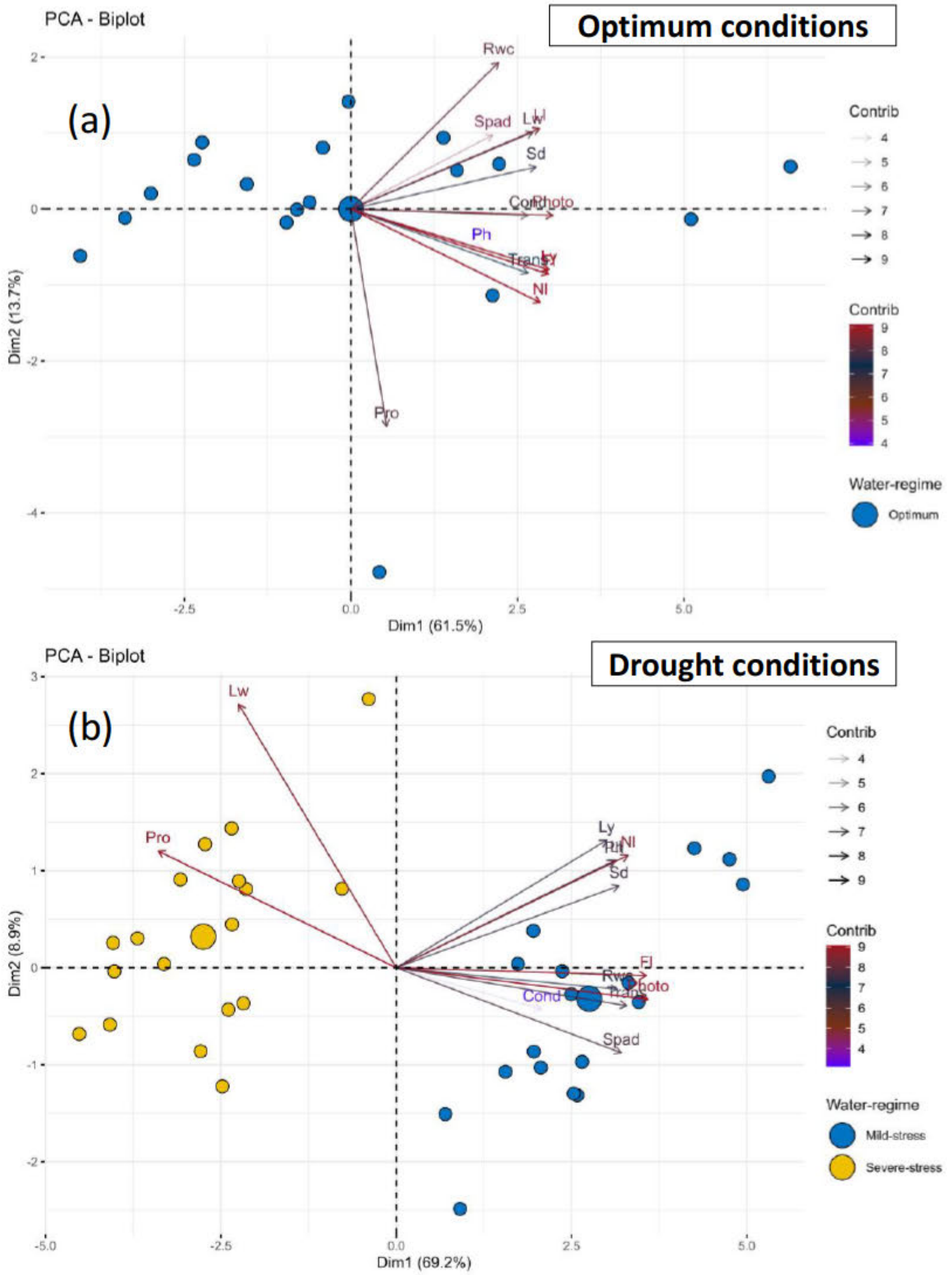
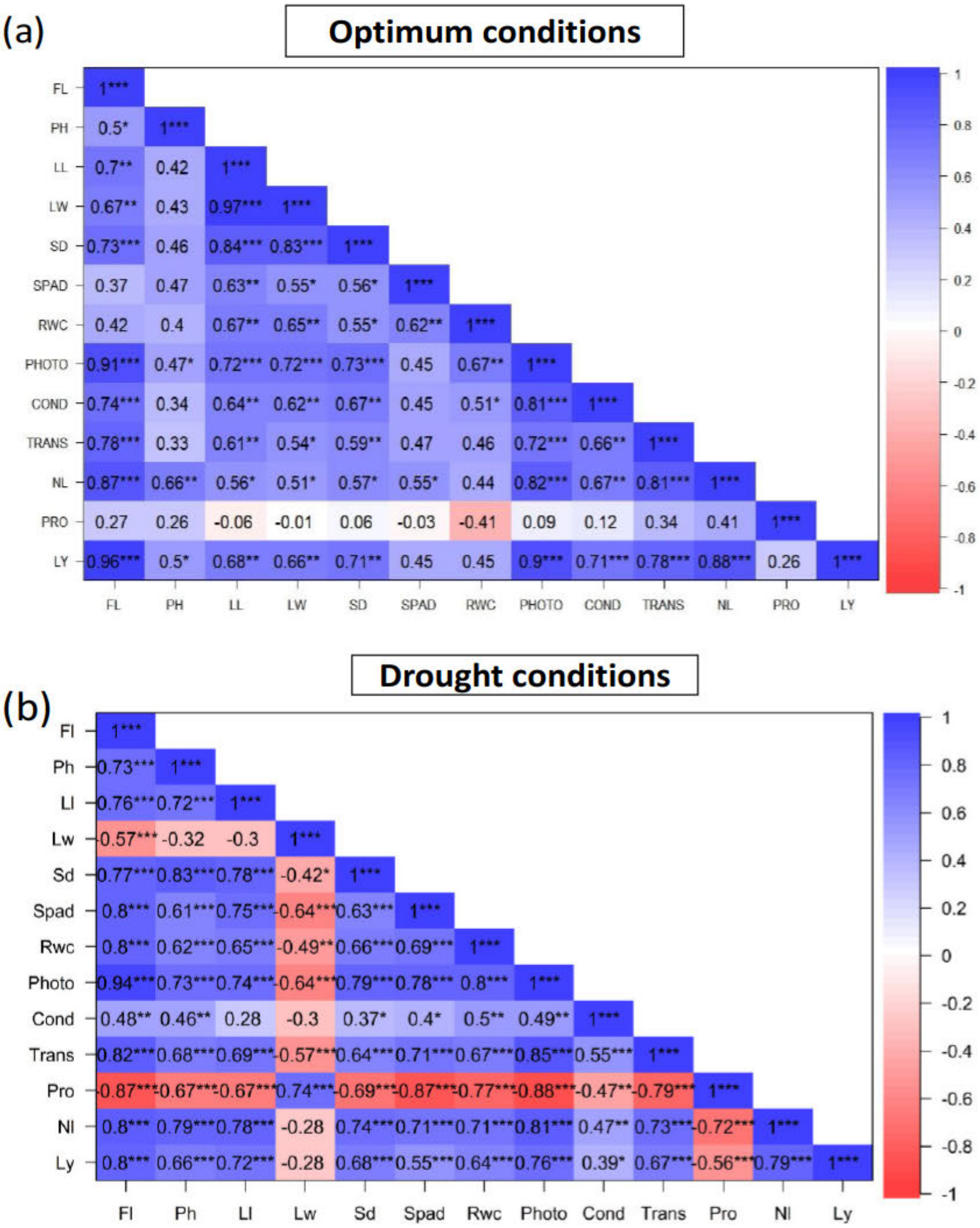


Figure 3. 3 Principal component analysis (PCA)-biplot of 18 African Spider plant accessions based on the variance in 13 morpho-physiological and biochemical traits grown under a) optimum and b) drought conditions. Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content, Rwc = Relative water content, Photo= Net photosynthesis rate, Cond = Stomatal conductance, Trans = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield, Pro = Proline content

3.5.4 Pearson's correlation coefficient analysis

Figure 3.3 illustrates Pearson correlation coefficients for the 13 characters studied. Under optimum conditions (Figure 3.4), morphological traits such as leaf length and leaf width were positively and significantly correlated with each other ($r = 0.97$; $P < 0.001$). Leaf length was also positively and significantly correlated with stem diameter ($r = 0.84$; $P < 0.001$). Physiological traits were also found to be correlated with each other under optimum conditions. Net photosynthesis was positively and significantly correlated with transpiration rate ($r = 0.72$; $P < 0.001$) and stomatal conductance ($r = 0.81$; $P < 0.001$). Yield and yield components were found to be high and significantly correlated with each other. Leaf yield was positively and significantly correlated with number of leaves per plant ($r = 0.88$; $P < 0.001$). Leaf yield was also positively and significantly correlated with Days to 50% flowering ($r =0.96$; $P < 0.001$), leaf length ($r =0.68$; $P < 0.001$), stem diameter ($r = 0.71$; $P < 0.001$), net photosynthesis ($r = 0.9$; $P < 0.001$), stomatal conductance ($r =0.71$; $P < 0.01$), transpiration rate ($r = 0.78$; $P < 0.001$) and leaf width ($r = 0.66$; $P < 0.01$). Furthermore, there was a negative correlation between Proline content and Relative water content ($r = -0.41$; $P = 0.09$).

In terms of correlation coefficients in drought conditions (Figure 3.3b), morphological traits such as leaf length and stem diameter were positively and significantly correlated with each other ($r = 0.78$; $P < 0.001$). Physiological traits such as chlorophyll content were positively and significantly correlated with transpiration rate ($r = 0.71$; $P < 0.001$), net photosynthesis ($r = 0.78$; $P < 0.001$) and relative water content ($r = 0.69$; $P < 0.001$). Leaf yield was positively and significantly correlated with Days to 50% flowering ($r = 0.80$; $P < 0.001$), plant height ($r = 0.66$; $P < 0.001$), leaf length ($r = 0.72$; $P < 0.001$), stem diameter ($r = 0.68$; $P < 0.001$), relative water content ($r = 0.64$; $P < 0.001$), net photosynthesis ($r = 0.76$; $P < 0.001$), transpiration rate ($r = 0.67$; $P < 0.001$) and number of leaves per plant ($r = 0.79$; $P < 0.001$). Proline content was significantly and negatively correlated with all studied traits except for leaf width ($r = 0.74$; $\mathrm{P} < 0.001$) the only positively correlated trait with proline content.



	FL	PH	LL	LW	SD	SPAD	RWC	PHOTO	COND	TRANS	NL	PRO	LY
FL	1***												
PH	0.5*	1***											
LL	0.7**	0.42	1***										
LW	0.67**	0.43	0.97***	1***									
SD	0.73***	0.46	0.84***	0.83***	1***								
SPAD	0.37	0.47	0.63**	0.55*	0.56*	1***							
RWC	0.42	0.4	0.67**	0.65**	0.55*	0.62**	1***						
PHOTO	0.91***	0.47*	0.72***	0.72***	0.73***	0.45	0.67**	1***					
COND	0.74***	0.34	0.64**	0.62**	0.67**	0.45	0.51*	0.81***	1***				
TRANS	0.78***	0.33	0.61**	0.54*	0.59**	0.47	0.46	0.72***	0.66**	1***			
NL	0.87***	0.66**	0.56*	0.51*	0.57*	0.55*	0.44	0.82***	0.67**	0.81***	1***		
PRO	0.27	0.26	-0.06	-0.01	0.06	-0.03	-0.41	0.09	0.12	0.34	0.41	1***	
LY	0.96***	0.5*	0.68**	0.66**	0.71**	0.45	0.45	0.9***	0.71***	0.78***	0.88***	0.26	1***

	Fl	Ph	Ll	Lw	Sd	Spad	Rwc	Photo	Cond	Trans	Pro	Nl	Ly
Fl	1***												
Ph	0.73***	1***											
Ll	0.76***	0.72***	1***										
Lw	0.57***	-0.32	-0.3	1***									
Sd	0.77***	0.83***	0.78***	-0.42*	1***								
Spad	0.8***	0.61***	0.75***	-0.64***	0.63***	1***							
Rwc	0.8***	0.62***	0.65***	-0.49**	0.66***	0.69***	1***						
Photo	0.94***	0.73***	0.74***	-0.64***	0.79***	0.78***	0.8***	1***					
Cond	0.48**	0.46**	0.28	-0.3	0.37*	0.4*	0.5**	0.49**	1***				
Trans	0.82***	0.68***	0.69***	-0.57***	0.64***	0.71***	0.67***	0.85***	0.55***	1***			
Pro	0.87***	-0.67***	-0.67***	0.74***	-0.69***	-0.87***	-0.77***	-0.88***	-0.47**	-0.79***	1***		
Nl	0.8***	0.79***	0.78***	-0.28	0.74***	0.71***	0.71***	0.81***	0.47**	0.73***	-0.72***	1***	
Ly	0.8***	0.66***	0.72***	-0.28	0.68***	0.55***	0.64***	0.76***	0.39*	0.67***	-0.56***	0.79***	1***

Figure 3. 4 Pearson 's correlation coefficient of the 13 measured traits of 18 African Spider plant evaluated under optimum (a) and drought stress (b) conditions. * Significant at $P < 0.05$, ** Significant at $P < 0.01$, *** Significant at $P < 0.001$. Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content, Rwc = Relative water content, Photo= Net photosynthesis rate, Cond = Stomatal conductance, Trans = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield, Pro = Proline content.

3.5.5 Cluster analysis

A hierarchical clustering characterized by significant fold-change values through a complete method and Euclidean distance measurement was conducted to provide an overview of the morpho-physiological and biochemical traits and identify major clusters across 18 accessions under both control and stress conditions (Figure 3.5). Based on cultivar-trait relationships, the various colors and intensities were adjusted. The lighter whitish-orange color represents lower values (drought-sensitive), whereas the darker red color represents higher values (drought-tolerant). Based on the cluster heatmap, four clusters were identified.

The 18 African Spider plant accessions were organized into four row-clusters, with cluster-1, cluster-2, cluster-3, and cluster-4 each consisting of 2, 1, 5 and 10 accessions, respectively, with the most closely related accessions within each cluster joining. Cluster 1 showed accessions with the highest drought tolerance based on leaf yield and other traits. Accessions L3 and L5 were found in cluster 1 thus representing genotypes with the highest drought tolerance. Cluster 2 also showed a great degree of drought tolerance with one accession L14 present in this cluster. Cluster 3 had accessions that were on average tolerant to drought stress with accessions L7, L4, L1, L8 and L6 representing this cluster. Cluster 4 had drought sensitive genotypes with 10 genotypes present in this cluster. Accessions L15, L11, L17, L9, L13, L10, L16, L2, L12, and L18 were all found in cluster 4.

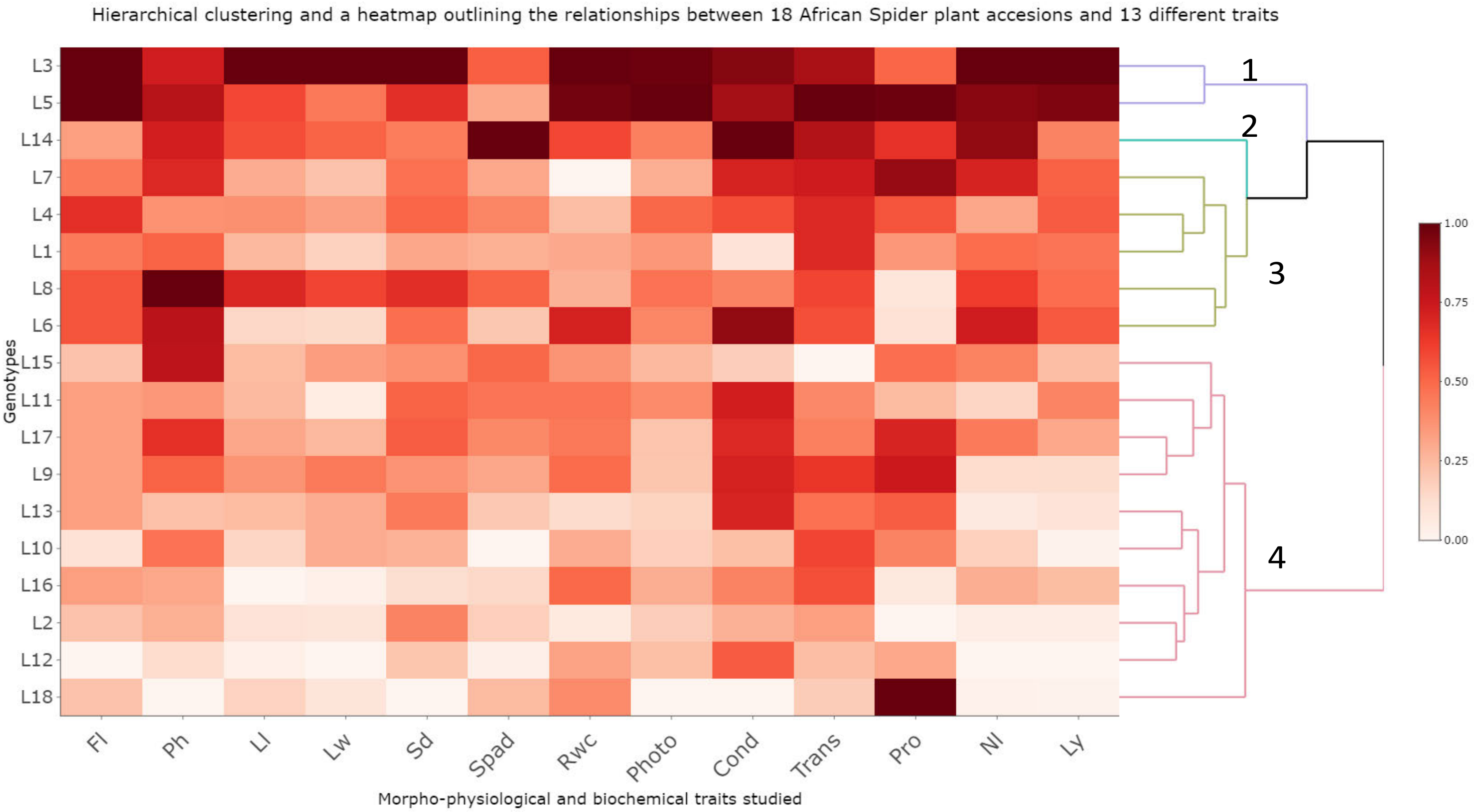


Figure 3. 5 Hierarchical clustering and heatmap illustrating the associations among 18 African Spider plant and 13 different traits in respect to drought tolerance. FL = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content, Rwc = Relative water content, Photo= Net photosynthesis rate, Cond = Stomatal conductance, Trans = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield, Pro = Proline content.

3.6 Discussion

The current study assessed the morpho-physiological and biochemical responses of African spider plant accessions to identify genotypes with a combination of traits that are adaptable to water-limited conditions. All the accessions used in the study were chosen from different pedigrees and most of the measured traits are quantitatively inherited and thus expected to have significant effects on the African spider plant genotypes, water regimes, and genotype by water regime interaction.

The study observed variable responses amongst the accessions for the measured morpho-physiological and biochemical traits varied considerably across the African spider plant accessions. Water stress affected several phenological processes (Table 3.5 and Figure. 3.1). For example, days to 50% flowering a significant phenological trait linked to present photosynthesis and assimilate displacement from reserve pools in vegetative tissues, was reduced. Drought, in particular, reduces a plant's life cycle and duration of flowering. The flowering period shortens under these conditions due to increased leaf senescence, reduced photosynthetic activity, and sink limitation (Fang and Xiong, 2015; Shavrukov et al., 2017; Paudel et al., 2021). Moreover, the relatively short flowering period has a direct impact on leaf number and leaf size, which accounts for a large portion of the decrease in African spider plant yields. Early flowering is a drought escape mechanism that has been developed by plants to complete its life cycle under water deficit stress. This phenomenon explains why accessions L12, L2 and L18 recorded extremely low leaf length, leaf width, number of leaves per plant and eventually very low leaf yields. Because the African spider plant is a facultative long-day plant, selecting for delayed bolting has possibility of increasing yield (Koevenig, 1973; Zorde et al., 2020).This phenomenon is backed by this study which showed that accessions L3 and L5 had more flowering days and because of that recorded the highest number of leaves and leaf yields.

Morphological traits such as plant height, leaf length, leaf width and stem diameter were significantly reduced in the study by drought stress. Water stress has a significant impact on cell expansion and growth, which is linked with a loss of cell turgor, resulting in a reduction in plant height. Similar patterns have previously been reported in legumes (Baroowa and Gogoi, 2012). Reduced leaf water status impairs cell division in dehydrated plants, causing a decrease in leaf morphological traits (Dale, 1988; Tardieu, 2013).

Leaf gas exchange parameters were reduced under both severe and mild stress conditions. The decrease in photosynthetic rates was caused by both stomatal and non-stomatal factors. Drought stress has been shown to reduce photosynthesis in faba bean (Girma and Haile, 2014), grain legumes (Farooq et al., 2017), and dry bean (Lanna et al., 2017). Furthermore, under drought stress, stomata close, causing a decrease in stomatal conductance and, as a result, a lower photosynthetic rate (Reddy et al., 2004; Chaves et al., 2009) . Drought stress causes a water shortage within the plant tissue, which significantly inhibits photosynthesis. It has been reported that stomatal closure reduces bean photosynthetic rates (Brestic et al., 1995). Berry and Bjorkman, (1980) claim that an integration of stomatal and non-stomatal effects on photosynthesis occurs, based on the severity of drought stress. Tezara et al., (1999) concluded that water stress restricts photosynthesis by reducing the supply of ribulose-1,5-bisphosphate (RuBP) because of low ATP-synthesis.

Furthermore, under water deficit conditions, stomata begin to close, resulting in decreased stomatal conductance, which may lead to decreased photosynthetic rate (Morales et al., 2020). Previous studies have shown that a decrease in stomatal conductance in drought-stressed plants reduces photosynthesis (Kirschbaum, 2004; Flexas et al., 2004; Reddy et al., 2004). Stomatal closure during water stress, as well as decreased CO_2 availability in chloroplasts, are major causes of reduced photosynthetic activity (Franks et al., 2015). Stomatal closure restricts CO_2 from entering the leaf and decreases photosynthetic carbon assimilation in favour of photorespiration. Drought stress has a similar effect on transpiration and photosynthetic rate (Li et al., 2017). When plants are stressed by drought, their stomata close, resulting in less transpiration and a restriction of gas exchange between the leaves and the environment.

Drought stress significantly reduced chlorophyll content, with a reduction of 14.4% under mild stress and 15.5% under severe stress conditions. The decrease in chlorophyll content could have stemmed from drought-damaged leaves turning yellowish. Drought stress has been shown to reduce chlorophyll content in wheat (Talebi, 2011), pea (Iturbe-Ormaetxe et al., 1998), maize (Mohammadkhani and Heidari, 2007), chickpea (Mafakheri et al., 2010), soybean (Makbul et al., 2011), and rice (Jnandabhiram and Sailen Prasad, 2012). The reduction in chlorophyll content is due to chloroplast damage stemming from active oxygen species (Golldack et al., 2014). Drought stress causes the formation of reactive oxygen species (ROS) such as O_2 and H_2O_2, which destroy chlorophyll (Smironff, 1993; Foyer et al., 1994).

The relative water content estimates in the drought stressed and optimum treatments were consistent with previous findings reported by (Goodarzian Ghahfarokhi et al., 2015; Soltys-Kalina et al., 2016). Drought stress reduced the relative water content of the leaves by 28.5% under mild stress and 47.3% under severe stress, but accessions differed in preserving their relative water content under both water stressed and optimum conditions. The water balance of a plant is interrupted during drought stress, resulting in a decrease in relative water content and water potential of leaves (Bogale et al., 2011). High relative water content values are widely regarded as an index of stress tolerance. Relative water content is also thought to be a reliable predictor of the severity of water stress as evidenced by accessions L3 and L5 which showed a high relative water content value across all water treatment.

As the stress increased, the proline content increased significantly. Plants can effectively protect cells from water stress by increasing proline accumulation and stabilizing osmotic potential with the external environment, as noted in wheat (Mwadzingeni et al., 2016). Elevated proline accumulation plays an adaptative role in conferring tolerance in plants (Bhaskara et al., 2015). During drought stress, proline accumulation performs the function of a compatible solute, limiting water loss from plant cells (Li et al., 2019). It also aids in the supply of energy for plant survival and growth (Furlan et al., 2020). As a result, accumulation of proline content can be employed as an effective selection criterion in germplasm screening studies for drought tolerance (Saddique et al., 2020; Arteaga et al., 2020; Belay et al., 2021).

Water stress causes defoliation and the cessation of new leaf production, resulting in fewer leaves. This explains why the number of leaves reduced significantly as the stress intensified from mild to severe stress. Selecting for higher yields under both stressed and non-stressed conditions allows accessions to retain high yield rankings because the same accessions will perform well in either situation.

The reported retention of high leaf yields under stressed and non-stressed conditions in some accessions, such as L3, and L5, supports (Foulkes et al., 2007) findings that accessions doing well under non-stressed conditions retain high leaf yield under stress. However, the strong cross-over associations found in this study, on the other hand, were caused by severe stress imposed on the accessions, resulting in yield losses of approximately 63.5% and 85.4% under mild and severe stress respectively in comparison to stress imposed by Foulkes et al., (2007).

In this study, a PCA was used to identify the most crucial morpho-physiological and biochemical traits for the distribution of a set of accessions in the three treatments (Table 3.5 Figure 3.3) and, as a result, the traits that may be most important for drought tolerance. PCA-biplot is a multivariate analytic technique that combines traits and variables in two or more dimensions while minimizing overlapping variations to make it simpler to discover key elements for selection (Kose et al., 2018; Huqe et al., 2021). Strong positive loading of leaf yield, days to 50% flowering, net photosynthesis, and the number of leaves per plant in the PC1 under optimum conditions indicates that they have a significant effect and can be selected for at the same time due to their direct influence on each other (Table 3.5). Under drought stressed conditions, PC3 had a favorable correlation with plant leaf count and relative water content. PCA results also showed that variables Leaf width and proline content clustered together in the PCA biplot, closely scattering around the accessions under severe stress conditions, indicating that their role in selecting best characters under severe stress conditions is critical.

Understanding the relationship between traits allows for effective and simultaneous selection. The moderate to high positive and significant correlations ($r > 0.50$) of leaf yield with days to 50% flowering, plant height, net photosynthesis, leaf length, stem diameter, transpiration rate and the number of leaves per plant under both optimum and stressed conditions (Figure 3.4) suggest these characteristics make a direct contribution to yield and should be characterized as influential target traits during selection.

Under optimum conditions, leaf yield was also positively and significantly correlated with Days to 50% flowering, leaf length, stem diameter, net photosynthesis, stomatal conductance, transpiration rate, leaf width, and number of leaves per plant which emphasizes the importance of those yield components in contributing to high leaf yield under optimum conditions. Previous research has found a positive and strong correlation between leaf yield and plant height, stem diameter, leaf length and leaf width (Zakaria et al., 2017). Similarly, (Kangai Munene et al., 2018; Houdegbe et al., 2022) discovered a positive and strong relationship between plant height and the number of leaves per plant.

Under drought stress conditions, leaf yield was positively and significantly correlated with days to 50% flowering, plant height, leaf length, stem diameter, relative water content, net photosynthesis, transpiration rate and number of leaves per plant. Such a positive correlation between these traits suggests that simultaneous and direct selection for these desired traits is feasible. Proline content was significantly and negatively correlated with all studied traits except for leaf width. Proline is an osmolyte that also functions as an osmotic regulator and has antioxidant activity by scavenging reactive oxygen species (ROS) and protecting plants from further oxidative damage and cell death. (Upadhyay et al., 2020).Various studies have indicated that a higher unrestricted accumulation of proline is related with drought-resilience and lower content with drought sensitivity (Asao, 2012; Upadhyay et al., 2020).

Although proline significantly increased in all African spider plant accessions studied when subjected to drought stress, the scale of accumulation differed, and the increase was observed on sensitive accessions such as L18. Corresponding research studies in wheat (Rampino et al., 2006) and maize (Ibarra-caballero et al., 1988) found that free proline levels multiplied as relative water content decreased in all sensitive wheat genotypes. Though proline levels rise during drought stress, the function of proline accumulation in conferring drought stress tolerance is debatable. As a result, more research is needed to avoid differences in opinion about whether an increase in proline levels can help plants cope with drought stress (Arteaga et al., 2020; Belay et al., 2021).

Ultimately, accessions in cluster 1 and, to a lesser extent, cluster 2 maybe valuable genetic stocks for breeding drought tolerance in African spider plant, considering the morpho-physiological traits. Accessions L3, L5 and L14 from these clusters were superior for several traits. According to the current study's findings, accessions such as L3, L5 and L14, can maintain notable high leaf yields in both optimal and stressful conditions, validating Foulkes' findings (Foulkes et al., 2007) .The accessions' tolerance response to drought stress is attributed primarily to their genetic constitution, which regulate the key traits in African Spider plant under drought stress which is consistent with previous research (Islam et al., 2007; Mohi-Ud-din et al., 2021).

Conclusion

In this study, we evaluated a panel of 18 African spider plant accessions based on 13 morpho-physiological, and biochemical character traits under three different water regimes. Overall, most traits studied were significantly affected by the seasons of study and accessions, whereas variability due to water regimes was significant for all traits studied. Significant changes in phenological, physiological, morphological, and yield traits were observed under the three different water regimes. Proline accumulates in response to stress, but when evaluated at a specific time point, proline may not be a reliable indicator or marker for indirect selection for water stress stressed yield. The present study also concluded that the investigated accessions contain valuable genetic diversity for drought resistance. The drought-tolerant accessions reported in this study based on discriminative analyses, L3, and L5, could be highly suggested as promising parents for spider plant drought tolerance improvement breeding programs, along with developing stable and high-performing lines. Future research can also look into the molecular facets of these promising inbred lines, such as the molecular mechanism and gene expression profile of candidate drought resistant genes.

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Chapter 4 Variance Components, Correlation and Path Coefficient Analysis of Morpho-Physiological and Yield Related Traits in Spider Plant (Gynandropsis gynandra (L.) Briq.) under Water-Stress Conditions.

Abstract

Drought is the most complex abiotic stress for crop production at the global level. Therefore, developing new African spider plant genotypes tolerant to drought stress is one of the best approaches to achieving and optimizing high yield potential with water use efficiency. Advances in the selection of this crop can be determined by an understanding of genetic variability, heritability, and the associations that exist among different traits. The aim of this study was to gather information that will aid in the breeding of African spider plant cultivars with improved drought tolerance. A randomized complete block design (RCBD) experiment with eighteen genotypes and four replications was carried out across three water regimes: severe drought (30% field capacity), intermediate drought (50% field capacity), and well-watered (100% field capacity), in two seasons. Data on twelve morpho-physiological traits were recorded. The analysis of variance showed significant differences among genotypes ($p < 0.05$) in leaf yield and yield-related traits. The phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) for all 12 traits evaluated. High to moderate heritability estimates combined with a high to moderate genetic advance were observed for number of leaves, leaf width, plant height and stem diameter under drought stress conditions, indicating that these traits are controlled by additive gene action. Because of their predominant additive gene effects, correlation with leaf yield and favourable direct and indirect effects via the other yield-attributing traits, number of leaves per plant, plant height, days to 50% flowering, relative water content, net photosynthesis and leaf length could be used as target traits to improve spider plant leaf yield under drought-stressed conditions..

Keywords: Morpho-physiological traits, water regimes, variance components, trait association, heritability, genetic advance

4.1 Introduction

Spider plant (*Gynandropsis gynandra* (L.) Briq.), also known as cat's whiskers, is a semi-wild indigenous African leafy vegetable (ALV) that has its natural habitat in Sub-Saharan Africa where it grows as a volunteer weed (Smith and Eyzaguirre, 2007). The crop is among the most essential primary food sources for several millions of people, particularly in the developing World. However, spider plant has received little or no effort in cultivation and domestication (Chataika et al., 2020b). Despite less effort on domestication and cultivation, spider plant has been reported to be rich in essential nutrients that include vitamins A and C, magnesium, calcium, iron, antioxidant enzymes (which include catalase, peroxidases, superoxide dismutase) together with non-enzymatic antioxidants (which include ascorbic acid, flavonoids, glutathione, tocopherols, and carotenoids) (Chataika et al., 2021). This makes it a good candidate for fighting several diseases and tackling malnutrition (WHO, 2005). Equally important, the spider plant is valuable as it can adapt to climate variability. Thus it is resilient to unfavorable environmental conditions and can effortlessly be cultivated in drought-prone areas (Bvenura and Afolayan, 2015). As a result, the leafy vegetable plays a vital role in nutrition, livelihood and health of populations residing in peripheral and marginal areas of Africa (Mishra et al., 2011). *Gynandropsis gynandra* has been reported to be mainly cultivated and commercialized by women, thus contributing to income generation and improvement of livelihoods (Weinberger and Pichop, 2009; Diouf and Ba, 2014; Olabode et al., 2017).

Nevertheless, to realize high leaf yields of spider plant, improved varieties are necessary. These varieties, should be able to produce high yields even under stress conditions such as drought (Chataika et al., 2020d). Drought remains a problem of global magnitude that influences the production and quality of many crops. It occurs when an area experiences below-average rainfalls leading to reduced moisture in the soil, decreased streamflow, and crop damage (Farooqi et al., 2020). Drought stress is an outcome of water loss from plants eclipsing the ability of roots to imbibe water when the plant's water content decreases enough to interrupt normal plant processes (Aroca, 2013). Drought adversely affects crops resulting in yield reduction by influencing their anatomy, morphology, physiology and biochemistry (Lima et al., 2015; Mukami et al., 2019). Drought tolerance is, therefore, an essential breeding objective in most crop breeding programs. However, breeding for yield, which is also a complex quantitative trait, under drought stress is not easy. It has been reported that when moisture stress increases, heritability and genetic variance of yield decrease (Rosielle and Hamblin, 1981b; Bolaños and Edmeades, 1996; Blum, 2018). Drought stress, thus, complicates the task of achieving genetic progress by intrinsically selecting for yield per se, hence utilization of secondary traits is highly recommended (Edmeades et al., 1997).

Drought tolerance in crops can be improved through direct or indirect selection. However, it is challenging to make genetic improvements under drought conditions by selecting for yield per se, and thus the use of secondary traits is recommended . These secondary traits are plant attributes, apart from yield, that give breeders additional knowledge on how the plant functions in a particular environment (Lafitte et al., 2003). Ideal secondary traits should have a high heritability and genetic correlation with yield under water stress conditions (Atlin et al., 2001). These secondary traits or yield components can be characterized into morphological and physiological components and are all under genetic and environmental influences (Talebi and Fayyaz, 2012). Thus, it is imperative to classify the total variation due to these traits into non-heritable and heritable components. This can be achieved with the aid of genetic parameters namely phenotypic and genotypic coefficients of variation, variance components and heritability estimates (Maniee et al., 2009; Kahrizi and Mohammadi, 2009; Aminzadeh et al., 2010). These estimates are important as they inform plant breeders on strategies that increase genetic gain during selection. Although correlation studies are useful in breeding programs, they do not provide adequate information and knowledge on the interrelationships of heritable traits. This, in turn, can result in inaccurate information. However, a combination of correlation studies and path coefficient analysis enable the determination of significant traits that influence economic traits such as yield. Path coefficient analysis estimates the direct and indirect effects of a parameter over the other and thus shows traits that can be used for selection of yield either directly or indirectly (Dewey and Lu, 1959).

Some studies have been conducted to establish the genetic diversity and genetic parameters of the spider plant to lay a scientific base for the preservation, evaluation and varietal enhancement of the species. Secondary morphological and physiological traits are presumed to be under simple genetic control compared with yield and are strongly correlated to it. This hypothesis has not been tested on the spider plant, especially under drought stress. Therefore, the objectives of this study were to (i) estimate variance components and heritability for yield and yield components under drought conditions, (ii) determine the relationship between yield and associated traits through correlation and path coefficient analysis under drought conditions and (iii) determine the secondary morpho-physiological traits to be used for selection in spider plant under drought situations.

4.2 Materials and Methods

4.2.1 Plant material

Eighteen accessions used in this study, were mainly landraces collected from various regions of the world and conserved at the gene bank of the Laboratory of Genetics, Horticulture, and Seed Science of the University of Abomey-Calavi, Benin (Table 1). The chosen accessions are grown mainly under rain-fed conditions by smallholder farmers and are frequently exposed to prolonged drought stress. These accessions were also selected based on germination percentage and ability to produce high leaf yield under optimum conditions.

Table 4. 1 List of genotypes used in the present study.

Genotype	Genebank of Origin	Country of origin	Region
L01	KENRIK*	Kenya	East Africa
L02	Universityof Ouagadougou	Burkina-Faso	West Africa
L03	GBioS/UAC	Benin	West Africa
L04	GBioS/UAC	Benin	West Africa
L05	GBioS/UAC	Togo	West Africa
L06	Universityof Ouagadougou	Burkina-Faso	West Africa
L07	World Vegetable Center	Thailand	Asia
L08	World Vegetable Center	Zambia	Southern Africa
L09	World Vegetable Center	South Africa	Southern Africa
L10	World Vegetable Center	Malaysia	Asia
L11	World Vegetable Center	Uganda	East Africa
L12	World Vegetable Center	Malaysia	Asia
L13	KENRIK	Kenya	East Africa
L14	World Vegetable Center	Uganda	East Africa
L15	LUANAR	Malawi	Southern Africa
L16	Otjiwarongo	Namibia	Southern Africa
L17	World Vegetable Center	Laos	Asia
L18	KENRIK	Kenya	East Africa

*KENRIK, Kenya Resource Centre for Indigenous Knowledge; LUANAR, Lilongwe University of Agriculture and Natural Resources; GBioS/UAC, Laboratory of Genetics, Biotechnology and Seed Science, University of Abomey-Calavi

4.2.2 Growing environments, experimental design, and agronomic practices

Two experiments were conducted in the tunnels at the University of KwaZulu-Natal, School of Agricultural, Earth, and Environmental Sciences in Pietermaritzburg, South Africa, in the Controlled Environment Research Unit (CERU). The experiments were carried out over two seasons from October 2020 to December 2020 (Season 1) and from February 2021 to April 2021 (Season 2).

The eighteen accessions were evaluated under three varying water regimes in each of the seasons. The water regimes were: severe stress (30% field capacity), moderate stress (50% field capacity), and well-watered (100% field capacity), defined based on the findings of (Masinde et al., 2005). The seeds of each accession were sown in dedicated seedling trays and nurtured into seedlings before transplanting into individual pots, two weeks after sowing, under each water regime. In each water regime, the eighteen genotypes were laid in a randomized complete block design with four replications and each plot constituting three plants for both experiments. The seedlings were irrigated to keep the soil moisture of the pots at 100% field capacity for the first seven days after transplanting. Drought stress was applied from the eighth day after transplanting by stopping the irrigation to 21 days after transplanting, coinciding with the harvest maturity stage. The quantity of water applied in the pots was determined by calculating the optimum amount of water (field capacity, FC) of the potting mixture using the procedure described by (Kesiime et al., 2016).

4.3 Data collection

4.3.1 Qualitative Characters

Some qualitative traits were recorded based on the index of revised spider plant descriptors (FAO Food and Agricultural Organisation, 1995). The traits recorded included flower color, petiole color, leaf hairiness, growth habit, stem hairiness, leaf color, petiole hairiness, and stem color (Table 4.2). All the genotypes in a plot per each replication were observed throughout plant growth. To determine the variation in qualitative traits, scoring was carried out on all accessions on the day they reached 50% flowering to prevent differences that might occur in the developmental phase of growth (Table 4.2).

Table 4. 2 Trait, descriptor, and codes exploited for evaluation of qualitative traits in African spider plant used in this study.

Character	**Descriptor and coding number**
Leaf Color	Dark green (1) and light green (2)
Leaf hairiness	Glabrous (1), weak or sparse (3), medium (5), and profuse (7)
Petiole color	Green (1), pink (2), violet (3), and purple (4)
Petiole hairiness	Glabrous (1), weak or sparse (3), medium (5), and profuse (7)
Stem color	Green (1), pink (2), violet (3), and purple (4)
Stem hairiness	Glabrous (1), weak or sparse (3), medium (5), and profuse (7)
Flower color	White (1), purple (2), and (3) pink
Growth habit	Erect (2), semi-erect (4), and prostrate (6)

Source; Food and Agriculture Organization (FAO Food and Agricultural Organisation, 1995). The figures in brackets rightwards are correspondent descriptor codes set out in the FAO publication with alterations throughout the improvement of the list.

4.3.2 Shannon Weiner diversity index

The Shannon Weiner diversity index H' was estimated using Microsoft Excel 2019 (Microsoft Corporation, 2018). The index was exploited to estimate the phenotypic diversity of each qualitative character as follows.

$$H' = -\sum_{i=1}^{R} Pi\, lnPi$$

Equation 4. 1

where H' is the diversity index and R is the number of samples, Pi is the proportion of individuals of each character belonging to the i^{th} character of the total number of individuals.

4.3.3 Quantitative Characters

Morphological traits

Data were collected for quantitative morphological traits as follows. Leaf yield (Ly) was recorded as weighed leaf yield per plot in grams, plant height (Ph) in cm was measured using a ruler from the surface of the soil to the tip of the flower for the three plants in each plot and an average was recorded. Number of days to 50% flowering (Fl) was recorded from planting date to the date when 50% of the plants started flowering in each plot. Leaf length (Ll) was measured in cm using a ruler as the length per each leaf from the pointy part at one end to the point at which the leaf joins the stalk at the other end; an average of three leaves per plant was recorded. Leaf width (Lw) in cm was measured using a ruler as the longest extension of any two points on the blade edge perpendicular to the leaf length axis. Stem diameter (Sd) was measured in mm on the base of the plant stem using a digital Vernier caliper and an average of three plants was recorded. Number of leaves per plant (Nl) was determined by counting all the leaves per plant and an average for three plants was recorded.

Physiological traits

Most of the physiological traits were recorded from 8:00 a.m. to 12:00 midday to prevent a sizable possibility of diurnal variation in stomatal conductance (Tardieu and Simonneau, 1998). Stomatal conductance to water vapor (Cond), net photosynthesis rate (Photo), and transpiration rate (Trans) were measured using an LI-6400XT Portable Photosynthesis System (Lincoln, Nebraska,) with an infrared gas analyzer (IRGA) connected to a leaf chamber fluorometer (LCF). Outward leaf CO_2 concentration (C_a) and constructed saturating photosynthetic active radiation (PAR) were set to $400\ \mu mol^{-1}$ and $1000\ \mu mol^{-2}\ m^{-2}\ s^{-1}$, respectively. The leaf temperature was maintained at 25 °C. The flow rate of water and relative humidity were kept constant at $500\ \mu mol$ and 43 %, respectively. To avoid stomatal closure due to low air humidity, the leaf-to-air vapor pressure deficit in the cuvette was kept constant at 1.7 kPa. Parameters were measured on the third half-formed leaf from the plant's tip by attaching the leaf inside the sensor head. For each accession, measurements were taken from three plants in both non-stressed and drought stressed conditions. Chlorophyll content (Spad) was measured for three flag leaves in each plot using a Biobase portable chlorophyll meter (Biobase, Jinan, China). Relative water content (Rwc) was estimated based on the following formula:

$(\%)\ RWC = \frac{FW-DW}{TW-DW} X \quad 100$ Equation 4. 2

Where FW = Sample of fresh leaf weight TW = Sample of turgid leaf weight and DW = Sample of dry leaf weight.

4.4 Data analysis

4.4.1 Analysis of variance

ANOVA for leaf yield in each water regime was computed in R software version R-4.1.1 using restricted maximum likelihood (REML) analysis with linear mixed models (R Core Team, 2021). The analysis was carried out using the following model. Seasons were treated as random effects, whereas Accessions were treated as fixed effects. The F statistic (P < 0.05) was used to test the significance of different items in the ANOVA, which is the ratio of the sum of mean squares to the mean square error.

$y_{ij} = \mu + t_i + b_j + e_{ij} \quad i = 1, 2, \cdots, 18, \quad j = 1, 2$ Equation 4. 3

where i is the general mean, t_i is the effect of the i^{th} treatment, b_j is the effect of the j^{th} block and e_{ij} is the random error term.

4.4.2 Evaluating genotypic and phenotypic variances

Genotypic V_G, genotype by environment interaction V_{GE} and error/environmental variances (V_E) were estimated by employing the lmer package in R software version R-4.1.1(R Core Team, 2021). Phenotypic variances (σ_p^2) were estimated using the following formula

$$V_p = V_G + \frac{V_{GE}}{l} + \frac{V_E}{rl}$$

Equation 4. 4

where, l is the number of growing environments and, r is the number of replications.

4.4.3 Evaluating genotypic and phenotypic Coefficients of variation

These components were estimated based on the formula proposed by (Burton, 1952).

Genotypic coefficient of variation, GCV % $= \frac{\sqrt{V_G}}{\bar{x}} \times 100$ Equation 4. 5

where Vg = Genotypic variance; and $\bar{x}$ is the mean for the population

Phenotypic coefficient of variation, PCV % = $\frac{\sqrt{V_P}}{\bar{x}} \times 100$ Equation 4. 6

where Vp = Phenotypic variance and $\bar{x}$ is the population mean.

The coefficients of variation (GCV and PCV) were classified as low: less than 10%, moderate: 10% to 20%, and high: greater than 20% as suggested by (Sivasubramanjan, S. Menon, 1973).

4.4.4 Estimation of heritability

Broad sense heritability (H^2) was determined for various traits as per the formula suggested by (Allard, 1999). The estimates for heritability for a single environment was done using the formula

$$\boldsymbol{H^2 = \sigma_g^2 / \sigma_p^2 \times 100}$$ Equation 4. 7

where σ_p^2 is the phenotypic variance and σ_g^2 is the genotypic variance.

Heritability percentage was classified as low: less than 30%, medium: 30% - 60%, and high: greater than 60% according to (Robinson et al., 1949)

4.4.5 Determination of genetic advance (GA) and genetic advance as percent of the mean (GAM)

The genetic advance for different characteristics was determined with the use of the formula proposed by (Johnson et al., 1983) using the formula

Genetic advance GA = $\boldsymbol{H^2 \cdot i \cdot \sigma_p}$ Equation 4. 8

where $\boldsymbol{H^2}$ is broad-sense heritability, i is the selection differential at 5% selection intensity, and σ_p is the phenotypic standard deviation.

The genetic advance as a percent of the mean was estimated using the following formula

$\boldsymbol{GA \,\%\, mean = \frac{GA}{\bar{x}} X\, 100}$ Equation 4.9

where GA = Genetic advance and $\bar{x}$ = Population means.

Following (Falconer and Mackay, 1996), the genetic advance (GA percent) values were classified as low: less than 10%, moderate: 10% - 20%, and high: greater than 20%.

4.4.6 Correlation analysis

Phenotypic correlation analysis was conducted following the Pearson's correlation coefficient method using ("corrplot") package in R software version R-4.1.1 (R Core Team, 2021).

$$\boldsymbol{r_p = \frac{p\ cov\ x\,.y}{\sqrt{\delta^2\ px\,.\ \delta^2 py}}} \qquad \text{Equation 4. 10}$$

where r_p is phenotypic correlation coefficients. $p\ cov\ \ x\,.y$ is phenotypic covariance between variables x and y. $\boldsymbol{\delta^2}\ px$ is the phenotypic variance for variable x and, $\boldsymbol{\delta^2}py$ is the phenotypic variance for variable *y*.

4.4.7 Path coefficient analysis

Path coefficient analysis was performed using correlation coefficients as suggested by (Dewey and Lu, 1959).

$$\boldsymbol{r_{ij} = p_{ij} + \sum r_{rk}\, p_{kj}} \qquad \text{Equation 4.11}$$

where r_{ij} = mutual relationship between independent character *i* and the dependent character *j* as can be seen by the phenotypic correlation coefficients. p_{ij} = elements of direct effects of the independent trait *i* on the dependent character *j* as evident by the path coefficients. $\sum_r rk\ \ p_{kj}$ = summation of indirect effects of a given independent character *i* on a given dependent character *j* via all other independent characters *k*.

A path analysis scale suggested by (Lenka and Mishra, 1973) was used to categorize the estimates as negligible with values ranging from 0.00 to 0.09, low with values ranging from 0.10 to 0.19, moderate with values ranging from 0.20 to 0.29, high with values ranging from 0.30 to 0.99, and more than 1 as very high path coefficients.

The involvement of additional unknown characters in the analysis is estimated as the residual $\boldsymbol{P_R}$ using the formula:

$$\boldsymbol{P_{R=}\sqrt{(1 - \sum P_{ij} r_{ij})}}. \qquad \text{Equation 4. 12}$$

4.5. Results

4.5.1. Qualitative traits

From the 18 spider plant accessions studied, variations within qualitative characters were observed. There was, however, no significant effect of growing environment observed for the qualitative traits studied.

4.5.1.1 Morphological descriptors

The data relating to morphological descriptors is presented in Table 4.3. White and purple flowers were the two colours exhibited by the accessions in the study (Figure 4.1). The white flower was the dominant colour on 72% of the accessions, whilst 28% displayed purple flowers (Figure 4.2). The growth habit also varied between erect (72%) and semi-erect plants (28%) (Figure 4.2). Petiole hairiness varied as sparse (50%), medium (28%), glabrous (6%) and profuse (17%), while stem hairiness varied as sparse (61%), medium (22%), profuse (11%) and glabrous (6%). Stem colours within the accessions studied were green (56%) and purple (44%), leaf colour varied from dark green (94%) to light green (6%), whereas petiole colors varied as green (83%), pink (11%) and violet (6%). Leaf hairiness also varied from glabrous (67%), sparse (28%), to medium (6%).

The proportion of accessions, which had white flowers and green stems was 44.4% whilst those possessing white flowers and purple stems was 27.7%. Accessions with purple flowers and purple stems were 16.6%, whilst 11.1% had purple flowers with green stems. As indicated in Table 4. 3, the green stem accessions with sparse stem hairiness (38.8%) were predominant over purple stem accessions with sparse stem hairiness (16.6%). Plants with purple stems and medium stem hairiness constituted 16.6% of the accessions whilst those with green stems and medium stem hairiness constituted 11.1%.

Figure 4. 1: Variations in growth and morphology of 18 African spider plant morphs.

Table 4. 3. Morphological descriptors reported for 18 African spider plant accessions.

Genotypes	**Growth habit**	**Stem color**	**Stem hairiness**	**Petiole color**	**Petiole hairiness**	**Leaf color**	**Leaf hairiness**	**Flower color**
L01	erect	green	sparse	green	sparse	dark green	glabrous	white
L02	semi-erect	purple	medium	green	sparse	dark green	glabrous	white
L03	erect	purple	medium	purple	medium	dark green	sparse	white
L04	erect	green	sparse	green	sparse	dark green	glabrous	white
L05	erect	green	sparse	green	sparse	dark green	glabrous	white
L06	semi-erect	green	medium	purple	medium	dark green	medium	purple
L07	semi-erect	purple	sparse	green	profuse	dark green	sparse	purple
L08	erect	green	sparse	green	profuse	dark green	sparse	white
L09	erect	green	sparse	green	sparse	dark green	sparse	white
L10	erect	purple	profuse	green	profuse	light green	sparse	purple
L11	erect	purple	medium	green	medium	dark green	glabrous	white
L12	erect	purple	sparse	green	medium	dark green	glabrous	white
L13	erect	green	sparse	green	sparse	dark green	glabrous	purple
L14	semi-erect	purple	sparse	violet	sparse	dark green	glabrous	white
L15	erect	green	medium	green	medium	dark green	glabrous	white
L16	erect	green	glabrous	green	glabrous	dark green	glabrous	white
L17	semi-erect	green	sparse	green	sparse	dark green	glabrous	white
L18	erect	purple	sparse	green	sparse	dark green	glabrous	Purple

Figure 4. 2 Distribution of African spider plant accessions by qualitative morphological descriptors

4.5.1.2 Shannon-Wiener diversity index

To compare phenotypic variation among the 8 qualitative traits in African spider plant accessions, Shannon-Weiner diversity indices (*H'*) were estimated. In general, the indices observed ranged from 0.21 ($H' < 0.50$) for leaf color to 1.16 ($H' > 0.50$) for petiole hairiness with an average of 0.70 (Table 4). Overall, the indices revealed that diversity for the qualitative characters measured was high among the accessions studied (Table 4.4).

Table 4. 4 Diversity index values for qualitative traits

Qualitative trait	Shannon-Wiener Diversity Index
Growth	0.59
Stem color	0.69
Stem	1.04
Petiole	0.56
Petiole	1.16
Leaf color	0.21
Leaf	0.79
Flower	0.59
Mean	0.70

4.5.2 Analysis of variance

The mean squares, means and coefficient of variations (CV) for morpho-physiological traits and yield components across two growing seasons under well-watered, mild stress and severe stress conditions are presented in Table 4.5. From the analysis of variance, highly significant differences ($p < 0.05$) were noted between the main effects of seasons, genotypes, replications, and their interactions for a good number of traits (Table 4.5). Under well-watered conditions, the seasonal effect was significant ($p < 0.05$) for a large number of traits including days to 50% flowering (Fl), plant height (Ph), leaf length (Ll), stomatal conductance (Cond), net photosynthesis rate (Photo) and transpiration rate (Trans). Under mild stress conditions, most traits were non-significant for the seasonal effect except for days to 50% flowering, leaf length, chlorophyll content and transpiration rate. Similarly, under severe stress conditions, the seasonal effects were non-significant on most traits except for days to 50% flowering, leaf width, stem diameter, chlorophyll content, stomatal conductance and leaf yield.

The mean squares for genotypes varied significantly ($p < 0.05$) in almost all the traits under the three water regimes. The mean squares for genotypes were highly significant ($p < 0.01$) for all the traits studied under well-watered conditions (Table 4.5). Under mild stress conditions, the genotype effect was significant ($p < 0.05$) for the majority of traits with chlorophyll content as the only non-significant trait. Similarly, under severe stress conditions (Table 4.5), the genotype effect was significant on most traits except for chlorophyll content (Spad), stomatal conductance (Cond) and transpiration rate (Trans).

Table 4. 5 Mean square of the 13 traits from a combined analysis of variance of 18 African spider plant accessions under 3 water regimes

Source	**Df**	**Fl**	**Ph**	**Ll**	**Lw**	**Sd**	**Spad**	***RWC***	**Cond**	**Photo**	**Trans**	**Nl**	**Ly**
Well-watered													
Season	1	220.03 ***	511.51 **	3.55 *	4.41	1.54	19.43	0.30	0.05 ***	350.91 ***	0.00 ***	10.6	3.5
Genotype	17	113.12 ***	223.22 ***	6.43 ***	8.37 ***	7.15 ***	19.88 ***	430.73 ***	0.01 ***	225.71 ***	0.00 **	6438.5 ***	8179.6 ***
Rep	3	18.06	333.62 ***	2.70 *	4.62 *	8.53 ***	14.75	12.48	0.02 ***	12.24	0.00 ***	284.8 **	435 ***
Season × Rep	3	336.68 ***	330.48 ***	3.94 **	8.98 ***	1.98	1.08	6.21	0.00	149.38 ***	0.00 ***	122.4	194.3 ***
Season × Genotype	17	7.23	83.80 *	2.76 ***	3.09 **	2.00 **	12.74 *	1.38	0.01 **	28.51 **	0.00	37.4	7.1
Residuals	102	17.97	47.41	0.75	1.20	0.83	6.52	3.54	0.00	11.67	0.00	68.4	19.6
Mean		58	52.78	5.96	7.64	6.75	40.49	74.38	0.2310	75.43	0.0178	81	68.16
CV		8.65	13.05	14.57	14.36	13.51	6.31	2.53	21.31	4.53	17.66	10.21	6.50
Mild stress													
Season	1	300.44 ***	60.32	2.56 *	0.93	1.36	45.68 *	0.11	0.00	0.19	0.00 ***	0.69	16.07
Genotype	17	31.48 ***	332.39 ***	2.52 ***	3.75 ***	3.61 ***	10.09	293 ***	0.01 ***	73.6 ***	0.00 *	1231.52 ***	1477.94 ***
Rep	3	80.15 ***	91.62	0.72537	0.79	2.28	10.34	1.26	0.01 *	5.36	0.00	42.35	34.89
Season × Rep	3	5.64	163.81 **	0.42796	0.56	2.00	2.51	1.39	0.01	347.42 ***	0.00	11.82	14.56
Season × Genotype	17	14.65	72.2 *	1.45 ***	2.00 **	1.11	5.34	1.19	0.01 *	24.76 *	0.00	5.55	1.61
Residuals	102	9.83	39.81	0.47	0.75	0.95	6.63	1.26	0.00	14.21	0.00	29.27	16.8
Mean		38	34.53	4.27	5.41	4.39	34.64	53.14	0.15	59.83	0.0124	43	24.85
CV		8.36	18.27	16.04	16.06	22.28	7.44	2.11	40.08	6.30	20.87	12.58	16.49
Severe stress													
Season	1	637.56 ***	142.80	0.56	4.03 **	7.47 ***	42.25 *	4.59	0.03 ***	64.66	0.00	0.44	7.27 *
Genotype	17	52.66 ***	295.30 ***	1.07 ***	1.99 ***	2.42 ***	14.20	196.32 ***	0.00	157.34 ***	0.00	616.47 ***	432.45 ***
Rep	3	38.53 *	167.82 **	0.27	0.50	0.25	8.72	3.27	0.01 *	21.88	0.00 *	14.28	25.86 ***
Season × Rep	3	81.69 ***	19.66	0.05	0.30	0.92	57.42 ***	1.57	0.00	70.12 *	0.00 ***	5.31	0.49
Season × Genotype	17	6.47	93.47 **	0.98 ***	0.56	1.34 ***	5.05	0.96	0.00	37.57 *	0.00	1.53	0.23
Residuals	102	12.41	37.36	0.28	0.44	0.46	9.26	4.28	0.00	17.98	0.00	9.43	1.61
Mean		29	22.89	3.36	4.16	3.20	30.15	39.20	0.1213	44.64	0.0094	22	9.89
CV		12.15	26.71	15.61	16.01	21.24	10.10	5.28	36.79	9.50	27.75	13.96	12.83

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Df= Degrees of freedom, Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content, Rwc = Relative water content, Photo= Net photosynthesis rate, Cond = Stomatal conductance, Trans = Transpiration rate, Nl = Number of leaves per plant, Ly= Leaf yield

4.5.3 Variance components and heritability estimates

The variance components estimates, heritability and genetic advances of 18 spider plant genotypes evaluated under three water regimes are presented in Table 4.6. Under well-watered conditions, genotypic variances ranged from 0.00 for transpiration rate (Trans) to 1020.20 for leaf yield (Ly). In addition to the above, the genotypic variance ranged from 0.00 for stomatal conductance (Cond) to 182.91 for leaf yield (Ly) under mild stress conditions and 0.00 for stomatal conductance (Cond) to 76.02 for number of leaves (Nl) under severe stress. The phenotypic variances observed ranged from 0.00 for stomatal conductance (Cond) to 1022.43 for leaf yield (Ly) under well-watered conditions, 0.00 for stomatal conductance to 184.74 for leaf yield (Ly) under mild stress conditions and 0.00 for stomatal conductance to 77.06 for number of leaves under severe stress conditions. For all yield and yield-contributing traits, phenotypic variance was greater than genotypic variance across the three water regimes.

The genotypic coefficient of variation estimates (GCV) ranged from 0.10% (relative water content) to 46.86% (leaf yield) under well-watered conditions, 0.11% (relative water content) to 54.42% (leaf yield) under mild stress conditions, and 0.00% (stomatal conductance) to 74.22% (leaf yield). In this study, it was observed that well-watered conditions had a phenotypic coefficient of variation (PCV) ranging from 0.10% (relative water content) to 46.91% ((leaf yield), whereas mild stress conditions had a PCV ranging from 0.11 (relative water content) to 46.86% (leaf yield) and lastly, severe stress conditions had a PCV ranging from 0.00% (stomatal conductance) to 74.34% (leaf yield). High GCV and PCV values of >20% were recorded for leaf yield (46.86% and 46.91%, respectively) and number of leaves (34.85% and 35.02, respectively), under well-watered conditions. The same traits, leaf yield and number of leaves, also showed a high (>20%) GCV (54.42% and 28.55%, respectively) and PCV (54.70% and 28.85%, respectively) under mild stress. Under severe stress conditions, GCV and PCV were high for leaf yield (74.22% and 74.34%, respectively), number of leaves (39.63% and 39.90%, respectively) and plant height (21.95% and 26.54%, respectively). As shown in Table 3a–c, moderate (10–20%) GCV and PCV values were recorded for leaf length (15.04% and 11.40%, respectively), leaf width (13.39% and 10.67%, respectively), stem diameter (14.01% and 11.92%, respectively) and stomatal conductance (18.37% and 13.63%, respectively) under well-watered conditions. Under mild stress conditions, moderate values were observed for plant height (18.67%, GCV) and (16.55%, PCV), stem diameter ((15.31%, GCV) and (12.75%, PCV)) and stomatal conductance (23.05%, GCV) and (11.95%, PCV). Leaf width (11.98%) and (10.15%) together with stem diameter (17.19%) and (11.48%) had moderate (10–20%) GCV and PCV values, respectively, under severe stress conditions.

Although most of the traits had minor differences between PCV and GCV values across all water regimes, a few traits showed a significant difference between the PCV and GCV values. Stomatal conductance (23.05–11.95) showed a significant difference between the PCV and GCV values under mild stress conditions. Under severe stress conditions, leaf length (10.89–3.61), stem diameter (17.19–11.48), stomatal conductance (15.77–0.00) and transpiration rate (10.91–4.89) exhibited large differences between the PCV and GCV values. The PCV was higher than its corresponding GCV for all the traits across water regimes.

Heritability estimates for all the studied traits varied from high (>60%), moderate (30–60%) and low (<30%) across all the water regimes (Table 3a–c). Under well-watered conditions, high heritability values (>60%) were observed for the vast majority of traits except for the transpiration rate (30–60%), chlorophyll content rate (30–60%), stomatal conductance (30–60%) and leaf length (30–60%). Plant height (78.63%), stem diameter (69.43%), relative water content (99.58%), net photosynthesis rate (66.65%), number of leaves (97.91%) and leaf yield (99.01%) exhibited high heritability (>60%) under mild stress conditions. Furthermore, days to 50% flowering (53.47%), leaf length (42.39%), leaf width (48.18%), chlorophyll content (36.04%) and transpiration rate (37.08%) showed moderate (30–60%) heritability estimates under mild stress conditions. In contrast, stomatal conductance (26.86%) showed a low heritability value under mild stress conditions. Under severe stress conditions, most traits had a high heritability (>60%), and these included leaf yield (99.67%), number of leaves (98.65%), plant height (68.40%), days to 50% flowering (78.05%), photosynthesis rate (76.13%), relative water content (98.10%) and leaf width (71.89%). In addition to the above, moderate heritability estimates (30–60%) were recorded for stem diameter (44.64%) and chlorophyll content (38.99%). Stomatal conductance (0.00%), transpiration rate (20.06%) and leaf length (10.96%) recorded low heritability (<30%) under severe stress.

Genetic advance as a percentage of the mean varied from low (0–10%) to moderate (10–20%) to high (20% and above) (Table 4.6). High values of genetic advance (20% and above) were reported for stem diameter (20.89%), number of leaves (71.43%), stomatal conductance (20.83%) and leaf yield (96.43%) under well-watered conditions. Moderate GAM values (10–20%) were also noted for days to 50% flowering (11.42%), plant height (12.88%), leaf length (17.80%), leaf width (17.51%) and net photosynthesis rate (12.67%) under well-watered conditions. Under mild stress conditions, high genetic advance estimates (20% and above) were observed for leaf yield (111.56%), number of leaves (58.20%), stem diameter (21.89%) and plant height (30.24%). In addition to high GAM values, moderate values (10–20%) were also observed for leaf length (11.49%), leaf width (12.55%) and stomatal conductance (12.75%) under mild stress conditions. Plant height (37.40%), number of leaves (81.09%) and leaf yield (152.64%) exhibited a high GAM (20% and above) values under severe stress. Furthermore, days to 50% flowering (14.22%), leaf width (17.73%) and stem diameter (15.81%) and net photosynthesis rate (15.58%) showed moderate (10–20%) GAM values under severe stress conditions.

A high heritability coupled with high genetic advance was observed for stem diameter, stomatal conductance, number of leaves and leaf yield under well-watered conditions. Moderate heritability coupled with high genetic advance was also noted for stomatal conductance under well-watered conditions. In addition, high to moderate heritability estimates accompanied with moderate genetic advance were observed for days to 50% flowering, photosynthesis, plant height, leaf length and leaf width under well-watered conditions. Additionally, a high heritability estimate coupled with a low genetic advance was observed for relative water content. Plant height, stem diameter, number of leaves and leaf yield had high heritability estimates coupled with high genetic advance under mild stress conditions. Furthermore, high heritability estimates coupled with low genetic advance were observed for relative water content and net photosynthesis under mild stress conditions. Moderate heritability accompanied with moderate genetic advance were observed for leaf length and leaf width under mild stress conditions. Under severe stress conditions, plant height, number of leaves and leaf yield showed a high heritability accompanied by high genetic advance. High to moderate heritability accompanied with moderate genetic advance were observed for days to 50% flowering, leaf width, stem diameter and net photosynthesis under severe stress. In addition, a high heritability coupled with a low genetic advance was also observed for relative water content.

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Table 4. 6. Variance components estimates and genetic parameters of eighteen spider plant genotypes evaluated over two seasons, under three water regimes a) well-watered b) mild stress c) severe stress

Trait	V_G	$V_{G x E}$	V_E	V_P	**PCV**	**GCV**	H^2	**GA**	**GAM (%)**
Well-watered									
Fl	12.09	0.00	16.44	14.14	6.48	5.99	85.47	6.62	11.42
Ph	17.43	9.10	47.41	27.90	10.01	7.91	62.46	6.80	12.88
Ll	0.46	0.50	0.75	0.80	15.04	11.40	57.43	1.06	17.80
Lw	0.66	0.46	1.20	1.05	13.39	10.67	63.49	1.34	17.51
Sd	0.65	0.29	0.83	0.89	14.01	11.92	72.39	1.41	20.89
Spad	0.89	1.55	6.52	2.48	3.89	2.33	35.90	1.17	2.88
Rwc	0.01	0.00	0.00	0.01	0.10	0.10	99.25	0.15	0.20
Photo	24.65	4.21	11.67	28.21	7.04	6.58	87.37	9.56	12.67
Cond	0.00	0.00	0.00	0.00	18.37	13.63	55.04	0.05	20.83
Trans	0.00	0.00	0.00	0.00	9.59	6.51	46.06	0.00	9.10
Nl	796.82	0.00	63.96	804.82	35.02	34.85	99.01	57.86	71.43
Ly	1020.20	0.00	17.83	1022.43	46.91	46.86	99.78	65.73	96.43
Mild stress									
Fl	2.10	1.20	9.84	3.94	5.22	3.82	53.47	2.19	5.75
Ph	32.67	7.79	39.87	41.55	18.67	16.55	78.63	10.44	30.24
Ll	0.13	0.25	0.47	0.32	13.15	8.56	42.39	0.49	11.49
Lw	0.23	0.30	0.75	0.47	12.66	8.78	48.14	0.68	12.55
Sd	0.31	0.04	0.96	0.45	15.31	12.75	69.43	0.96	21.89
Spad	0.45	0.00	6.45	1.26	3.24	1.95	36.04	0.83	2.41
Rwc	0.00	0.00	0.00	0.00	0.11	0.11	99.58	0.12	0.23
Photo	6.13	2.58	14.22	9.20	5.07	4.14	66.65	4.16	6.96
Cond	0.00	0.00	0.00	0.00	23.05	11.95	26.86	0.02	12.75
Trans	0.00	0.00	0.00	0.00	10.14	6.17	37.08	0.00	7.74
Nl	150.72	0.00	25.74	153.94	28.85	28.55	97.91	25.02	58.20
Ly	182.91	0.00	14.63	184.74	54.70	54.42	99.01	27.72	111.56
Severe stress									
Fl	5.14	0.00	11.56	6.58	8.85	7.82	78.05	4.13	14.22
Ph	25.25	13.99	37.37	36.91	26.54	21.95	68.40	8.56	37.40
Ll	0.01	0.17	0.27	0.13	10.89	3.61	10.96	0.08	2.46
Lw	0.18	0.03	0.44	0.25	11.98	10.15	71.89	0.74	17.73
Sd	0.14	0.22	0.46	0.30	17.19	11.48	44.64	0.51	15.81
Spad	0.69	0.00	8.66	1.77	4.42	2.76	38.99	1.07	3.55
Rwc	0.00	0.00	0.00	0.00	0.13	0.13	98.10	0.10	0.26
Photo	14.97	4.89	17.98	19.67	9.93	8.67	76.13	6.96	15.58
Cond	0.00	0.00	0.00	0.00	15.77	0.00	0.00	0.00	0.00
Trans	0.00	0.00	0.00	0.00	10.91	4.89	20.06	0.00	4.51
Nl	76.02	0.00	8.30	77.06	39.90	39.63	98.65	17.84	81.09
Ly	53.88	0.00	1.41	54.06	74.34	74.22	99.67	15.10	152.64

V_G = genotypic variance, V_{GxE} = genotype x environment interaction variance, V_E = error variance, V_P = phenotypic variance, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation, H^2 = heritability, GA = genetic advance, GAM =genetic advance as a percentage of the mean, Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content, Rwc = Relative water content, Photo = Net photosynthesis rate, Cond = Stomatal conductance, Trans = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield

4.5.4. Correlation coefficient estimates

Figure 4.3 shows Pearson's correlation coefficients (r) among morphological and physiological traits of 18 spider plant accessions in non-stressed and water-stressed conditions. In this study, the degree of association was divided into three categories: weak (0–0.3), moderate (0.3–0.7) and strong (0.7+). Under well-watered conditions, leaf yield showed a strong positive correlation with the number of leaves ($r = 0.86$; $P < 0.001$), moderate positive association with net photosynthesis rate ($r = 0.70$; $P < 0.001$), stem diameter ($r = 0.50$; $P < 0.001$), days to 50% flowering ($r = 0.59$; $P < 0.001$) leaf length ($r = 0.47$; $P < 0.001$), leaf width ($r = 0.43$; $P < 0.001$), relative water content ($r = 0.43$; $P < 0.001$) and stomatal conductance ($r = 0.40$; $P < 0.001$). Weak and positive correlations were observed between leaf yield and plant height ($r = 0.30$; $P < 0.001$), transpiration rate ($r = 0.26$; $P < 0.001$) and chlorophyll content ($r = 0.21$; $P < 0.05$). A strong and positive correlation was also observed between leaf length and leaf width ($r = 0.89$; $P < 0.001$) under well-watered conditions (Figure 4.3).

Under mild stress conditions, leaf yield showed a moderate and positive correlation with the number of leaves per plant ($r = 0.60$; $P < 0.001$), net photosynthesis rate ($r = 0.41$; $P < 0.001$), plant height ($r = 0.39$; $P < 0.001$), Days to 50% flowering ($r = 0.39$; $P < 0.001$) and relative water content ($r = 0.38$; $P < 0.001$) were observed. Weak and positive associations were observed between leaf yield and stem diameter ($r = 0.30$; $P < 0.001$) and leaf length ($r = 0.29$; $P < 0.001$). Leaf length and leaf width also showed a strong and positive correlation ($r = 0.82$; $P < 0.001$) under mild stress conditions.

Strong and positive correlations were also observed between leaf yield and number of leaves per plant ($r = 0.87$; $P < 0.001$), moderate and positive correlations with net photosynthesis rate ($r = 0.54$; $P < 0.001$), Days to 50% flowering ($r = 0.50$; $P < 0.001$), relative water content ($r = 0.32$; $P < 0.001$), leaf length ($r = 0.30$; $P < 0.001$) under severe stress. A weak and positive correlation was also noted between leaf yield and plant height ($r = 0.21$; $P < 0.05$). Similar to the other two water regimes leaf length and leaf width had a strong and positive association ($r = 0.78$; $P < 0.001$).

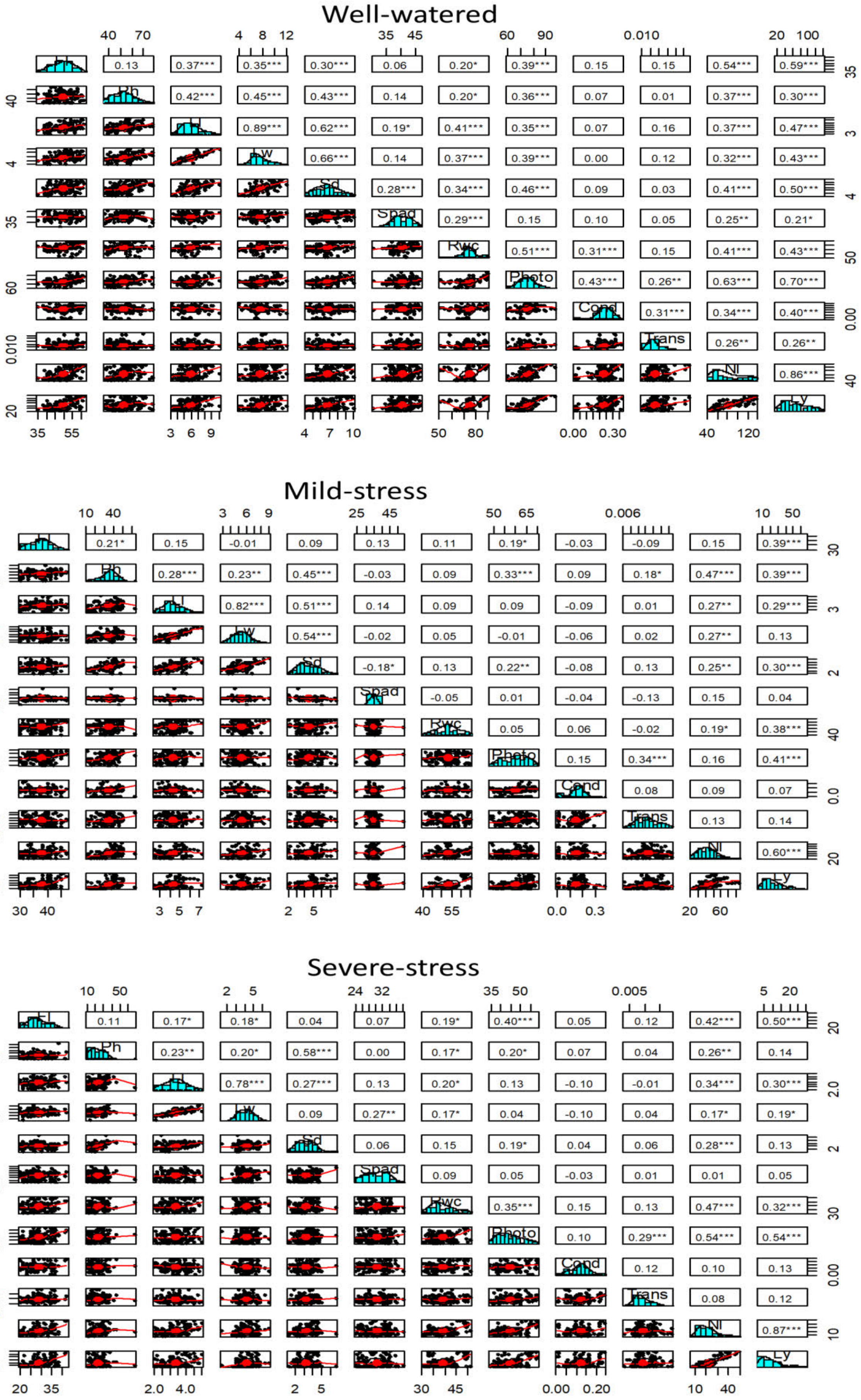

Figure 4. 3 Pearson correlation coefficients for twelve morpho-physiological traits of 18 eighteen African Spider plant accessions evaluated under three water regimes a) well-watered b) mild stress c) severe stress* P < 0.05, ** P < 0.01, *** P < 0.001, Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spad = Chlorophyll content, Rwc = Relative water content, Photo = Net photosynthesis rate, Cond = Stomatal conductance, Trans = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield.

4.5.5 Path coefficient analysis

Path analysis was used to assess the direct and indirect effects of yield attributing traits on leaf yield across all three water regimes as reported in figure 4.4. A scale for path analysis was used to categorize the estimates into negligible with values ranging from 0.00 to 0.09, low from 0.10 to 0.19, moderate from 0.20 to 0.29 and high from 0.30 to 0.99. The residual effects were 0.17 for well-watered conditions figure 4.4, 0.37 for mild stress conditions (figure 4.4), and 0.18 for severe stress conditions. Under well-watered conditions, the number of leaves (0.62) showed a high positive direct effect on leaf yield. The net photosynthesis rate (0.21) showed a moderate direct effect on leaf yield whereas leaf length (0.14) and days to 50% flowering exhibited (0.11) a low but positive direct effect on leaf yield. However, plant height (−0.11) had a negative direct effect on leaf yield. The remaining traits were recorded as negligible with some positive, stem diameter (0.09) and stomatal conductance (0.09). Chlorophyll content (−0.02), relative water content (−0.03) and transpiration rate (−0.02) were negatively negligible under well-watered conditions.

Under well-watered conditions, the indirect effects of several traits observed were mostly positive and high through the number of leaves. Net photosynthesis rate (0.39) had a high and positive indirect effect on leaf yield through the number of leaves. The indirect effects of relative water content (0.25), stem diameter (0.25) plant height (0.23), leaf length (0.23), leaf width (0.20), and stomatal conductance (0.21) through the number of leaves were positive and moderate. Transpiration rate (0.17) and chlorophyll content (0.16) showed a low and positive indirect effect on leaf yield through the number of leaves. Under mild stress conditions, the number of leaves (0.50) and leaf length (0.33) showed a high positive direct effect on leaf yield. Net photosynthesis rate (0.25), relative water content (0.23) and days to 50% flowering (0.20) exhibited a moderate direct effect on leaf yield. Leaf width (−0.32) showed a very high but negative direct effect on leaf yield. Plant height (0.24) showed a moderate indirect effect whereas leaf length (0.14), stem diameter (0.13), and relative water content (0.10) showed a positive and low indirect effect via the number of leaves.

The results for path coefficient analysis estimates under severe stress conditions are presented in . Number of leaves (0.86) had a high and positive direct effect on leaf yield. Days to 50% flowering (0.11) had a low and positive direct effect on leaf yield. In addition to the above, relative water content (−0.14) and stem diameter (−0.09) had a negative but direct effect on leaf yield. Net photosynthesis rate (0.46), relative water content (0.40), leaf length (0.31) and days to 50% flowering (0.36) exhibited a high and positive indirect effect via the number of leaves. A positive and moderate indirect effect via number of leaves was observed for plant height (0.26) and stem diameter (0.24).

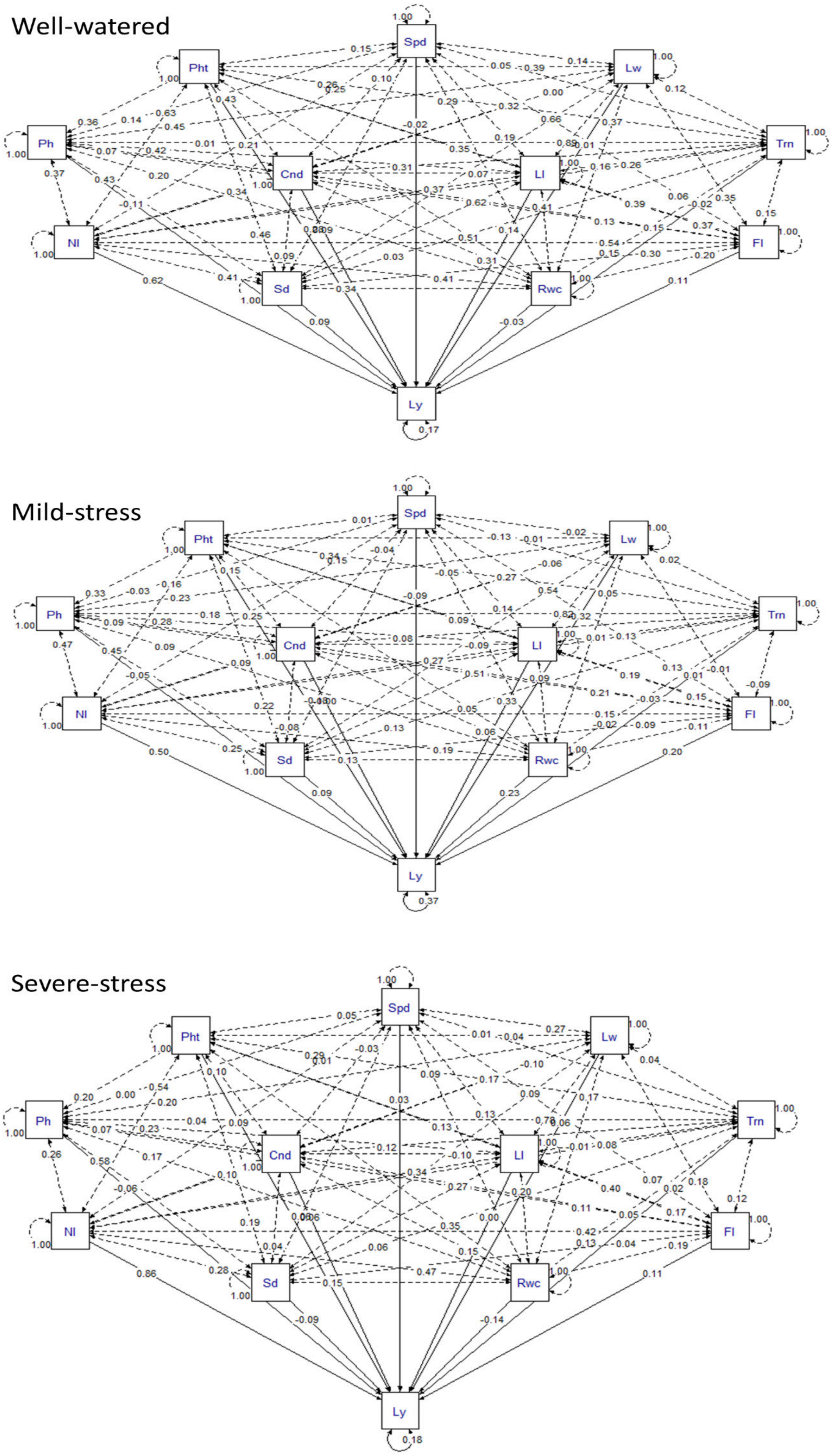

Figure 4. 4 Direct (diagonal) and indirect effects of different metrical traits on leaf yield in African spider plant accessions * P < 0.05, ** P < 0.01, *** P < 0.001, Fl = Days to 50% flowering, Ph = Plant height, Ll = Leaf length, Lw = Leaf width, Sd = Stem diameter, Spd = Chlorophyll content, Rwc = Relative water content, Pht = Photosynthesis, Cnd = Stomatal conductance, Trn = Transpiration rate, Nl = Number of leaves per plant, Ly = Leaf yield

4.6 Discussion

4.6.1 Qualitative traits

The descriptors developed by the Food and Agriculture Organization (FAO Food and Agricultural Organisation, 1995) outline three primary growth behaviours in spider plant; erect, semi-erect, and prostrate, but only two of these (72% erect and 28% semi-erect; Figure 2) were observed in this study, probably due to a small number of accessions evaluated. This observation is consistent with the findings of Wasonga et al., (2015) who found over 84% of the spider plant accessions to be erect. In addition to the above, previous research conducted by (Mnzava and Chigumira, 2004) also revealed that most of the spider plant morphotypes grow upright (erect growth habit). Growth habit plays a vital role in the breeding of spider plant since erect growth coupled with primary and secondary branches has been found to lead to the maximization of leaf area (Wasonga et al., 2015). This suggests that yield improvement in the spider plant could be achieved by selecting genotypes with an erect growth habit and thus big leaf size. Mnzava, (1997) found growth habit to be highly essential in the cropping system of spider plants and has an impact on its harvesting. Masuka et al., (2012) suggested that farmers could use the semi-erect variety in mixed cropping whilst the erect types are best for intercrop adaptation. K'Opondo, (2011) also reported that erect spider plants have high reproductive efficiency, whereas semi-erect spider plants have a greater total pod output.

From this study, green stem accessions were generally sparse in stem hairiness (38.8%), while purple stem accessions showed abundant pubescence on the stem (16.6%). These results are in contrast to the findings of Wasonga et al. (2015) who observed green stemmed accessions to be more glabrous. However, our results are in agreement with those of Makgakga. ,(2011) who observed an abundance of pubescence in purple stemmed accessions. This is because plants with purple stems have more glands than those with green stems.

The color variations noted on the plants' stems, flowers, and petioles included green, purple, pink, and violet. This is attributable to anthocyanins accumulating in the plant tissues (Chalker-Scott, 1999; Chataika et al., 2021). Anthocyanins are water-soluble phenolic pigments that play a significant role in plant improvement and environmental interaction (Chalker-Scott, 1999; Ahmed et al., 2015; Kamjad et al., 2021). Anthocyanins have been shown to reduce the risk of cardiovascular disease, cancer, neurodegenerative disorders (Mishra et al., 2011; Chen et al., 2021), and bone loss associated with aging as well as lower blood pressure, improve visual acuity, and prevent diabetes (Naseri et al., 2018). Anthocyanins and other flavonoids also help plants cope with stress. There has been an increasing desire to understand the mechanisms by which anthocyanins assist plants in dealing with abiotic stress, particularly in the context of crop yield reductions caused by climate change (Naing and Kim, 2021). Anthocyanins are frequently produced in plant vegetative tissues in response to a wide range of abiotic stresses such as drought, excess light, salinity, sub- or supra-optimal temperatures, and nitrogen and phosphorus deficiency (Kovinich et al., 2014; Miki et al., 2015; Zhang et al., 2019; Cirillo et al., 2021).

4.6.2 Quantitative traits

4.6.2.1 Performance of Accessions under Different Water-Regimes

The analysis of variance of the spider plant revealed that genotypic effects were highly significant ($P< 0.01$) for leaf yield and yield components under well-watered conditions which showed that genotypes differed significantly for all yield and yield contributing traits. Under mild stress, genotypic effects were highly significant for all traits except for chlorophyll content. Under severe stress conditions, only chlorophyll content, stomatal conductance and transpiration rate were not significant ($P < 0.05$) indicating the existence of genetic variation among the spider plant accessions studied. According to Mosenda et al (2020), a wide and varied source of tolerance to water stress has been discovered in various African spider plant genotypes. In any plant breeding program, variability is critical for selecting superior genotypes. This also reaffirms that the concept of single-trait selection is not the most reliable way to improve a trait in any crop improvement program.

Under stressed conditions, the significantly large genotype mean squares obtained for the number of leaves, plant height and leaf yield revealed that the accessions varied widely in their potential for these traits (Kang, 2009). According to the findings of this study, the selection for leaf yield improvement under drought stress conditions should include simultaneous selection for several traits such as photosynthesis, number of leaves, days to 50% flowering, relative water content, plant height and leaf length. As evidenced by the results, this can be attributed to the influence of the accessions on the interaction with the water treatment. The considerably higher CV% for some traits could be attributed to the higher variability of genotype x treatment in accession performance. This appears to suggest that evaluating the drought tolerance traits in African spider plant populations under varying levels of water stress rather than just one level was satisfactory (Kang, 2009; Badu-Apraku et al., 2017; Apala Mafouasson et al., 2018).

4.6.2.2 Variance components and heritability

The selection of genotypes on the basis of their phenotypic variation (mean and range) is often complicated by environmental factors that may obscure the actual genetic variation. Therefore, apportioning phenotypic variation into genotypic and environmental factors is critical for genotype selection. In table 4.6, phenotypic variance estimates were higher than genotypic variance estimates across all the water regimes, which indicates the impact of environmental factors on these traits (Semahegn et al., 2021). Zakaria et al., (2017) reported similar findings in all the traits studied in spider plant. The genetic element is fundamental for breeding purposes because it influences the amount of variation passed down to offspring during the breeding process. High genotypic variance and phenotypic estimates were recorded for the number of leaves per plant and leaf yield under all three water regimes. The high genotypic and phenotypic variance of the abovementioned traits revealed that there was more variability, indicating a greater scope of selection for the traits (Hasan-Ud-Daula and Sarker, 2020).

Estimates of genotypic and phenotypic coefficients of variation are required to determine the magnitude of environmental influence on various traits. The genotypic coefficient of variation

(GCV) determines the best relative amount of genetic variation. The number of leaves per plant and leaf yield had higher PCV and GCV values (>20%) under all three water regimes. In addition, plant height had higher PCV and GCV values (>20%) under severe drought stress. These traits with the highest PCV and GCV values have a high level of genetic variability, whereas traits with the lowest PCV and GCV values have a low level of genetic variability (Terfa and Gurmu, 2020). The moderate (10–20%) GCV and PCV values recorded for leaf length, leaf width, stem diameter and stomatal conductance under well-watered conditions, plant height, stem diameter and stomatal conductance under mild stress conditions and leaf width and stem diameter severe stress conditions suggests that selection based on these traits could be effective and their phenotypic expression can be a favourable indicator of genotypic potential. In general, selection will be beneficial for the development of traits related to the degree of preferable variation. Lower PCV and GCV (<10%) values of traits indicate that environmental factors influenced the expression of these traits more than genetic factors. As a result, development through selection cannot be effective based on these traits.

The magnitude of the differences between genotypic and phenotypic coefficients of variation indicates the environmental effect on any trait. Large differences indicate a strong environmental influence, whereas small differences indicate a strong genetic influence. Stomatal conductance showed a significant difference between the PCV and GCV values under mild stress conditions. Under severe stress conditions, leaf length, stem diameter, stomatal conductance and stomatal conductance exhibited large differences between the PCV and GCV values in this study. This suggests that the environmental effect on the expression of those characteristics is greater and that selection based on these traits would be ineffective for further yield improvement (Kushwah et al., 2021).

Heritability assessment calculates the relative influence of genetic and non-genetic discrepancies to overall phenotypic variance in a population. It is a vital feature in quantitative genetics, especially in selective breeding. High heritability values (>60%) were observed for the vast majority of traits except for the transpiration rate, chlorophyll content rate, stomatal conductance and leaf length under well-watered conditions. Plant height, stem diameter, relative water content, photosynthesis rate, number of leaves and leaf yield exhibited high heritability (>60%) under mild stress conditions. Under severe stress conditions, most traits had a high heritability (>60%), and these included leaf yield, number of leaves, plant height, days to 50% flowering, photosynthesis rate, relative water content and leaf width. In this study, relatively high heritability (>60%) was noted in drought stress environments for leaf yield and yield-related traits such as plant height, number of leaves, leaf yield, days to 50% flowering and photosynthesis rate when compared to the non-stress environment. This suggests that environmental variability had less impact on these traits. This inferred that these characteristics were expressed more clearly under drought stress conditions, and therefore, phenotypic selection for such traits would be favourable, as also explained by Zakaria et al., (2017). Furthermore, the high heritability estimates for those characters demonstrate a strong selection response in these characters. Similar findings were reported by (Munene et al., 2018c) under well-watered conditions which support the current study.

High estimates of genetic advance as a percentage of the mean (20% and above) were recorded for leaf yield, number of leaves per plant, stomatal conductance, and stem diameter under well-watered conditions. Under mild stress conditions, leaf yield, number of leaves per plant, stem diameter, and plant height had a high GAM. Plant height, number of leaves per plant and leaf yield also recorded high genetic advance as a percent of the mean values under severe stress. Under both mild and severe stress conditions, plant height, stem diameter, number of leaves per plant and leaf yield showed high to moderate heritability and genetic advance. High genetic advance as a percentage of the mean values indicate additive gene action, whereas low values indicate non-additive gene action(Muhammad et al., 2015).

Heritability and genetic advance estimates are critical in predicting the reliability of phenotypic value as a breeding value guide. Stem diameter, stomatal conductance, number of leaves per plant, plant height, leaf length, leaf width, photosynthesis and stomatal conductance had high/moderate heritability with a high/moderate genetic advance as a percentage of the mean under normal conditions. Under mild stress conditions, plant height, stem diameter, number of leaves per plant, leaf length and leaf width recorded a high/moderate heritability with a high/moderate genetic advance as a percentage of the mean values. Under severe stress conditions, plant height, number of leaves per plant, leaf width, stem diameter, days to 50% flowering and photosynthesis showed a high/moderate heritability accompanied with a high/moderate genetic advance. This suggests that these traits were influenced by additive genes favoring their improvement through direct selection even under water stress conditions (Ali et al., 2009; Semahegn et al., 2021). High heritability in combination with low genetic advance as a percentage of the mean indicates the presence of non-additive gene effects in the inheritance of the specific trait. Traits with low heritability estimates and poor genetic advance as a percentage of the mean are difficult to select directly and should be selected indirectly through related traits.

4.6.2.3 Correlation coefficient

Knowledge of correlation coefficients among traits is useful to breeders because it indicates which traits should be targeted for selection to increase yield under certain environmental conditions. The correlation coefficient results (figure 4.3) revealed that traits such as number of leaves per plant, photosynthesis, and days to 50% flowering had a high to moderate positive correlation with leaf yield under all three water regimes. This suggests that an increase in leaf yield can be obtained by an increase in the number of leaves per plant, leaf photosynthesis rate and late flowering. As a result, these traits could be used as indirect selection criteria to improve leaf yield in African spider plant under both stressed and non-stressed conditions (Kangai Munene et al., 2018c).

Under both mild and severe stress conditions, days to 50% flowering, relative water content, photosynthesis and number of leaves had moderate to high positive correlation with leaf yield. The correlations varied between water regimes, indicating that the level of association between the traits is influenced by environmental factors such as drought stress. Selection strategies must therefore account for such variations in trait association (Long et al., 2006). Correlation findings between yield and yield-attributing traits revealed that selecting for photosynthesis rate, number of leaves per plant, relative water content and days to 50% flowering would be effective in achieving higher leaf yield in African spider plant under drought stress environments.

4.6.2.4 Path coefficient analysis

Correlation coefficients do not provide precise information about the interrelationship between the causal and resultant variables. Path coefficient analysis is an effective way of determining direct and indirect bases of correlations through the use of correlation coefficients of various plant traits. Therefore, correlation coefficient estimates were partitioned into direct and indirect effects to determine the intensity of independent variables' effects on dependent variables. The residual effects in the study were 0.17 for well-watered conditions, 0.37 for mild stress conditions, and 0.18 for severe stress conditions. This indicates that the causal traits explained approximately 83% of the variability in leaf yield, leaving 17% unexplained under well-watered conditions. Under mild stress, 63% of the variability was explained by the casual traits leaving 37% unexplained. Furthermore, under severe stress, 82% of the variability was explained by the characters studied leaving 18% of the variability unexplained. According to Hasan-Ud-Daula and Sarker (2020), these residual effects on leaf yield could be due to other factors not studied such as environmental errors or sampling errors.

The number of leaves per plant had a high and positive direct effect on leaf yield across all water regimes. Under well-watered conditions, the photosynthesis rate had a moderate and positive direct effect on leaf yield. In the mildly stressed environment, leaf length had a high and positive direct effect on leaf yield. relative water content and photosynthesis rate had a moderate and positive direct effect. This suggests that more attention should be placed on the genetic improvement of these traits in order to increase leaf yield through indirect selection (Kumar et al., 2017; Mubai et al., 2020). Plant height had a negative direct effect on leaf yield across all water regimes, but this trait had a positive correlation with leaf yield. Belay, (2018) reported similar findings in maize. The positive correlation of this trait with leaf yield was because of the positive indirect effect of this trait through other traits.

Plant height had a moderate and positive indirect effect on leaf yield via the number of leaves per plant in all three water regimes. The photosynthesis rate had a high and positive indirect effect on leaf yield under well-watered conditions. Plant height, stem diameter, leaf length, leaf width, relative water content and stomatal conductance all had a moderate and positive indirect effect on leaf yield via the number of leaves per plant under well-watered conditions. Plant height had a moderate and positive indirect effect on leaf yield via the number of leaves per plant under mild stress conditions. In the severe stress regime, relative water content, photosynthesis rate and leaf length had a high and positive indirect effect on leaf yield via the number of leaves per plant. Stem diameter and plant height had a moderate and positive indirect effect on leaf yield via the number of leaves per plant. This suggests that selecting for traits such as plant height, days to 50% flowering, relative water content, net photosynthesis and leaf length under stress conditions could be effective and can improve leaf yield indirectly by influencing the number of leaves per plant.

Conclusions

The accessions used in this study showed significant genetic variation for all traits measured, providing essential genetic resources for African spider plant improvement and opportunities to identify genotypes and traits with water regime stability. Traits with high to moderate genetic variance, heritability, and genetic advances, such as the number of leaves per plant, plant height and stem diameter could be improved through direct selection under drought stress conditions. These traits can also be used to indirectly select for yield under water stress conditions. The number of leaves per plant had the highest positive direct effect and strongest positive correlation with leaf yield, providing a foundation for leaf yield selection and improvement under drought stress conditions. Other traits including plant height, days to 50% flowering, relative water content, net photosynthesis and leaf length should also be considered for selection for leaf yield improvement especially under drought stress. In addition to data generated in this study, molecular genotyping of the accessions with selected molecular markers is essential to select complementary accessions for drought tolerance breeding.

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Chapter 5 Assessment of drought tolerance indices and root traits to identify tolerant African Spider plant (*Gynandropsis gynandra (*L.) Briq.) genotypes.

Abstract

Root components affect the assimilation of nutrients and water and are essential in retaining yield under stressed conditions. Identifying and selecting genotypes with drought tolerance is part of the primary objectives of crop improvement in arid and semi-arid areas. The current study sought to characterize root traits of 18 African spider plant accessions under varying moisture regimes, evaluate the accessions' response to drought stress and identify drought-tolerant accessions that can be used as parents for breeding. The experiments were laid out in a randomized complete block design with three replications and two water regime treatments. There were significant differences in leaf yield between spider plant genotypes under stressed and non-stressed conditions .Six leaf yield based drought tolerance indices and six root traits were estimated under non-stressed and severe stress environments. Correlation and principal component analysis showed the importance of yield-based stress tolerance indices such as tolerance index (TOL) , geometric mean productivity (GMP), stress tolerance index (STI), mean productivity (MP) , and yield index (YI), which allowed discrimination of drought-tolerant spider plant genotypes. Accessions such as L3 and L5 were identified as useful for drought-tolerance breeding programs.

Keywords; drought stress, genotypes, root traits, tolerance indices,

5.1 Introduction

African leafy vegetables are crops that in the past have not been considered to be major crops, have beforehand been understudied, presently occupying a small degree of utilization and predominantly limited to small-holder farmers (Azam-Ali, 2010). Various names have been used to describe these crop species with the popular names being forgotten crops, underutilized crops, minor crops neglected crops, and orphan crops (Chivenge et al., 2015b). Unlike the majority of essential crops, African leafy vegetables are most often well suited to local environments (Padulosi and Hodgkin, 1999). These environments are usually marginal and characterized by harsh conditions which make leafy vegetables a good alternative for sustained food production (Idowu, 2009).

One such vegetable with enormous potential is the African Spider plant (*Gynandropsis gynandra* (L.) Briq.) It is among the most essential and highly regarded African leafy vegetables consumed in most African countries including South Africa, Zimbabwe, Ghana, Benin, and Kenya (Abukutsa-Onyango, 2007; Dansi et al., 2012; Chweya & Eyzaguirre, 1999). African Spider plant is native to tropical and subtropical areas (Bala et al., 2010b). African Spider plant is generally cultivated in marginal areas that are quite desolate and lacking in agricultural inputs or irrigation. Research carried out on this vegetable revealed that its leaves are more nutritious as compared to other exotic leafy vegetables (Van Den Heever and Venter, 2007). African Spider plant is a great source of nourishment particularly minerals in the form of Calcium and iron and Vitamins A and C (Jinazali* et al., 2017; Omondi et al., 2017c). This indigenous vegetable is an essential food source in rural areas where over 75% of the people reside in most African countries thus contributing significantly to food security in families during droughts. Efforts are being made all over the world to increase leaf yield and other characteristics by creating novel genotypes and varieties together with better agronomic practices.

Drought is amongst the most predominant abiotic stress constraining the growth and survival of plants in arid and semi-arid environments around the world. Approximately a third of the world's arable land is located in arid and semi-arid regions (Atlin and Frey, 1990; Blum, 2011). Drought stress triggers plants to act in response and acclimatize through response mechanisms such as drought escape, drought tolerance, and drought avoidance (Wang and Huang, 2004). Hypothetically, the exhibition of drought tolerance characteristics is impressionable and enables plants to acclimatize to seasonal changes in rainfall under water-stressed environments. For this reason, a better understanding of plant response to drought stress can be useful in selecting drought-tolerant varieties (Herder et al., 2010).

Root Characteristics play a crucial role in soil exploration and the absorption of nutrients and water. They are essential for crop advancement under low moisture conditions (Manschadi et al., 2007a; Gupta et al., 2012). A deep root system aids a plant's drought avoidance by drawing water preserved in deep soil layers (Ludlow and Muchow, 1990; Bonos et al., 2004a). Studies have demonstrated that total root length is highly correlated with drought tolerance due to its effect on root distribution in the soil and its effect on the significant degree of water uptake (Manschadi et al., 2006). Enhanced root diameter was strongly correlated to drought tolerance in rice *(Oryza* *sativ*a L.) for the reason that thicker roots possess extensive xylem vessels characterized by elevated axial conductance and effectively penetrate the bottom soil layers to draw out water (Fukai and Cooper, 1995; Clark et al., 2008). Root dry weight together with root to shoot ratio showed an association with drought tolerance in Tall fescue (*Festuca arundinacea* Schreb.) (Pirnajmedin et al., 2014).

Developing drought-tolerant varieties continues to be one of the most important objectives in various breeding programs. However, its success has been restricted by ineffective screening methods, and an insufficient number of genotypes exhibit a noticeable difference in response to low soil moisture stress. In order to discriminate genotypes for drought tolerance, some selection indices have been proposed based on mathematical relations between non-stressed and drought-stressed environments (Huang. 2000). Some selection indices namely stress tolerance index (STI) and Geometric mean productivity (GMP), (Fernandez, 1992), drought susceptibility index (SSI) (Fischer and Maurer, 1978a), Tolerance (TOL), and Mean production (MP) (Rosielle and Hamblin, 1981c), and Yield index (YI) (Gavuzzi et al., 1997).

The Stress Tolerance Index (STI) has been established as a tool for identifying genotypes with high yield and stress tolerance potential (Fernandez, 1992). Stress tolerance (TOL) as reported by (Rosielle and Hamblin, 1981c) is characterized by variations in yield between non-stress and stress conditions. The mean yield of genotypes under stress and non-stress environments is referred to as mean productivity (MP). Plant breeders are most often interested in the comparative performance of genotypes given the fact that water stress varies in intensity under field conditions over a period of years Thus they use the geometric mean productivity (GMP) (Khodarahmpour et al., 2011). It was demonstrated by (Drikvand et al., 2012) that MP, GMP, and STI were the most appropriate indices utilized for identifying genotypes under rainfed and irrigated conditions. Strong and positive correlations between yield and GMP, MP, and STI were discovered under irrigated and drought-stressed environments (Anwar et al., 2011).

Although roots traits are essential in stress tolerance, no studies have been conducted to incorporate drought adaptive traits of roots in breeding for drought-tolerant genotypes of African Spider plant. Most of the studies on this leafy vegetable have focused on above-ground factors. Most breeding programs have extensively neglected root traits to a great degree because of the challenges closely linked with root recovery and assessment of these traits in situ. Investigating the genetic diversity of root traits could advance African Spider plant improvement programs in establishing varieties with desirable root components for drought tolerance and target environment (Poorter and Nagel, 2000; Manschadi et al., 2007b). An insight into the association of root and shoot traits that advance leaf yield is critical to realize development and productivity (Hodge et al., 2009). Furthermore, there is presently no information on the association between drought tolerance indices and root traits. The objective of this study was to characterize root traits among African Spider plant accessions and investigate numerous drought tolerance indices and identify drought-tolerant genotypes in African Spider plant.

5.2 Material and methods

5.2.1 Plant material, growing environments, experimental design, and agronomic practices

Eighteen African Spider plant accessions described in Chapter 3 were used for this study. Information on these accessions is given in table (3.1) in Chapter 3 under material and methods.

5.2.2 Growing environments, experimental design, and agronomic practices

This study was carried out in controlled environment facilities (greenhouses) at the University of KwaZulu-Natal's School of Agricultural Earth, and Environmental Sciences in Pietermaritzburg, South Africa. Two separate pot experiments were carried out in 2021 and 2022 to assess the variability of root traits among African Spider plant accessions.

The experiment included two factors (moisture levels and accessions), with three replications arranged in a complete block design. Each accession had two seeds planted per pot and later thinned to one plant. For the moisture stress conditions, each accession was assigned to three pots, giving rise to three replications per accession. The accessions were grown following the steps described in chapter 3. Drought stress was imposed at 30% as described in the material and methods in chapter 3.

5.3 Data collection

5.3.1 Root morphological traits

To quantify root phenes, the shovelomics approach was used (Bucksch et al., 2014; Arifuzzaman et al., 2019). The shovelomics technique captures much of the biomass from the root system within the excavation site. Following excavation, the shoot was separated from the root with secateurs 5 cm just above soil level and rinsed in 5% sodium hypochlorite to eliminate soil and microbiome from the roots and root wet weight was recorded (RWW). Following the detachment of shoots, roots were set on a flat surface and stretched to determine their length (from the base of the stem to the tip of the root system) as an estimate of rooting depth. For 8 days, the rinsed roots were covered with a paper towel and pressed onto newsprint. The roots were then scanned with a root scanner (Epson Perfection V 700 Photo/V 750 Pro, Seiko Epson Corporation, Nagano-ken, Japan). Upon scanning the roots, the root image for each plant was analyzed using the WinRHIZO Pro image analysis system (Regent Instruments, Inc., Quebec City, QC) to calculate root characteristics. Archimedes' recommended method of measuring root volume (RV) was used. Root dry weight (RDW) was determined after roots were dried in an oven at 85 degrees Celsius for 48 hours. The root to shoot ratio (RR) was then computed.

5.4 Statistical Analysis

5.4.1 Data analysis

The analysis of variance was used to statistically examine all of the data acquired for root morphological traits using the *Agricolae* package. The LSD test was used to separate treatment means ($P < 0.05$). A principal component analysis (PCA) test was also performed using the packages *FactoMineR*", and "*factoextra*". The correlation coefficient for all morphophysiological traits was done using the *corr* package. All the above-mentioned analyses were performed in R software version R-4.1.1 (R Core Team, 2021). The analysis of variance was performed using the following model.

$$\boldsymbol{y_{ij} = \mu + t_i + b_j + e_{ij} \quad i = 1, 2, \cdots, 18, \quad j = 1, 2} \qquad \text{Equation 5. 1}$$

where i is the general mean, t_i is the effect of the i^{th} treatment, b_j is the effect of the j^{th} block and e_{ij} is the random error term.

5.4.2 Drought tolerance indices

Six drought tolerance indices namely stress tolerance index (STI), Tolerance (TOL), mean productivity (MP), drought susceptibility index (SSI), geometric mean productivity (GMP), and Yield Index (YI) were estimated using the below formula (Table 5.1).

Table 5. 1 list of drought indices used in the studies.

Name of Index	**effect**	**Equation**	**Reference**
Stress Tolerance Index (STI)	The accessions with a high STI values are regarded to be tolerant to drought stress.	$STI = \frac{(Y_{nS})(Y_{ds})}{(X_{ns})^2}$	(Fernandez, 1992)
Geometric Mean Productivity (GMP)	The accessions with a higher value of this index are considered more suitable	$GMP = \sqrt{(Y_{nS})(Y_{ds})}$	(Schneider et al., 1997)
Mean Productivity (MP)	The accessions with a higher MP value is more desirable	$MP = \frac{Y_{nS} + Y_{ds}}{2}$	(Rosielle and Hamblin, 1981c)
Tolerance (TOL)	The accessions with a higher TOL value is more desirable	$TOL = Y_{nS} - Y_{ds}$	(Hossain et al., 1990)
Stress Susceptibility Index (SSI)	The Accessions with a higher value of this index is considered. more suitable	$SSI = \frac{Y_{nS} - Y_{ds}}{Y_{ns} \, x \, DSI}$	(Fischer & Maurer,1978)
Yield Index (YI)	Accessions with the highest value of YI is desirable for drought stress Condition	$YI = \frac{Y_{ds}}{\bar{X}_{ds}}$	(Gavuzzi et al., 1997)

Y_{ds} = yield for stressed, Y_{nS} = yield for non-stress, STI = Stress Tolerance Index, GMP = Geometric Mean Productivity, MP = Mean Productivity, TOL = Tolerance, SSI= Stress Susceptibility Index YI = Yield Index

5.5 Results

5.5.1 Effect of Ascensions, Environments, and Water Regimes on root traits

The ANOVA results in Table 5.1 demonstrated the effects of all growing seasons, water regimes, and genotype factors, as well as their interactions and coefficients of variation (CVs), on the 7 traits studied. For most traits, the effect of planting season was not significant except for root dry weight (RDW) which was significant ($P< 0.05$). The analysis of variance also revealed that drought stress had a highly significant effect on all root traits ($P < 0.001$). Most of the studied traits were not affected by the accessions except for Total root volume (TRV), average root diameter (ARD) and leaf yield (LY). The genotype by water-regime interaction was not significant for all the traits studied.

Intense drought stress significantly decreased root area (RA), root volume (RV), root dry weight (RDW), root dry weight (RDW), and leaf yield as compared to the control condition, according to a comparison of the means of measured traits (Table5.2). Furthermore, under severe drought stress, the root-to-shoot (RR) ratio and root length (RL), increased (Table 5.2); (Figure 5.2).

Table 5. 2 Analysis of variance for root traits in 18 African Spider plant genotypes under moisture environments

Source of variation	**Df**	**RDW**	**RR**	**TRL**	**TRV**	**TRA**	**ARD**	**LY**
Season	1	0.90*	0.00398	0.35	2.1	1090866***	0.108	21
Water regime	1	80.57***	1.05937***	1154.59***	4464***	29690127***	60.346***	206922***
Genotype	17	0.137	0.00215	0.88	13.4**	30985	0.238***	3499***
Block	2	0.882**	0.01015**	0.99	5.3	113197	0.172	3
Water_regime×Genotype	17	0.184	0.00270	1.48	9.1	34549	0.125	1723***
Season×Genotype	17	0.129	0.00117	1.25	7.0	16119	0.087**	129
Season×Water_regime×Genotype	18	0.131	0.00098	1.02	4.2	40017	0.029	167
Residuals	142	0.173	0.00186	1.14	6.4	44502	0.057	161

* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, Df = Degrees of freedom TRL= Total root length, TRV= Total root volume, TRA= Total root area, RDW = Root dry weight, RR = Root to shoot ratio, ARD = Average root diameter, LY = Leaf yield

Table 5. 3 Means of root traits in 18 African Spider plant genotypes under two levels of moisture environments.

	RDW	RR	TRL	TRV	TRA	ARD	LY
Non-stressed	1.97^{a}	0.11^{a}	8.49^{a}	27.57^{a}	1734.11^{a}	1.70^{a}	73.028^{a}
Stressed	0.74^{b}	0.25^{b}	18.11^{b}	18.48^{b}	987.69^{b}	0.64^{b}	10.741^{b}

TRL= Total root length, TRV= Total root volume, TRA= Total root area, RDW = Root dry weight, RR = Root to shoot ratio, ARD = Average root diameter, LY = Leaf yield

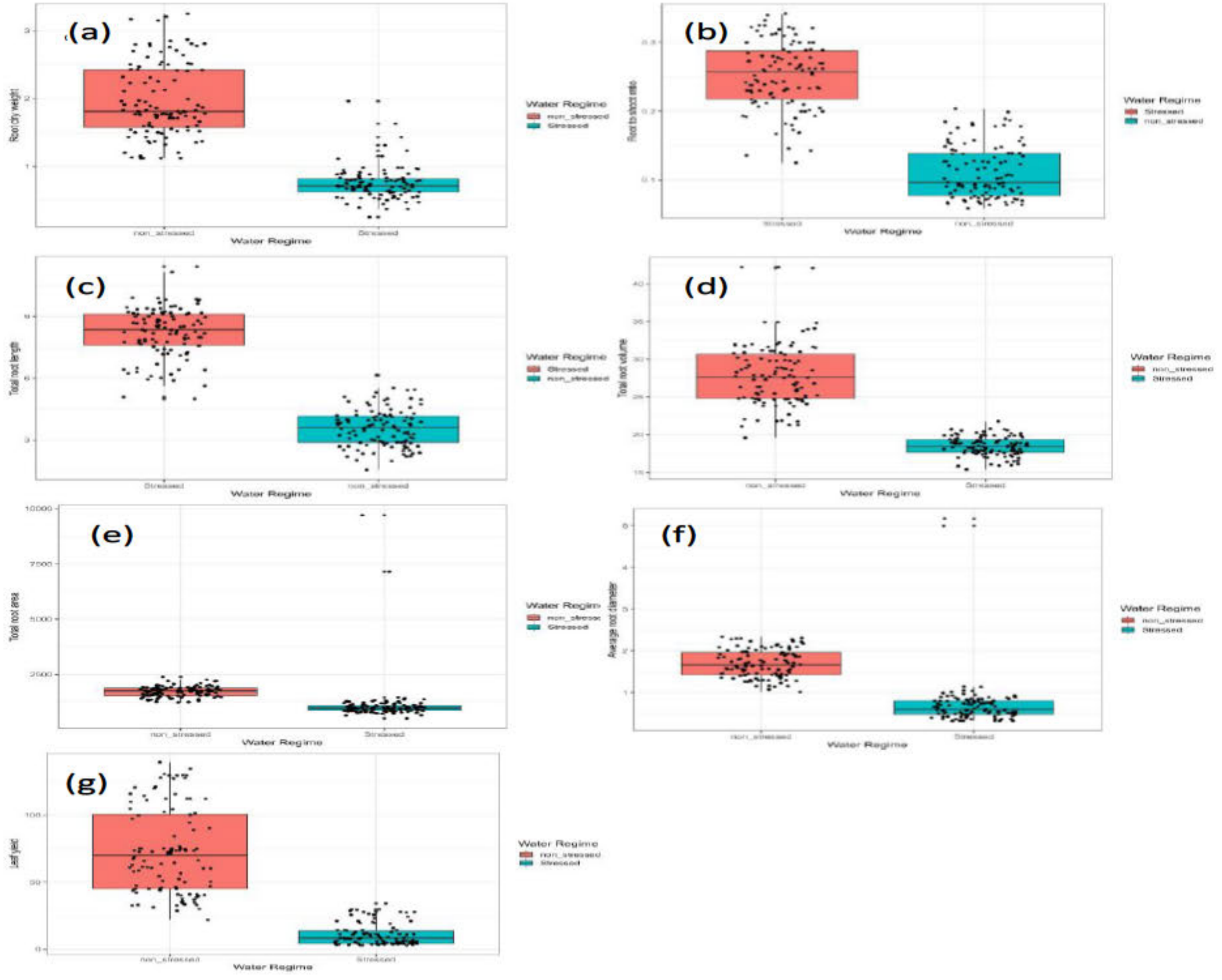


Figure 5. 1 Response of spider plant accessions for root traits under non-stress and drought-stressed conditions. On a) root dry weight b) root to shoot ratio c)Total root length d) total root volume e) root area f) average root diameter g) leaf yield

5.5.2 Yield-based indices of drought tolerance

Various indices based on leaf yield under stressed and non-stressed conditions were estimated to identify suitable drought tolerance indices for screening African spider plant accessions under drought stress conditions (Table 5.4). Leaf yield under non-stressed ranged from 33,412-132,162grams. Accessions L3 and L5 had the highest leaf yield under non-stress conditions whereas accessions L18 and L12 recorded the lowest leaf yield under non-stress. Leaf yield under drought stress ranged from 3.833- 29,232 grams with accessions L3 and L5 recording the highest leaf yield under stressed conditions. Accessions L2 and L18 recorded the lowest leaf yield under stressed conditions.

Mean Productivity (MP) ranged from 19,15 to 80,69. Accessions L5 and L3 recorded the highest Mean Productivity (MP) whilst Accessions L18 and L12 recorded the lowest Mean Productivity (MP). Regarding (TOL) tolerance, its mean values ranged from 28,51- 102,93. Accessions L3 and L5 recorded the highest (TOL) tolerance whilst accessions L18 and L12 recorded the lowest (TOL) tolerance. The Stress Susceptibility Index (SSI) ranged from 0,87 - 1,103 with accessions L6 and L7 recording the highest Stress Susceptibility Index (SSI) whilst accessions L1 and L3 recorded the lowest Stress Susceptibility Index (Table 5.4).

Regarding Geometric Mean Productivity (GMP), its values ranged from 12,795- 62,155. The highest Geometric Mean Productivity was observed in accessions L3 and L5 whilst the lowest Geometric Mean Productivity was observed for accessions L18 and L2. Stress Tolerance Index (STI) ranged from 0,031- 0,724. Accessions L3 and L5 had the highest Stress Tolerance Index values whilst accessions L2 and L18 recorded the lowest Stress Tolerance Index. With regards to yield index (YI), its values ranged from 0,357- 2,721. Accessions L3 and L5 recorded the highest yield index (YI) whereas, accessions L2 and L12 recorded the lowest yield index (YI).

Table 5. 4 Mean values of Y*d*s, Y*n*s, and stress tolerance indices of 18 African Spider plant genotypes.

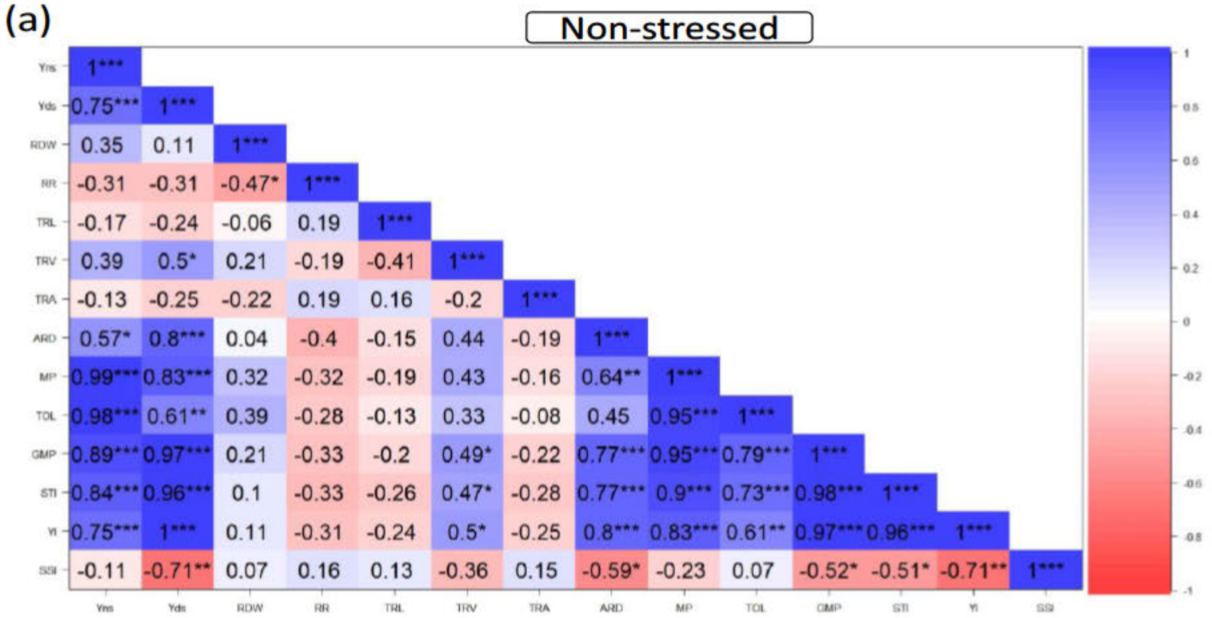
Genotype	Y_{ns}	Y_{ds}	**MP**	**TOL**	**SSI**	**GMP**	**STI**	**YI**
L1	75.108	19.378	47.243	55.730	0.873	38.151	0.273	1.804
L2	43.537	3.833	23.685	39.703	1.073	12.919	0.031	0.357
L3	132.162	29.232	80.697	102.930	0.916	62.155	0.724	2.721
L4	105.262	12.178	58.720	93.083	1.040	35.804	0.240	1.134
L5	123.373	23.697	73.535	99.677	0.951	54.070	0.548	2.206
L6	104.405	8.395	56.400	96.010	1.082	29.605	0.164	0.782
L7	86.597	5.414	46.005	81.183	1.103	21.653	0.088	0.504
L8	83.440	11.797	47.618	71.643	1.010	31.374	0.185	1.098
L9	50.268	8.162	29.215	42.107	0.985	20.255	0.077	0.760
L10	42.363	8.008	25.186	34.355	0.954	18.419	0.064	0.746
L11	74.700	7.872	41.286	66.828	1.052	24.249	0.110	0.733
L12	41.098	4.415	22.757	36.683	1.050	13.470	0.034	0.411
L13	56.003	9.085	32.544	46.918	0.986	22.556	0.095	0.846
L14	71.972	8.667	40.319	63.305	1.035	24.975	0.117	0.807
L15	63.485	6.160	34.823	57.325	1.062	19.775	0.073	0.573
L16	54.193	11.812	33.002	42.382	0.920	25.300	0.120	1.100
L17	73.128	10.337	41.732	62.792	1.010	27.494	0.142	0.962
L18	33.412	4.900	19.156	28.512	1.004	12.795	0.031	0.456
Mean	71.79	10.73	41.26	61.06	1	26.57	0.17	1
LSD	17.52	6.43	9.45	18.43	0.14	8.43	0.09	0.60

Y_{ds} = yield for stressed, Y_{nS} = yield for non-stress, STI= Stress Tolerance Index, GMP = Geometric Mean Productivity, MP = Mean Productivity, TOL = Tolerance, SSI= Stress Susceptibility Index ,YI =Yield Index.

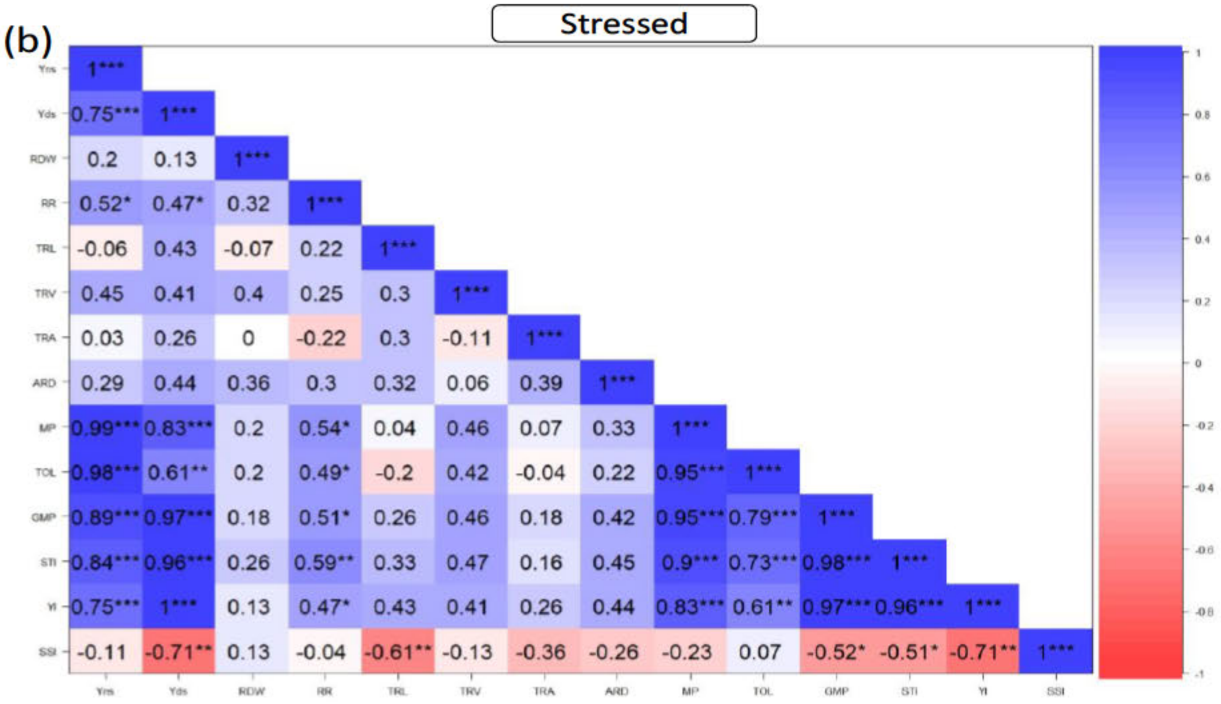
5.5.3 Correlation coefficients

Pearson’s correlation coefficients between various root traits and drought tolerance indices calculated in the pot experiments and are shown in (Figure 5.2). Under optimum conditions, Y_{ns} was positively and strongly correlated with yield index YI (r = 0.75; $P < 0.001$), Stress Tolerance Index (STI) (r = 0.84; $P < 0.001$), Geometric Mean Productivity (GMP) (r = 0.89; $P < 0.001$), Mean Productivity (MP) (r = 0.99 ; $P < 0.001$) and Tolerance (TOL) (r = 0.98 ; $P < 0.001$). In addition to the above Y_{ns} was moderate and positively correlated with (ARD) average root diameter (r = 0.57 $P < 0.05$). Negative correlations between Y_{ns} and (TRL) total root length (r =-0.17: $P = 0.08$), Y_{ns} and (RR) root to shoot ratio (r = -0.31; $P = 0.06$) were also observed. Similarly, Y_{ds} was positively and strongly correlated with yield index YI (r = 1; $P < 0.001$), Stress Tolerance Index (STI) (r = 0.96; $P < 0.001$), Geometric Mean Productivity (GMP) (r = 0.97; $P < 0.001$), Mean Productivity (MP) (r = 0.83; $P < 0.001$) and Tolerance (TOL) (r = 0.6; $P < 0.01$)1Furthermore, Y_{ds} was positive and strongly correlated with (ARD) average root diameter (r = 0.8; $P < 0.001$), moderate and positively correlated with (TRV) total root volume (r =0.5; $P < 0.05$) (Figure 5.2).

Under drought stress conditions, similar trends were also observed between Y_{ns} and Y_{ds}. These traits also had similar associations with drought tolerance indices as observed in (figure 5.2). However, Y_{ns} had a moderate and positive correlation with root to shoot ratio RR (r = 0.52; $P < 0.05$). Furthermore, Y_{ds} had a positive and moderate association with root to shoot ratio under drought stress conditions (r = 0.47; $P < 0.05$). It can be noted that Y_{ns} had a negative and weak correlation with total root length (TRL) (r = -0.06; $P = 0.07$) (Figure 5.2).



	Yns	Yds	RDW	RR	TRL	TRV	TRA	ARD	MP	TOL	GMP	STI	YI	SSI
Yns	1***													
Yds	0.75***	1***												
RDW	0.35	0.11	1***											
RR	-0.31	-0.31	-0.47*	1***										
TRL	-0.17	-0.24	-0.06	0.19	1***									
TRV	0.39	0.5*	0.21	-0.19	-0.41	1***								
TRA	-0.13	-0.25	-0.22	0.19	0.16	-0.2	1***							
ARD	0.57*	0.8***	0.04	-0.4	-0.15	0.44	-0.19	1***						
MP	0.99***	0.83***	0.32	-0.32	-0.19	0.43	-0.16	0.64**	1***					
TOL	0.98***	0.61**	0.39	-0.28	-0.13	0.33	-0.08	0.45	0.95***	1***				
GMP	0.89***	0.97***	0.21	-0.33	-0.2	0.49*	-0.22	0.77***	0.95***	0.79***	1***			
STI	0.84***	0.96***	0.1	-0.33	-0.26	0.47*	-0.28	0.77***	0.9***	0.73***	0.98***	1***		
YI	0.75***	1***	0.11	-0.31	-0.24	0.5*	-0.25	0.8***	0.83***	0.61**	0.97***	0.96***	1***	
SSI	-0.11	-0.71**	0.07	0.16	0.13	-0.36	0.15	-0.59*	-0.23	0.07	-0.52*	-0.51*	-0.71**	1***



	Yns	Yds	RDW	RR	TRL	TRV	TRA	ARD	MP	TOL	GMP	STI	YI	SSI
Yns	1***													
Yds	0.75***	1***												
RDW	0.2	0.13	1***											
RR	0.52*	0.47*	0.32	1***										
TRL	-0.06	0.43	-0.07	0.22	1***									
TRV	0.45	0.41	0.4	0.25	0.3	1***								
TRA	0.03	0.26	0	-0.22	0.3	-0.11	1***							
ARD	0.29	0.44	0.36	0.3	0.32	0.06	0.39	1***						
MP	0.99***	0.83***	0.2	0.54*	0.04	0.46	0.07	0.33	1***					
TOL	0.98***	0.61**	0.2	0.49*	-0.2	0.42	-0.04	0.22	0.95***	1***				
GMP	0.89***	0.97***	0.18	0.51*	0.26	0.46	0.18	0.42	0.95***	0.79***	1***			
STI	0.84***	0.96***	0.26	0.59**	0.33	0.47	0.16	0.45	0.9***	0.73***	0.98***	1***		
YI	0.75***	1***	0.13	0.47*	0.43	0.41	0.26	0.44	0.83***	0.61**	0.97***	0.96***	1***	
SSI	-0.11	-0.71**	0.13	-0.04	-0.61**	-0.13	-0.36	-0.26	-0.23	0.07	-0.52*	-0.51*	-0.71**	1***

Figure 5. 2 Figure 5. 2 correlation coefficient for root traits and stress tolerance indices under a)non-stress b) stress conditions Y_{ds} = yield for stressed Y_{ns} = yield for non-stress STI= Stress Tolerance Index GMP = Geometric Mean Productivity MP = Mean Productivity TOL = Tolerance SSI= Stress Susceptibility Index YI =Yield Index , TRL= Total root length, TRV= Total root volume, TRA= Total root area, RDW = Root dry weight , RR = Root to shoot ratio, ARD = Average root diameter.

5.5.4 Principal component analysis

A Principal component analysis was performed to examine the connection between root traits, accessions, and yield-based indices of drought tolerance. Under non-stressed conditions, three principal components (PCs) with a cumulative variance of 77.26% were identified (Table 5.5). PC1 positively related to SSI, TRL, TRA and RR, accounting for 54.56 % of total variation. Y_{ns}, RDW, TOL, SSI and MP were positively correlated with PC2 which accounted for 12.83% of the total variation. PC3 positively correlated with RDW and TRV which explained 9.87% of total variation.

Under water stressed conditions, three PCs with a cumulative variance of 78.52% were identified. All traits except for SSI negatively correlated with PC1 and accounted for 52.38% of total variation. PC2 positively correlated with Y_{ns}, SSI, TOL, MP, RDW, RR, and TRV accounting for 16.85% of total variation. PC3 negatively correlated with RDW, RR, TRV, ARD, TRL and SSI accounting for 9.29% of total variation (Table 5.5).

The PCA biplot was used to identify the relationships between root traits, yield and yield-based drought tolerance indices (Figure 5.3 a-b). Under non-stressed conditions, the biplot was created using PC1 (54.6%) and PC2 (12.8%) (Figure 5.3a). The biplot results revealed that most traits clustered together in the biplot's rightmost part region except for SSI, TRL, TRA and RR. Most of the accessions, however, were scattered in the biplot's leftmost region.

The biplot was created under drought stressed conditions using PC1 (52.4%) and PC2 (16.9%). (Figure 5.3b). The biplot results confirmed that traits like SSI were concentrated in the biplot's leftmost region. Similarly, majority of the accessions, were concentrated in the biplot's leftmost region.

Table 5. 5 Principal component loadings for the traits measured on 18 African Spider plant genotypes under two moisture levels

	Non-stressed				**Drought-Stressed**	
Traits	**PC1**	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Y_{ns}	-0.31	0.30	-0.17	-0.32	0.25	0.14
Y_{ds}	-0.34	-0.19	-0.05	-0.35	-0.15	0.09
RDW	-0.09	0.45	0.43	-0.09	0.17	-0.71
RR	0.15	-0.16	-0.45	-0.21	0.17	-0.25
TRL	0.103	0.079	-0.41	-0.11	-0.47	-0.18
TRV	-0.20	-0.10	0.29	-0.19	0.10	-0.26
TRA	0.09	0.04	-0.45	-0.06	-0.38	-0.04
ARD	-0.28	-0.21	-0.009	-0.17	-0.18	-0.43
MP	-0.33	0.21	-0.15	-0.345	0.18	0.14
TOL	-0.28	0.41	-0.18	-0.29	0.35	0.14
GMP	-0.35	-0.01	-0.11	-0.365	-0.005	0.11
STI	-0.35	-0.06	-0.09	-0.36	-0.025	0.014
YI	-0.34	-0.19	-0.05	-0.35	-0.15	0.09
SSI	0.18	0.55	-0.09	0.17	0.49	-0.15
Explained variance (eigenvalue)	2.76	1.34	1.17	2.70	1.53	1.14
Proportion of variance (%)	54.56	12.83	9.87	52.38	16.85	9.29
Cumulative Proportion (%)	54.56	67.39	77.26	52.38	69.23	78.52

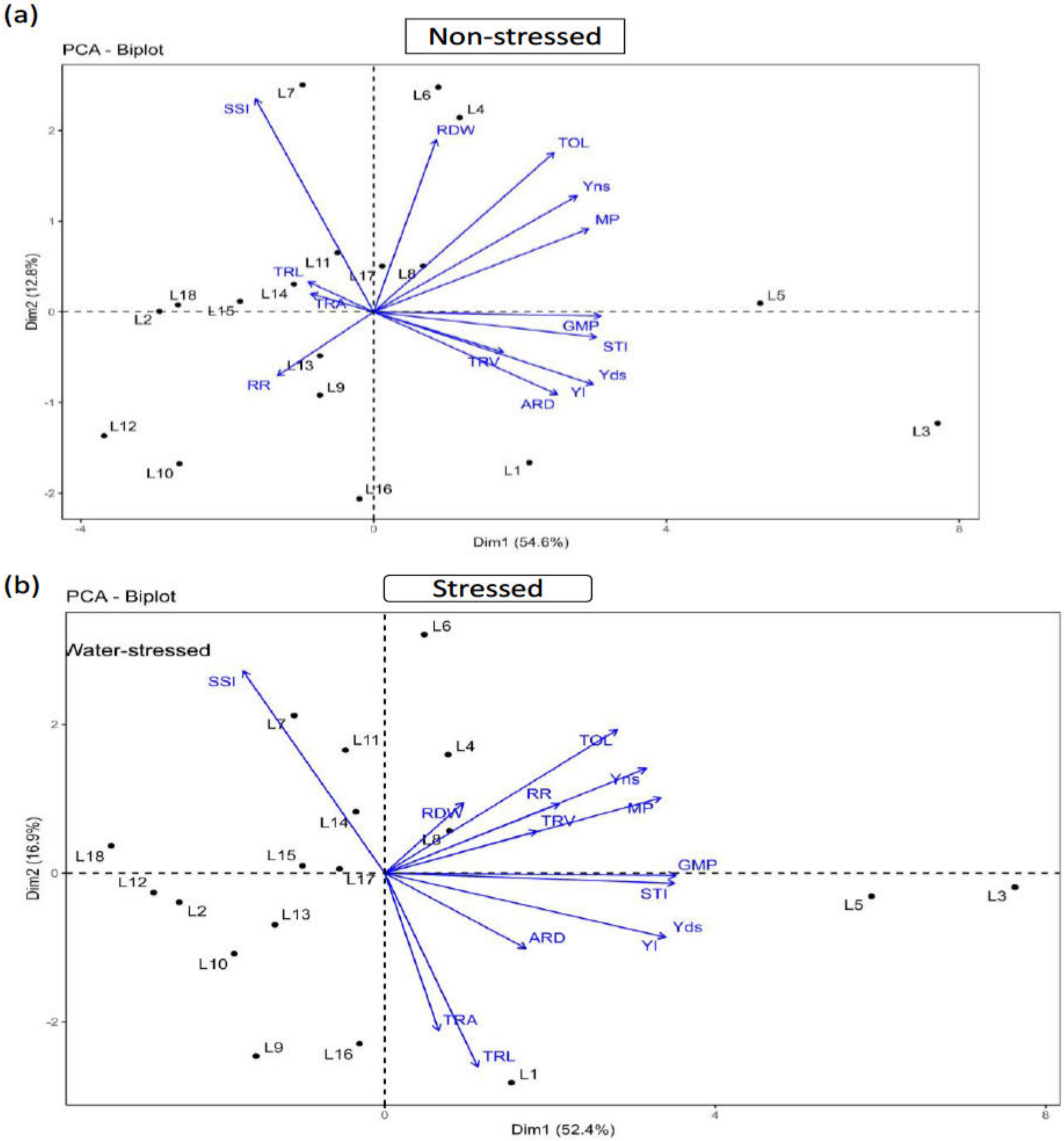


Figure 5. 3 Biplot of root and stress tolerance indices in 18 African spider plant under a) non-stressed b) stressed conditions. $\boldsymbol{Y_{ds}}$ = yield for stressed $\boldsymbol{Y_{ns}}$ = yield for non-stress STI= Stress Tolerance Index GMP = Geometric Mean Productivity MP = Mean Productivity TOL = Tolerance SSI= Stress Susceptibility Index YI =Yield Index TRL= Total root length, TRV= Total root volume, TRA= Total root area, RDW = Root dry weight , RR = Root to shoot ratio, ARD = Average root diameter.

5.5.5 Cluster analysis

The 18 African Spider plant accessions were divided into four column-clusters, with cluster-1, cluster-2, cluster-3, and cluster-4 each containing two, one, five and ten accessions (Figure 5.4). Cluster 1 had the most drought-tolerant accessions based on leaf yield, root traits and drought tolerance indices. L3 and L5 accessions were discovered in cluster 1, representing genotypes with the highest drought tolerance. Cluster 2 also demonstrated a high level of susceptibility, with one accession L12 present. Cluster 3 had moderately tolerant genotypes with 5 genotypes in this cluster. Accession L7, L11, L15, L4 and L6 were observed in cluster 3. Cluster 4 contained the most accessions and was the most stress sensitive cluster with 10 accessions observed in this cluster. Accessions L10, L9, L2, L18, L13, L1, L16, L8, L14 and L17 were observed in this cluster (Table 5.5).

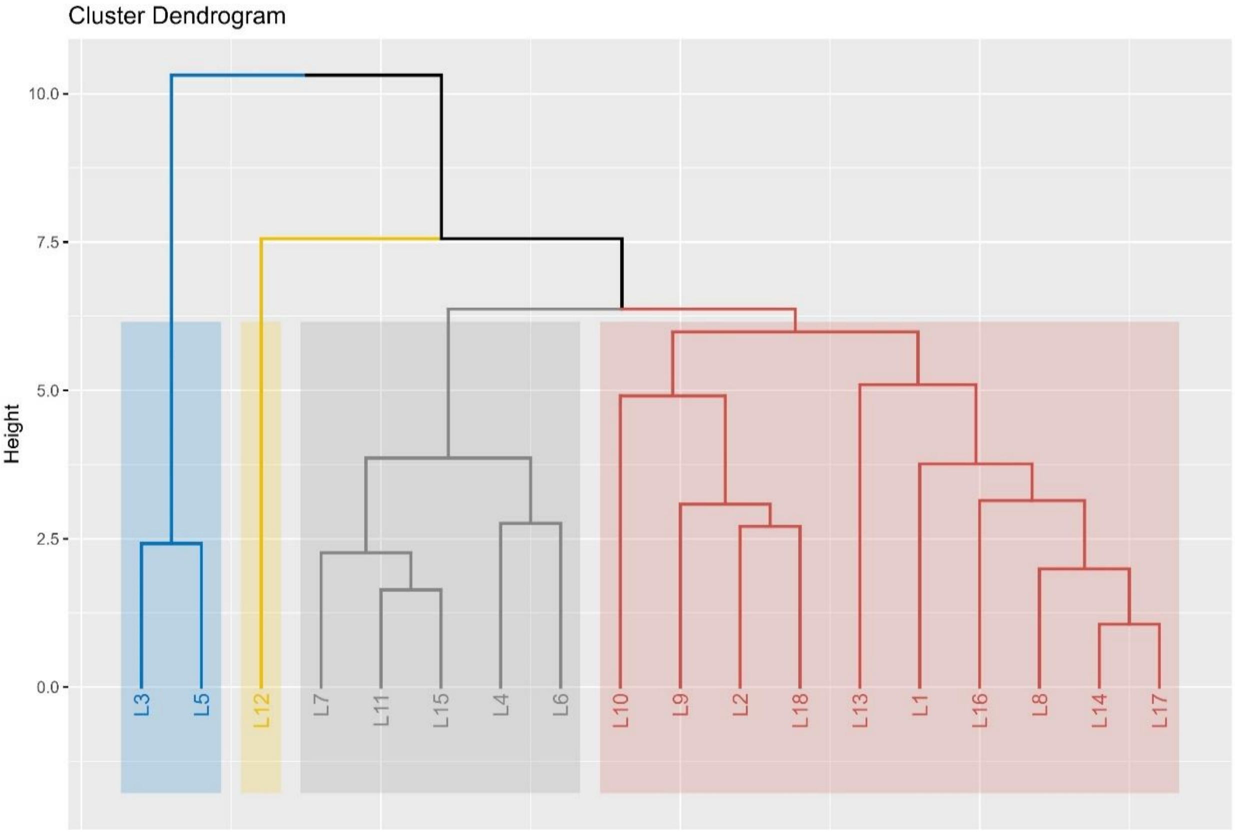


Figure 5. 4 Cluster dendrogram of 18 African Spider plant accessions. different colors represent different clusters with cluster 1-4 represented by blue, yellow, grey and red respectively

Figure 5. 5 18 African spider plant genotypes in different clusters.

Genotypes	**LY**	**ARD**	**TRA**	**TRV**	**RDW**	**RR**	**TRL**	**MP**	**TOL**	**GMP**	**STI**	**YI**	**SSI**	**cluster**
L1	47.24	1.18	1385.81	23.11	1.36	0.184	6.07	47.243	55.730	38.151	0.273	1.804	0.873	4
L2	23.69	1.03	1355.68	22.69	1.29	0.163	6.21	23.685	39.703	12.919	0.031	0.357	1.073	4
L3	80.70	1.45	1266.74	21.77	1.33	0.176	6.16	80.697	102.930	62.155	0.724	2.721	0.916	1
L4	58.72	1.02	1345.16	24.17	1.43	0.192	5.85	58.720	93.083	35.804	0.240	1.134	1.040	3
L5	73.54	1.49	1428.60	21.89	1.45	0.183	5.82	73.535	99.677	54.070	0.548	2.206	0.951	1
L6	56.40	1.09	1374.53	22.61	1.53	0.169	5.71	56.400	96.010	29.605	0.164	0.782	1.082	3
L7	49.70	1.10	1372.09	22.39	1.42	0.205	6.22	46.005	81.183	21.653	0.088	0.504	1.103	3
L8	47.62	1.14	1294.99	23.40	1.42	0.190	5.53	47.618	71.643	31.374	0.185	1.098	1.010	4
L9	29.22	1.32	1375.46	24.61	1.36	0.170	6.14	29.215	42.107	20.255	0.077	0.760	0.985	4
L10	25.19	1.41	1431.68	22.80	1.16	0.157	5.69	25.186	34.355	18.419	0.064	0.746	0.954	4
L11	41.29	1.16	1393.03	22.80	1.44	0.185	6.10	41.286	66.828	24.249	0.110	0.733	1.052	3
L12	22.76	1.07	2635.82	22.31	1.14	0.179	5.75	22.757	36.683	13.470	0.034	0.411	1.050	2
L13	32.54	1.57	1283.19	26.46	1.42	0.202	6.17	32.544	46.918	22.556	0.095	0.846	0.986	4
L14	40.32	1.19	1360.67	23.46	1.31	0.175	5.65	40.319	63.305	24.975	0.117	0.807	1.035	4
L15	37.43	1.14	1418.87	22.82	1.32	0.194	5.94	34.823	57.325	19.775	0.073	0.573	1.062	3
L16	33.00	1.24	1401.00	24.15	1.25	0.182	5.28	33.002	42.382	25.300	0.120	1.100	0.920	4
L17	41.73	1.22	1423.68	23.48	1.36	0.175	5.43	41.732	62.792	27.494	0.142	0.962	1.010	4
L18	19.16	0.99	1282.12	23.99	1.50	0.170	6.28	19.156	28.512	12.795	0.031	0.456	1.004	4

STI= Stress Tolerance Index GMP = Geometric Mean Productivity MP = Mean Productivity, TOL = Tolerance SSI= Stress Susceptibility Index YI =Yield Index TRL= Total root length, TRV= Total root volume, TRA= Total root area, RDW = Root dry weight , RR = Root to shoot ratio, ARD = Average root diameter.

5.6 Discussion

5.6.1 Yield performance, root traits and stress indices performance

This study examined the roots' response to drought stress and drought tolerance indices of African spider plant accessions to identify genotypes that are drought tolerant. The study found genetic variability for water stress tolerance in African spider plant accessions collections. Under stressed and non-stressed conditions, genotypic variability for yield performance was noted, which may be beneficial in breeding for improved African spider plant yield. Accessions L3 and L5 recorded the highest leaf yield under both stressed and non-stressed conditions and can be regarded as drought tolerant. According to Blum (2011), genotypes should be screened for drought based on their good performance in stressed and non-stressed conditions. Drought stress had a significant impact on root traits and leaf yield in the present study. This condition reduced root area, and root volume by

43.04%, and 32.97% respectively when compared to the non-stressed treatment. This suggested that drought stress improved root mortality, resulting in decreased root area and volume. (Asseng et al., 1998; Huang and Gao, 2000; Wang et al., 2009) all reported similar findings.

Under severe drought conditions, a decrease in root dry weight (RDW) was noted. This could have been caused by reduced water potential and, as a result, a decrease in net assimilation (Khalid., 2006). Crop yield declines more than root production during periods of extreme drought stress, and thus the root-to-shoot ratio increases (Polania et al., 2017). Increased root-to-shoot to shoot ratio during drought stress has also been proposed as a drought avoidance mechanism (Kmoch et al., 1957; Bonos et al., 2004b; Karcher et al., 2008).

The distribution of roots within a soil profile varies depending on crop species, soil type, moisture, soil fertility, and other environmental factors. While water stress caused a sharp increase in TRL in our study, no such change was observed under non-stress conditions. This means that , African spider plant spread their roots on the surface using less energy for water uptake in well-watered conditions (Merewitz et al., 2010). Increased TRL during drought conditions has been observed in other crop species and is therefore regarded as an essential coping mechanism for improving plant water uptake efficiency (Molyneux and Davies, 1983; Gallardo et al., 1996).

To identify useful stress tolerance indices for screening genotypes under drought stress conditions, leaf yield of accessions was estimated under both non-stress and stress conditions to calculate different drought tolerance indices (Table 5.4). In this study, accessions L3 and L5 recorded the highest Stress Tolerance Index (STI), Geometric Mean Productivity (GMP), Mean Productivity (MP), Tolerance (TOL) and Yield Index (YI) values; as a result, they could be the most productive accessions in both stressed and non-stressed conditions (Table 5.3). Stress Tolerance Index (STI) and Geometric Mean Productivity (GMP) have been suggested for screening accessions that produce high yield in stressed and non-stressed conditions (Bahrami et al., 2014). Similar findings were also observed by (Mashilo et al., 2017) in bottle gourd landraces. The greater the Stress Tolerance Index (STI) estimates for a specific genotype, the greater its water stress tolerance and yield potential (Kumar et al., 2014).

A genotype with a fairly high GMP value has a higher yield performance under both stressed and non-stressed conditions (Fernandez, 1992). Selecting higher MP values could also increase crop yields in both stressed and non-stressed conditions. Therefore, screening African Spider plant accessions based on high Stress Tolerance Index (STI), Geometric Mean Productivity (GMP), Mean Productivity (MP), Tolerance (TOL) and Yield Index (YI) will lead to drought stress resistance and leaf yield improvement because they have been used effectively for drought tolerance selection in different crops species (Ganjeali et al., 2011; Naderi and Emam, 2014; Belko et al., 2014). The accessions L6 and L7 had the highest SSI values, indicating that these lines are more susceptible to drought conditions. According to (Bruckner and Frohberg, 1987; Solomon and Labuschagne, 2003; Subhani et al., 2015) genotypes with a high SSI value are more sensitive to stress, whereas genotypes with a low SSI value are more stable.

5.6.2 Correlation analysis, principal component analysis and cluster analysis

The correlation coefficients between Y_{ds}yield for stressed, Y_{nS}, and other statistical stress tolerance indices were determined to identify the most desirable drought resistant criteria. in other terms, a Pearson's correlation of yield and stress tolerance indices can serve as a useful criterion for identifying the best genotypes and indices to use (figure 5.2 a-b). A desirable index should have a significant correlation with yield in both stressed and non-stressed conditions (Mitra, 2001). Under both stressed and non-stressed conditions Y_{ns} was positively and strongly correlated with yield index YI, Stress Tolerance Index (STI), Geometric Mean Productivity (GMP), Mean Productivity (MP) and Tolerance (TOL). Similarly Y_{ds} was also positively and strongly correlated with yield index YI , Stress Tolerance Index (STI), Geometric Mean Productivity (GMP) , Mean Productivity (MP) and Tolerance (TOL) suggesting that these characteristics were more efficient in identifying high-yielding accessions under varying water conditions. However, under stressed conditions, there was a significant and negative correlation between SSI and yield Y_{ds}. The reported relationships were consistent with those reported in maize by (NAGHAVI et al., 2013). This suggests that SSI is not a reliable index and therefore cannot be utilized in selecting African Spider plant accessions. The association of root to shoot ratio with both yield under stressed and non-stressed conditions implies that this trait is a reliable marker and can be used to select for leaf yield under drought stress conditions.

One of the most effective multivariate techniques for screening suitable genotypes is principal component analysis (Drikvand et al., 2012). Following the distribution of studied genotypes on the biplot of PCA and cluster analysis, genotypes with superior root traits, and higher drought tolerance indices were identified. Accessions associated with PC1 values in this study were L3 and L5. These accessions were also the most drought tolerant genotypes.

Conclusion

This study found genetic variability for drought tolerance among African Spider plant accessions. Landraces such as L3 and L5 have been identified as useful for African Spider plant drought tolerance breeding and development programs. The absence of association between most root traits and leaf yield suggests that while root traits may aid in the extraction of water from deeper soil strata to sustain plant growth in water-stressed environments, they may not necessarily contribute to higher leaf yield. Root to shoot ratio was discovered to be an important root trait that can aid in the selection of drought tolerant genotypes. Furthermore, water stress yield indices such as TOL, GMP, STI, MP, and YI, allowed for the discrimination of drought-tolerant African Spider plant accessions.

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Chapter 6. Overview and implications of study findings

Summary

Eighteen African spider plant accessions were evaluated in the greenhouse in 2020 and 2021. Thirteen morphological, physiological, and biochemical character traits were measured under three different water regimes. Proline content rose exponentially under stress, but it was only weakly correlated with morpho-physiological and biochemical traits under both optimum and water-stressed conditions. Several morphological and physiological parameters, including days to 50% flowering, leaf length, net photosynthesis, and number of leaves per plant were correlated with leaf yield under stressed conditions. Two genotypes, L3 and L5, with high leaf yield and favourable adaptive traits were chosen for breeding as potential parents.

Estimates of heritability were found to be extremely high for relative water content, number of leaves per plant, and leaf yield under all three water regimes. High heritability estimates combined with a high genetic advance were noted for number of leaves and Plant height under stress conditions. Number of leaves per plant had a strong to moderate positive correlation with leaf yield under all three water regimes. Number of leaves per plant, Plant height, leaf width, and stem diameter could be used as target traits to improve Spider plant leaf yield under drought-stressed conditions.

Drought stress had a significant effect on all the root traits in the study. Drought stress increased total root length and root-to-shoot ratio but decreased most of the root traits. Yields for stressed and non-stressed conditions were correlated with all drought tolerance indices except for the Stress susceptibility index. Accessions L3 and L5 were identified as drought-tolerant genotypes for breeding.

Implications of findings of this study for drought tolerance breeding and germplasm development.

The existence of genetic variability for target traits is critical to the success of any breeding program. The World Vegetable Centre is fully committed to pre-breeding programs that establish and distribute germplasm for worldwide evaluation and breeding. Such materials may contain a mix of genes that provide adaptability to the target water stress scenario. Under stress, the plant's capacity to retain cell stability as well as extract and save water is dependent on compatible osmolytes such as proline. As a result, screening germplasm for morpho-physiological and biochemical markers assists in selecting candidate genotypes for stress tolerance breeding. The observed strong association between several morphological and physiological parameters, including days to 50% flowering, leaf length weight, net photosynthesis, and plant number with leaf yield under stressed conditions provides some evidence that these traits are useful and can be used to effectively select drought-tolerant genotypes. The accessions evaluated in this study revealed adequate phenotypic variability that breeders could use to improve drought tolerance when developing African spider plant varieties. African spider plant accessions L3 and L5, which have high leaf yield under drought stress and desirable adaptive traits, are important genetic stocks for drought-tolerance breeding.

The reproducibility or heritability of key traits in target environments determines the effectiveness of breeding materials. This reveals on the ability to pass on such traits to future generations through breeding. This also demonstrates the importance of such germplasm samples in locating genes for specific traits. High heritability estimates combined with a high genetic advance were noted for number of leaves and Plant height. Based on the variance components, correlation and path coefficient analysis, Number of leaves per plant, Plant height, leaf width and stem diameter could be used as target traits to improve Spider plant leaf yield under drought-stressed conditions. Molecular techniques can be used to track the genetic components of these traits.

Root traits are important in soil exploration and nutrient and water absorption. Investigating the genetic diversity of root traits could help African Spider Flower breeding programs develop varieties with desirable root components for drought tolerance and target environment. The study showed that yields for stressed and non-stressed conditions were strongly associated with all drought tolerance indices except for the Stress susceptibility index. This implies that these indices are efficient and can be utilized in selecting drought-tolerant genotypes. Furthermore, accessions L3 and L5 have been identified as drought-tolerant genotypes which can be used as parents in breeding programs.

Recommendations

In conclusion, future research should concentrate on i) Field experiments to validate this greenhouse study; ii) Include more genotypes in order to capture a wider genetic base: iii) Molecular studies to validate these studies and identify genes that confer drought tolerance: and, iv) more indices and more root traits to be used in future studies to identify more traits that are useful in screening African Spider plant genotypes for drought tolerance.