DROUGHT TOLERANCE IN MALAWIAN SOYBEAN (GLYCINE *MAX* L.) GERMPLASM

By

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Declaration

“I, Margaret Kondowe Chiipanthenga, declare that the thesis that I herewith submit for the Doctoral Degree Philosophiae Doctor in Plant Breeding at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education”.

17 January 2020

Margaret Kondowe Chiipanthenga Date

Dedication

To my daughter Faith and my son Peter Junior; you are every reason I go on.

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List of abbreviations and SI units

ABA	Abscisic acid
AEC	Average environment axis
AICC	African Institute of Corporate Citizenship
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of variance
BARS	Bvumbwe
Ca	Calcium
cm	Centimeter(s)
CAB	Centre for Agriculture and Bioscience International
CV	Coefficient of variation
R^2	Coefficient of determination
ETc	Crop water consumptive use
°C	Degrees Celsius
DARS	Department of Agricultural Research Services
DI	Drought resistance index
FAO(STAT)	Food and Agriculture Organization (Statistics)
GCA	General combining ability
GEI	Genotype by environment interaction
GGE	Genotype main effects and genotype by environment interaction
GMP	Geometric mean productivity
g	Gram(s)
HM	Harmonic mean
IITA	International Institute for Tropical Agriculture
KAS	Kasinthula
kg ha^{-1}	Kilogram per hectare
kg ha^{-1} m^{-3}	Kilogram per hectare per cubic meters
LSD	Least significant difference
L	Litre(s)
L/s	Litres per second
Mg	Magnesium
MAD	Maximum allowable depletion
MAS	Masenjere
MP	Mean productivity
MT	Metric tons
MMT	Million metric tons

nm	Nanometers
Non-WL	Non-water-limited stress
$\bar{R}$	Mean rank
NPK	Nitrogen, potassium and phosphorus
ΦNO	Non-photochemical quenching basal dissipation of light energy
%	Percentage
PSII	Photosystem II photochemistry
ΦNPQ	Photoprotective non-photochemical quenching
Yp	Potential yield
PC	Principal Component
PCA	Principal Component analysis
QTL	Quantitative trait loci
ΦII	Quantum yield of efficiency for photosystem II photochemistry
RCBD	Randomised complete block design
SPAD	Relative chlorophyll content
R	Regression coefficient
R1-R8	Reproductive growth stages
SCA	Specific combining ability
SE	Standard error
SADC	Southern Africa Developing Countries
SSA	Sub-Sahara Africa
SSI	Stress susceptibility index
TOL	Stress tolerance
STI	Stress tolerance Index
t ha^{-1}	Tons per hectare
T	Total irrigation
USDA	United States Department of Agriculture
WLS	Water-limited stress
WUE	Water use efficiency
YI	Yield index
Yp	Yield under non-water-limited stress
YR	Yield reduction ratio
Ys	Yield under water-limited stress
YSI	Yield stability index
Zn	Zinc

ABSTRACT

Water-limited stress (WLS) is associated with adverse changes at morphological, physiological, biochemical and molecular levels among genotypes, which consequently affects crop growth and productivity. These changes are useful indicators in breeding of drought tolerant genotypes. This study was, therefore, carried out to identify genotypes that are good performing under WLS conditions and to determine traits' response to WLS using a combination of morphological traits, physiological traits, water use efficiency and grain yield. In addition, interrelationships among morphological traits, physiological traits and water use efficiency were determined in order to identify traits that contribute to grain yield under WLS conditions. The study also elucidated the association between drought tolerance indices and grain yield under WLS conditions in separating tolerant genotypes from sensitive genotypes. It further looked at the impact of drought on grain yield of soybean with change in environment and season. The study also tried to understand the mode of gene action considering that the majority of the characteristics of importance in a crop are inherited quantitatively.

Genotypes showed significant variability in tolerance levels to WLS. Genotypes with a high drought tolerance level generally exhibited a higher grain yield, 100-seed weight, plant height, number of pods per plant, minimal grain yield reduction, maintained a higher relative chlorophyll content, quantum yield of efficiency for photosystem II and water use efficiency under severe WLS compared to genotypes with a low drought tolerance level. Among the physiological traits, relative chlorophyll content was most significantly associated with genotype, while quantum yield of efficiency for photosystem II, photoprotective non-photochemical quenching and non-photochemical quenching basal dissipation of light energy for other unregulated process were more frequently and significantly associated with WLS. Plant height, number of nodes per plant, 100-seed weight, water use efficiency and relative chlorophyll content were less affected by change in WLS levels.

Results showed that selection criteria would differ across different WLS regimes. Morphological traits 100-seed weight, number of pods per plant, and bomass per plant significant directly contributed to grain yield under non-WL and moderate (50%) WLS. Water use efficiency showed the highest direct contribution to the variation in grain yield across WLS regimes of all the traits. Both significant positive and negative correlations were observed between morphological and physiological traits. The physiological trait relative chlorophyll content was strongly positively associated with morphological traits and contributed directly to grain yield variation under WLS. This showed that chlorophyll content can be used as physiological marker for identifying drought tolerant genotypes under WLS conditions. It was also observed that the tolerance indices mean productivity, geometric mean productivity, harmonic mean, drought resistance index and yield index correlated positively with both grain yield under non-WL (Yp) and WLS (Ys) conditions. This proved that these indices can be useful in screening for WLS tolerance in soybean.

The WLS negatively impacted on grain yield of soybean with change in environment and season but the extent varied from one growth stage to the other. Grain yield was most sensitive to environment, followed by environment by season interaction effects. Effects of WLS were severe at flowering, indicating that the most critical growth stage to soil WLS is between flowering and pod-filling stages when plants partition assimilates for seed formation. Results further displayed the role of both additive and dominance gene effects in expression of tolerance to drought in soybean using grain yield and yield components such as number of pods per plant, number of seeds per plant per plant, and 100-seed weight. However, non-additive gene effects were more important for WLS for the studied traits than additive gene effects in a current study.

Keywords: Soybean, morphological traits, physiological traits, grain yield, water-limited stress

CHAPTER 1

General introduction

Soybean (*Glycine max* L.) is referred to as a double duty crop (Goldschein 2011) furnishing nutrition to both humans and soil (Kananji et al. 2013). It is one of the major cash crops for more than 700 million smallholders in the developing countries and it contributes 83.8% of an economic value from the export in the developing countries (Daryanto et al. 2015). In Malawi, soybean consumption can combat severe nutritional deficiency as the seed contains protein, carbohydrates, oil, dietary fiber, vitamins and minerals. Soybean is also believed to improve maize yields by 10 to 20% when rotated with maize, since it has the ability to fix nitrogen in the soil (TechnoServe 2011).

Drought is a dry weather condition, characterised by a shortage of water supply to plants for an extended period (Rukundo et al. 2013, 2014). Water plays a crucial role in the life of a plant since it is the main constituent of plant tissues. Water contributes, in mass, 80 to 95% in growing tissues, 85 to 95% in vegetative tissues, 35 to 75% in wood with dead cells and 5 to 15% in dried seeds (Taiz and Zeiger 2006). Shortage of water supply to plants for an extended period adversely affects plant growth and its productivity (Rukundo et al. 2014). Drought stress affects both vegetative growth and grain yield of soybean (Mwenye 2018) and can decrease grain yield with about 43 to 44% (Kobraei et al. 2011). Lei et al. (2006) have observed a rapid drop in leaf water potential and the net photosynthesis ratio when soil water content dropped below 47% of field water capacity.

Drought is a general problem across all countries of sub-Sahara Africa (SSA) (Couttenier and Soubeyran 2013; Cervigni and Morris 2016). Literature has shown that soybean is affected by drought stress since it has a relatively high water requirement and it is sensitive to water deficiency (Hossaina et al. 2014). This has led to the identification of drought tolerant sources among global soybean germplasm and in SSA (Pathan et al. 2010; Fenta et al. 2012; Mabulwana 2013). Despite the availability of tolerant genotypes worldwide and knowledge on the occurrence of both intermittent and terminal droughts, all farmers in Malawi still grow soybean genotypes with no prior knowledge of their level of tolerance to drought.

Malawi occupies 12.3 million hectares of land. It is located in the south-east of Africa (FAO 2015), between latitudes 9° and 17° south of the Equator, and longitudes 33° and 36° east. It shares boundaries with Mozambique to the east, south and south-west, Zambia to the west, and Tanzania to the north (Yaron et al. 2011). Malawi has an agro-based economy of which the agriculture sector contributes 28.1% to the gross domestic product (Gondwe and Chagunda 2017). The main cash crops are sugarcane, cotton and tea. Maize is the main food crop and occupies 70% of cultivated land (FAO 2015). Over 86% of Malawians living in rural areas depend on rain-fed agriculture (Gondwe and Chagunda 2017) and relying on a single maize harvest for their livelihoods. This makes them more vulnerable to climate-related natural disasters, such as floods and droughts, which directly affect agricultural productivity (FAO 2015).

Soybean is being advocated as alternative food and cash crop for smallholder farmers and farmers have started growing the crop on a large scale (Sopo and Mulekano 2014). Soybean has a ready domestic and export market that is not fully exploited in Malawi; hence, it is a source of income. The crop, therefore, has the ability to reduce poverty in Malawi which is widespread and deep, with 70.9% of the population living below the poverty line (Gondwe and Chagunda 2017). However, the recommended genotypes currently used by Malawian farmers were all selected for yield, disease resistance and pod shattering resistance, among other factors, and no genotypes have been specifically recommended for drought tolerance in Malawi despite recurrent drought effects and irregular distribution of rainfall (Sopo and Mulekano 2014; Akaogu et al. 2017). Use of drought tolerant varieties gives an opportunity to farmers to have their crop escape drought effects by maturing early as well as displaying mechanism to tolerate drought with minimum economic losses (Kivuva 2013; Fuganti-Pagliarini et al. 2017). As such use of drought tolerant varieties is an ideal option to deal with drought especially under smallholder farmers' conditions as they may not need to supplement their fields with irrigation in times of drought which may be costly. Therefore, to contribute to improvement of income, food and nutritional security levels, development of appropriate soybean genotypes with agronomic and key traits that are preferred by farmers is regarded critical.

1.1 Aim and objectives

This study aimed at identifying genotypes that are tolerant to drought, and determining the role that drought plays in the interaction of genotype and environment overs years in soybean production. The specific objectives were to:

i. Evaluate soybean genotypes for morphological traits, physiological traits and grain yield under different (Water-limited stress) WLS levels;
ii. Determine interrelationships among morphological and physiological traits under WLS conditions;

iii. Evaluate the ability of several drought tolerance indices in identifying drought tolerant genotypes under different WLS conditions;
iv. Determine the effect of drought on grain yield at two growth stages of soybean genotypes evaluated across environments and seasons;
v. Determining combining ability and mode of gene action in soybean for drought tolerance related characteristics

1.2 References

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CHAPTER 2

Drought stress and its implications for soybean crop improvement

2.1 Soybean

2.1.1 Origin of soybean

Soybean (*Glycine max* L.) is a leguminous vegetable of the Fabaceae family (Singh 2017) and sub-family Faboideae (Moghadam and Alaei 2014). The crop was originally domesticated from wild soybean (*Glycine soja Sieb*. and *Zucc*.) in the eastern half of north China (CAB International 2010; Guo et al. 2010; Tian et al. 2010). Its production was initially localised in China and then spread throughout east and south-east Asia as source of food, animal feed and medicine after the Chinese-Japanese war (Mwenye 2018b). The soybean crop is believed to have been introduced to Africa in the 19th century by Chinese traders and missionaries along the east coast of Africa (Khojely et al. 2018). Soybean is currently grown throughout the world specifically in tropical, sub-tropical and temperate climates (IITA 2009; Kolapo 2011).

2.1.2 Botany of soybean

Cultivated soybean, just like wild soybean belongs to sub-genus Soja (Tian et al. 2010) within the genus Glycine of the family Leguminosae, which includes alfalfa (*Medicago sativa*), pea (*Pisum sativum*), common bean (*Phaseolus vulgaris*), peanut (*Arachis hypogaea*) and lentil (*Lens culinaris*) (Kim et al. 2010). Soybean has 20 chromosomes ($2n = 40$) (Walling et al. 2006; Gill et al. 2009; Singh 2017; Mwenye 2018b), hybridises easily, exhibits normal meiotic chromosome pairing and generates viable fertile hybrids (Kim et al. 2010). It has a genome size of 1.1 to 1.15 Gb (Walling et al. 2006; Cannon and Shoemaker 2012). The soybean genome structure is the product of a diploid ancestor ($n = 11$), which is believed to have undergone aneuploid loss ($n = 10$), polyploidisation ($2n = 20$) (CAB International 2010) and diploidisation ($n = 20$) (Shultz et al. 2006; Walling et al. 2006).

Soybean is grown from seed (Casteel 2010; Kim et al. 2010) that grows rapidly upward soon after emergence under optimum conditions. The leaves are categorised into unifoliolates, which are the first two-blade leaves that develop, trifoliolates, which consist of three leaflets and compound leaves, which are the remaining leaves (K-State Research and Extension 2016). The flowers are small, either white, pink or purple in colour and resembles the flowers of pea or clover (Casteel 2010). The root system of soybean is fibrous (Mwenye 2018b). The young fibrous roots tend to develop root nodules (Kananji et al. 2013) with the ability to fix nitrogen in the soil (Chaudhary et al. 2015). Soybean sets most of its pods within three weeks after first flower set with three to four seeds per pod (Naeve 2011). The newly formed seeds contain about 90% moisture, which decreases as the seed matures and the moisture content of a fully mature seed is 45 to 55% (Purcell et al. 2014).

2.1.3 Flowering in soybean

Soybean is a self-pollinating crop (Kiryowa et al. 2008; Fasahat et al. 2016). Its reproductive stage starts after six to 10 trifoliate leaves have been produced (Casteel 2010). Soybean growth type can be categorised into two main types namely indeterminate and determinate, based on its flower development (Mwenye 2018b). Indeterminate plants continue growing upward from the tip of the stem for several weeks after flowering has begun lower on the stem. Upper nodes will not flower until later. Flowers occur in axillary racemes (Endres and Kandel 2015). In contrast, determinate plants complete their growth in height and then produce all the flowers at more or less the same time. They do not initiate new leaves after flowering has begun. Flowers occur in both axillary and terminal racemes (Tian et al. 2010).

A major factor that controls flowering in soybean is photoperiod (Kumawat et al 2016). Soybean is a short-day plant (De Avila et al. 2013) but its response to day length varies with genotype and temperature (Endres and Kandel 2015; Khojely et al. 2018). Day length has an influence on the rate of development of the crop. Increased day length results in the delay of flowering and taller plants with more nodes. Short days hasten flowering, particularly for late-maturing genotypes. Depending on the genotype, some genotypes flower earlier when the days are shorter while others flower later when the days are longer (Kumawat et al. 2016).

2.1.4 Growth stages of soybean

Soybean development goes through two main stages, namely vegetative [VE-V(n)] and reproductive growth stages (R1-R8) (Table 2.1). These stages are determined through classifying leaf, flower, pod and seed development (Endres and Kandel 2015). The vegetative growth stage covers development from emergence up to flowering (K-State Research and Extension 2016). The reproductive stage commences at flowering (R1) and ends at full maturity when 95% of the pods have reached their mature pod colour (R8) (Casteel 2010; Naeve 2011).

At R2, flowers are found at most nodes throughout the plant and drought stress at this stage will result in flower abortion instead of developing viable pods. Drought stress can limit pod number per plant, number of seeds per plant per pod as well as seed size when the crop is exposed to drought stress at R3 (Endres and Kandel 2015; K-State Research and Extension 2016). The R4 stage is the most crucial for grain yield because at this stage the plant reaches the full pod stage where pod growth is rapid and seed development begins (Endres and

Kandel 2015). The pods are filled at a maximum rate and senescence is about to begin, making the plant most susceptible to drought stress (Purcell et al. 2014). When drought occurs at seed-filling (R5), the plant hardly compensates for the effect of stress because at this stage the plant requires water for nutrient redistribution and dry weight accumulation (Endres and Kandel 2015). Plant senescence and leaf loss become rapid while seed growth rapidly slows down when drought stress occurs at the peak of total pod weight (R6). Drought stress has little effect when it occurs at vegetative, R1, R7 and R8 stages (Mwenye 2018b).

Table 2.1: Growth and developmental stages of soybean

Growth stage	Description
VE	From emergence to appearance of cotyledons above the soil surface
VC	From cotyledon to full spread of unifoliolate leaves where leaf edges are not touching
V1	From first node development to fully developed leaves at the unifoliolate node
V(n)	Plant with sufficient number of nodes on the main stem with fully developed leaves
R1	When flowering begins and one flower opens at any node on the main stem
R2	Full flowering where a fully developed flower opens at one of the two uppermost nodes on the main stem
R3	Beginning of pod development
R4	Full pod where a fully developed pod appears at one of the four uppermost nodes on the main stem with a fully developed leaf
R5	Beginning seed where seed development commences in a pod at one of the four uppermost nodes on the main stem with a fully developed leaf
R6	Full seed where a pod contains a green seed that fills the pod cavity at one of the four uppermost nodes on the main stem with a fully developed leaf
R7	Beginning maturity when one normal pod on the main stem has reached its mature pod colour
R8	Full maturity where 95% of the pods have reached their mature pod colour

Sources: Kobraei et al. (2011); Naeve (2011); K-State Research and Extension (2016)

2.1.5 Growth requirements for soybean

Soybean is a hardy plant and is, therefore, well-adapted to a wide range of soil types and soil conditions (Makbul et al. 2011). For optimum yield it requires a loose, well-drained loam soil (Kananji et al. 2013). Soybean is relatively tolerant to both low and high temperatures because it is a hot weather crop (CAB International 2010) that is suitable for year-round growth in most tropical regions. However, its growth rate may decrease with temperatures above 35°C and below 18°C (Thuzar et al. 2010; FAO 2015). The crop requires a soil temperature of around 15°C to germinate (K-State Research and Extension 2016) and warmer weather to mature. In cooler growing regions, the rate of development slows down (De Avila et al. 2013). However, soybean plants can withstand temperatures as low as -2.8°C for a short period of time.

Soybeans can tolerate a wide range of soil pH if they have adequate nutrients but do best in slightly acid soil with pH of 6.0 to 7.0. Soybean is moderately tolerant to soil salinity. Yield decreases, due to soil salinity, are in the range of 0% at ECe 5 mmhos/cm, 10% at 5.5, 25% at 6.2, 50% at 7.5 and 100% at ECe 10 mmhos/cm (FAO 2015).

For maximum soybean production, water requirements vary between 450 and 700 mm per season (Hossaina et al. 2014) depending on climate and length of the growing period (FAO 2015; K-State Research and Extension 2016). Soybean requires adequate water of 15 to 50% soil water depletion for germination (FAO 2015) in order to obtain its yield potential. Water deficiency or excess water during the vegetative period will retard growth (Mwenye 2018b). Soybean is most sensitive to water-limited stress (WLS) during flowering and pod development stages (Kobraei et al. 2011). Its sensitivity is critical at the later part of flowering and early part of the yield formation stage (Purcell et al. 2014; FAO 2015). Water deficits at flowering and pod development stages may cause excess flower and pod abortion (Endres and Kandel 2015; FAO 2015). Even though its daily water use depends on stage of growth and weather conditions, the typical peak rate of soybean plant is 8 mm per day (De Avila et al. 2013). This mostly occurs at the onset of the pod-filling stage (K-State Research and Extension 2016).

2.1.6 Importance of soybean

Soybean is one of the most economically important crops across the world (Tian et al. 2010; TechnoServe 2011; Weber et al. 2014). Worldwide soybean is mainly used as a source of foreign exchange since it is the number one export crop in terms of whole soybeans, soybean meal and soybean oil. Soybean provides complete protein (Moghadam and Alaei 2014), vitamin C, thiamine, lipid, fatty acids and calcium for humans and animals in comparison with other major crops of the world (Guo et al. 2010; Zilic et al. 2011). The soybean seed contains about 40% protein (Chaudhary et al. 2015), 20% oil (CAB International 2010; Kole et al. 2015), 35% carbohydrates and 5% ash (Rajcan et al. 2005). Soybean as source of protein for human food can be used to balance the nutrient deficiencies of other grains such as maize and wheat, which are low in the important amino acids, lysine and tryptophan (Gibson and Garren 2005). Soybean has shown to have health benefits such as protection against bowel and kidney disease (Zilic et al. 2011), type 2 diabetes (Miraghajani et al. 2012), lowering of plasma cholesterol and prevention of cancer (Applegate et al. 2018). Oil can also be pressed from soybeans (Moghadam and Alaei 2014) and made into shortening, margarine, cooking oil and salad dressings. Soybean oil is further used in industrial paint, varnishes, caulking compounds, linoleum, printing inks and other products (CAB International 2010). In Africa, soybean has gained importance due to the increased demand of soybean cooking oil, soy-fortified food and animal feed (Fenta et al. 2012; Rurangwaa et al. 2018).

2.1.7 Soybean production

Soybean is referred to as a crop of both the developed and developing world with half of its total production coming from the developing world (FAO 2015). A total land of 120 million hectares globally is under soybean production (Table 2.2). The three major soybean producing countries are the USA, Brazil and Argentina and they occupy 27.89%, 28.25% and 15.29% respectively of the total world soybean growing area (Manavalan et al. 2009; USDA 2017) (Table 2.2).

In Sub-Sahara Africa (SSA), soybean covers around 1.5 million hectares with an annual estimated production of 2.3 million tons (Khojely et al. 2018). However, the southern Africa region produces <1% of global soybean output, which has turned the region into a significant net importer of soybean products, importing 55% of its total demand (TechnoServe 2011). One of the reasons for low soybean production in SSA is that it is marked as one of the regions across the world that is severely affected by climate change (Couttenier and Soubeyran 2013). The region has been experiencing adverse weather conditions including erratic rain fall, intermittent dry spells and extreme heat (Serdeczny et al. 2017). Farming systems across countries and the food supply of more than 300 million people in southern Africa are affected by these adverse weather conditions (Cervigni and Morris 2016). The sub-region of southern Africa has been particularly susceptible to drought and land degradation and it is expected that these problems will increase in future (Hoerling et al. 2006).

Malawi is one of the six major soybean producing countries in SSA besides South Africa, Nigeria, Uganda, Ghana, Zambia and Zimbabwe (Kolapo 2011; Table 2.2*)*. Since soybean is well-adapted for production across all agro-ecological zones in Malawi (Kananji et al. 2013), it is grown in all eight Agricultural Development Divisions (ADD) (Monyo 2013; Kananji et al. 2013; Nzima and Dzanja 2015). Across the country, the crop is grown by both commercial and smallholder farmers. However, soybean production in Malawi is severely constrained by biotic and abiotic factors (Kananji et al. 2013). This has led to low yields realised by farmers of around 0.9 t ha^{-1} which is 40% less than reported potential yields of 2.0 to 2.5 t ha^{-1} (Monyo 2013). Among the abiotic stresses, drought is one of the most critical factors in reducing yield (De Carvalho 2008; Evers 2010; Rukundo et al. 2013). Malawi has had years of droughts and computer models have projected that the west part of Malawi will be severely impacted by drought due to climate change by 2050 (Cervigni and Morris 2016). This, therefore, calls for an urgent need to identify strategies that could mitigate the impact of droughts.

Table 2.2: World soybean production in the 2016/2017 growing season

Country/ Region	Area (million hectares)	Yield (metric t ha^{-1})	Production (MMT)	Production change (2016 season MMT)	Source
World	120.00	2.92	351.32	-2.85	USDA (2017)
USA	33.47	3.49	116.92	3.52	USDA (2017)
Brazil	33.90	3.37	114.10	-6.10	USDA (2017)
Argentina	18.35	3.15	57.80	-0.80	USDA (2017)
South Africa	0.57	2.29	1.32	-0.07	USDA (2017)
Nigeria	0.70	0.96	0.68	0.00	USDA (2017)
Zambia	0.14	1.94	0.27	0.08	USDA (2017)
Uganda	0.05	0.6	0.03	0.00	USDA (2017)
Malawi	0.15	0.88	0.13	0.01	FAOSTAT (2016)

MMT = million metric tons

2.2 Drought stress

Drought can be categorised as meteorological, agricultural, hydrological and socioeconomic (Horion et al. 2012; Lweendo et al. 2017). The most important category to breeders is agricultural drought stress. Agricultural drought stress can be defined as a short-term dryness in the root zone, which occurs at a critical time during the growing season and that can result in severe crop yield reduction (Horion et al. 2012). The duration of drought is variable as it can last either for a short time without severe adverse physiological impact, or last throughout an entire growing season or even years, resulting in complete devastation of crops. The response to drought stress varies with genotype (Lewthwaite and Triggs 2012).

Drought is one of the most important adverse abiotic stress factors that affect plant growth and productivity (Xiao et al. 2008; Makbul et al. 2011; Fenta et al. 2012; Chowdhury et al. 2016). Drought stress is inevitable especially when the water that is available in the soil is reduced and the demand for water exceeds supply (Fandika et al. 2011). The supply of water is determined by the amount of water held in the soil to the depth of crop root system (Fandika et al. 2014). The demand for water is determined by plant transpiration rate or crop evapotranspiration (Harb et al. 2010; Fandika et al. 2011; FAO 2015). The rate of transpiration is influenced by solar radiation, ambient air temperature, relative humidity and wind at the single leaf level. Rim (2013) observed that both geographical and climatic factors such as air temperature, wind speed, relative humidity and solar radiation have significant effects on the occurrence of drought. DaMatta and Ramalho (2006) noted that water requirements depend on the retention properties of the soil, atmospheric humidity, cloud cover as well as cultivation practices.

Drought stress has been associated with other stresses such as salt, cold, heat, acidity and alkalinity (Manavalan et al. 2009; Boutraa et al. 2010). Drought stress tends to be accelerated in the presence of salt, acidity and alkalinity as they affect root growth and the absorption of water and nutrients (Joris et al. 2013). The interaction between drought and these other stresses therefore have an effect on plant growth, leaf water relations, membrane stability, photosynthetic activity, proline content, sugars as well as enzymatic activities which are physiological and biochemical characteristics (Liu et al. 2014; Abid et al. 2018). Joris et al. (2013) observed a negative influence on shoot biomass production, root length density and nutrient uptake when maize and soybean were exposed to WLS in acidic soils. The exposure of plants to drought, coupled with high salinity and low temperature leads to cellular dehydration (Van Oosten et al. 2016).

Drought stress can lead to a change in the physiological metabolism process (Wu et al. 2011). Peroxidase and phenoloxidase activities in plant leaves are negatively affected under drought conditions (Hossaina et al. 2014). Leaf water potential is one of the reliable parameters in quantifying plant water deficit response (Wu et al. 2011). Reports have shown that growth characteristics, relative water content (Fandika et al. 2014), photosynthetic pigments (Chowdhury et al. 2016), total soluble sugars, total carbohydrates, total free amino acids, enzyme activities (Hossaina et al. 2014) and minerals (NPK% and uptakes) (Wu et al. 2011) are negatively affected by water deficit conditions. Several studies in numerous crops have been conducted to identify drought tolerance (Pathan et al. 2010; Fenta et al. 2012; Mabulwana 2013), to understand drought coping mechanisms under different stress levels, and to identify morphological (Fenta et al. 2012; Joris et al. 2013), physiological (Fenta et al. 2012; Talebi et al. 2013; Chowdhury et al. 2016; Van Oosten et al. 2016), biochemical (Hossaina et al. 2014), molecular (Farooq et al. 2009; Fenta et al. 2011) and ecological (Lweendo et al. 2017) characteristics of the plant crop. Crop simulation models have also been developed to estimate and quantify the impact of drought stress on crop productivity (Horion et al. 2012).

2.3 Effect of drought on important plant traits

In nature, all plant life processes are affected by drought (Shashidhar et al. 2013). Some of the major plant traits that play a role in drought response include phenology (Manavalan et al.

2009), plant development and size (Clauw et al. 2016), plant root characteristics (Mwenye 2018b), plant surface (Denny 2007), non-senescence (Wehner et al. 2016), water use efficiency (WUE) (Fandika et al. 2011) and photosynthetic systems (Fandika et al. 2014).

2.3.1 Phenology

Botanical phenology can be defined as the study of timing on vegetative activities, flowering, fruiting and their relationship to environmental factors (Tuberosa 2012). Drought occurs at all phenological stages of plant growth (Farooq et al. 2009). A plant's response to drought may vary depending on the species, genotype, age, stage of development, length and severity of stress (Xiao et al. 2008; Mabulwana 2013). Drought stress occurring during flowering or early stages of pod development, will result in soybean yield loss (Kobraei et al. 2011; Ku et al. 2013; Moloi et al. 2016). A yield loss of up to 88% can occur when drought occurs between growth stages R2- R6 (Mwenye 2018b). One of the effective strategies for minimising yield loss due to drought is developing short-duration genotypes. Early maturity helps the crop to avoid the period of stress (Farooq et al. 2009; Anithakumari 2011).

2.3.2 Plant development and size

Drought stress at early leaf development impairs leaf and rosette areas (Clauw et al. 2016). Boutraa et al. (2010) observed plant growth reduction, which was reflected in plant height, leaf area and dry weight when wheat genotypes were subjected to drought stress. Clauw et al. (2016) reported a negative effect on cellular parameters defining leaf area, such as pavement cell area and pavement cell number of up to fourfold in *Arabidopsis thaliana* due to drought stress. Soybean genotypes have shown significant decline in leaf expansion rates, plant height (Hossaina et al. 2014), shoot size (Mwenye et al. 2018a) and plant biomass (Joris et al. 2013) when exposed to drought stress. This shows that both cell division and expansion are negatively affected by drought stress.

2.3.3 Plant root characteristics

Roots provide water and nutrients to the aboveground tissues as well as mechanical support (Mwenye 2018b). Roots are the only source to acquire water from soil, hence root growth, its density (Hossaina et al. 2014), proliferation and size (Farooq et al. 2009) are key primary responses of plants to drought stress. Drought affects root depth, root quality in terms of distribution and structure (Kumawat et al. 2016), which are the most efficient strategies for extracting water (Manavalan et al. 2009; Denny 2007). Drought stress also tends to decrease root development (Fried et al. 2019), root hair growth and xylem diameter, which all have an effect on water movement from soil to root (Wasson et al. 2012). Drought effects on roots in soybean have been reported by Farooq et al. (2009), Fenta et al. (2014), Mwenye (2018b) and Hossaina et al. (2014). The effect of drought on the root system has a direct impact on overall yield because selection for yield is directly linked to the root system for delivering high yield potential (Wasson et al. 2012; Fried et al. 2019).

2.3.4 Plant surface

Plants interact with the ambient environment through their surface such as stomata and cuticular properties (Denny 2007; Barthlott et al. 2017). The reflective properties of leaves and resistance to transpiration depend on plant surface. The stomatal activity (Hossaina et al. 2014) primarily determines the resistance of plant leaves to transpiration because crop water loss directly involves stomata. Stomatal density is greatly affected by drought, which consequently affects leaf gas exchanges (Barthlott et al. 2017). This has an impact on WUE as well as the photosynthesis process (Kim et al. 2007).

2.3.5 Non-senescence

Senescence can be defined as the inability of a plant to stay green under adverse growth conditions. Drought stress not only has an impact on physiological changes but also changes in gene expression. Drought stimulates both down and upward regulation of genes responsible for photosynthesis, chloroplast development and degradation respectively, which result in leaf senescence (Wehner et al. 2016). Le et al. (2012) observed that many soybean genes that are related to photosynthesis are down-regulated under drought stress. This makes soybean more prone to leaf senescence under drought stress. Genotypes that delay leaf senescence under drought stress have the capacity to maintain transpiration as well increase cumulative photosynthesis (Tuberosa 2012). Early leaf senescence has been reported in cereal crops as a result of drought stress (Gupta et al. 2011). Drought stress is one of the detrimental environmental factors since it induces premature leaf senescence, which in turn has an impact on biomass production and yield formation (Burke et al. 2010; Chen et al. 2015; Wehner et al. 2016).

2.3.6 Water use efficiency

Boutraa et al. (2010) defined water use efficiency (WUE) as the ratio of units of plant growth to units of evapotranspiration. It is determined through direct measurement of instantaneous gas exchange rates at the leaf level (Fandika et al 2014; Medranoa et al. 2015). Water use efficiency is an initial but most common measure of plant response to drought stress (Tuberosa 2012). Besides drought tolerance traits, a good genotype needs to have a high level of control of WUE (Fandika et al. 2011) while still maintaining the yield required (Van Oosten et al. 2016). Water use efficiency is a genetically linked trait and plant species show large variations for the trait (Farooq et al. 2009; Fandika et al. 2014). To improve WUE in crop plants the key processes would be increasing the uptake of available water (Harb et al. 2010), improving biomass production per unit transpired water and partitioning of produced biomass towards the harvested product (Farooq et al. 2009). However, drought stress affects WUE by increasing total seasonal transpiration and reducing crop harvest index (Manavalan et al. 2009; Fandika et al. 2014; Van Oosten et al. 2016).

2.3.7 Photosynthetic systems

The photosynthetic ability of the plant is highly sensitive to drought stress (Yooyongwech et al. 2013). Drought effects on photosynthesis are either reversible or irreversible (Feller and Vaseva 2014; Abid et al. 2018). In the reversible condition, photosynthetic processes fully recover from the drought stress effect after re-watering, while in the irreversible condition the photosynthetic processes do not recover even after re-watering (Abid et al. 2018). The irreversible effect could be due to membrane damage of affected tissues as a result of high production of reactive oxygen species (Pinheiro and Chaves 2011) as well as the inhibition of enzyme activity (Souza et al. 2013).

Inadequate water supply can inhibit canopy development and limit photosynthetic activity (Lewthwaite and Triggs 2012; Fandika et al. 2014). Drought stress affects photosynthesis rate by decreasing leaf expansion, impairing photosynthetic machinery (Denny 2007), pre-mature leaf senescence (Chowdhury et al. 2016), reduced stomatal conductance (Fandika et al. 2014) and lowered transportation of photosynthate (Hossaina et al. 2014). Photosynthesis rate reduction, as a result of drought stress, is also associated with reduction in food production processes such as reduction in protein concentration (Chen et al. 2015), decline in photosynthetic pigment concentration, reduced carboxylase activity, diminishing activities of Calvin cycle enzymes, and inhibition of the light reaction mechanism (Hossaina et al. 2014). Drought stress negatively affects enzymes involved in photosynthesis and consequently inhibits metabolism (González-Pérez 2015).

Chlorophyll fluorescence has efficiently been used as a tool to project the extent of damage drought causes in photosystems in various crops (Pinheiro and Chaves 2011; Ghassemi-Golezani and Lotfi 2012; Mwale et al. 2017). Narina et al. (2014) reported a higher photosynthetically active radiation (PAR) in tolerant genotypes compared to susceptible field bean genotypes under WLS. Drought stress has a negative impact on the amount of light that has been absorbed and used by photochemistry, known as quantum yield efficiency for photosystem II photochemistry (ΦII) (Narina et al. 2014). Drought tolerant genotypes of different crops have been shown to give higher yield with stable quantum yield potential compared to drought sensitive genotypes. The maximum quantum efficiency of PSII photochemistry (Φmaxp) is equally negatively affected by WLS. Low maximum quantum yield of PSII in the chloroplastic organelle is restricted (Yooyongwech et al. 2013) under WLS.

The relative greenness of the leaf (SPAD) has also been used to estimate chlorophyll (Fenta et al. 2014) and nitrogen content, which directly correlates to photosynthesis efficiency (Kuhlgert et al. 2016). Total chlorophyll content of soybean genotypes tends to decline when soybean leaves are exposed to drought stress (Hossaina et al. 2014; Chowdhury et al. 2016).

Other crops such as cowpea (Mwale et al. 2017), poplar (Silim et al. 2009), wheat (Abid et al. 2018) and Arabidopsis (Harb et al. 2010) have also experienced reduced photosynthesis (Farooq et al. 2009; Feller and Vaseva 2014) due to drought stress.

2.4 Ways of managing drought stress in crop plants

Drought effects can be managed by use of irrigation, adjustment of agronomic practices (Joris et al. 2013) and use of drought-resilient genotypes (Weber et al. 2014; Kole et al. 2015). Irrigation is one of the primary means of managing drought in crop production (Neumann 2008; Fandika et al. 2014; Cervigni and Morris 2016). The common practice is to use supplemental irrigation when rainfall is inadequate (K-State Research and Extension 2016). The irrigation water can be sourced from ground cover and water harvesting (Manivannan et al. 2017), which can be used when there is dry spell.

Adjustment of agronomic practices would include sowing time and soil management. An appropriate sowing time ensures that sensitive crop stages such as flowering and pod-filling occur at the time when drought is minimal (Farooq et al. 2009). Soil management practices, such as soil cover, can help to reduce water loss by evaporation and provides more available moisture in the surface layers (Joris et al. 2013). In addition, increasing lime rates, P fertiliser and planting in undisturbed soil columns, respectively can help to increase nutrient uptake and root length density by plants grown under WLS (Rurangwaa et al. 2018). Joris et al. (2013) also observed that applying lime in acidic soils under WLS resulted in greater uptake of N, P, K, Ca, Mg and Zn in maize and soybean.

However, the irrigation and adjustment of agronomic practices strategies tend to be costly for smallholder farmers as they require capital, intensive labour and special expertise which may be lacking in smallholder farmers. As such development and use of appropriate plant genotypes are the most effective strategies of managing drought in developing countries where majority of farmers are smallholders (Mwije et al. 2014; Cervigni and Morris 2016; Akaogu et al. 2017). Drought-resilient genotypes have mechanisms to tolerate drought stress, which could range from the ability to mature early, restrict stomatal opening and produce smaller leaves (Farooq et al. 2009). In addition, other measurements and traits such as leaf rolling (Denny 2007; Le et al. 2012), improved root characteristics (Wasson et al. 2012) and anatomical adjustments (Makbul et al. 2011) may further assist in genotype protection and adaptation to drought stress.

2.5 Mechanisms plants use to cope with drought stress effects

Shashidhar et al. (2013) defined drought resistance as the plant's ability to sustain the least injury to life functions at decreasing levels of tissue water status or turgor. Plants have developed defensive mechanisms to cope with drought (Neves-Borges et al. 2012). These mechanisms can be categorised into drought escape, dehydration avoidance and dehydration tolerance (Denny 2007; Xu et al. 2010; Kivuva 2013). Plants can adapt to drought stress and are able to produce optimal yields by using important drivers such as water uptake, WUE and harvest index (Farooq et al. 2009; Fuganti-Pagliarini et al. 2017).

A plant is said to have escaped drought when it has the ability to complete its lifecycle before the onset of severe drought (Harb et al. 2010; Abid et al. 2018). Flowering time is a major trait related to the drought escape mechanism. Plants escape drought by adjusting its phenology so that the critical developmental stages such as flowering and pod-filling escape the adverse impacts of drought through a short lifecycle (Anithakumari 2011). This drought escape mechanism works well when phenological development successfully coincides with periods of water availability. In addition, the genotype has to have a shorter growing season with terminal drought stress predominating (Farooq et al. 2009). Some plant species tend to shorten their lifecycles by entering the reproductive phase in times of soil water decrease, coupled with increased soil evaporation (Gupta et al. 2011). However, genotypes with shortened lifecycles, tend to lose more opportunity for partitioning of photosynthetic products (Fenta et al. 2014), resulting int a shorter reproductive phase and consequently lowering yield potential and protein content in soybean (Kole et al. 2015).

Dehydration avoidance is the mechanism where the plant maintains a high level of water status or turgor under conditions of increasing soil moisture deficit (Tuberosa 2012). One such dehydration avoidance measure is osmotic adjustment (Denny 2007) through a biochemical reaction (Hossaina et al. 2014). Osmotic adjustment plays a key role as a drought tolerance mechanism (Harb et al. 2010) by delaying dehydrative damage through continued maintenance of cell turgor and physiological processes (Abid et al. 2018). During osmotic adjustment there is an accumulation of compatible solutes (Chen et al. 2015) such as amino acids, glycine and betaine, sugars or sugar alcohols in the protoplasm (Xiao et al. 2008). The accumulation of these solutes facilitates the enlargement of the cell and plant growth by allowing the cell to decrease osmotic potential (Abid et al. 2018) and, thereby, increasing the gradient for water influx (Farooq et al. 2009; Kivuva 2013). This consequently allows stomata to remain partially open to avoid excess water loss (Hossaina et al. 2014), while carbon dioxide assimilation is limited (Yooyongwech et al. 2013) and thus plant turgor is maintained.

Maintenance of high turgor leads to higher photosynthetic rate and increased plant growth (Farooq et al. 2009). Dehydration avoidance is also possible when the plant has the ability to maximise water uptake (Tuberosa 2012). This is done through the development of deep roots (Kivuva 2013) with a high density, and also a highly prolific and thick size root system, which is able to extract ground water more efficiently (Denny 2007; Farooq et al. 2009; Talebi et al. 2013). Leaf area of single leaves also significantly affects the amount of water used by plants (Fandika et al. 2011; Hossaina et al. 2014). Drought tolerant genotypes tend to reduce water loss by reducing the leaf area (Fandika et al. 2014) through leaf shedding, leaf rolling (Denny 2007) and production of smaller leaves (Fenta et al. 2014).

In addition, cuticular properties such as wax load, pubescence and leaf colour also play role in drought tolerance (Tuberosa 2012). Plants tend to develop waxy bloom (Farooq et al. 2009) on leaves. This helps in maintenance of high tissue water potential as it protects plants from water loss (Harb et al. 2010). Drought induces increased wax deposition on the leaf surfaces of a plant, which in turn enhances drought tolerance (Fuganti-Pagliarini et al. 2017). Kim et al. (2007) reported an increase in wax deposition in soybean genotypes under drought stress. This phenomenon confirms the connection between drought tolerance and cuticle properties. Leaf pubescence is a xeromorphic trait (Denny 2007) that helps protect leaves from excessive heat load. Hairy leaves have reduced leaf temperatures and transpiration (Barthlott et al. 2017).

Maintenance of physiological processes under drought stress is another dehydration avoidance method. For instance, certain genotypes have the capacity to delay senescence or stay green under drought stress conditions. Such genotypes have high chlorophyll content and leaf reflectance (Manavalan et al. 2009). Chlorophyll pigment content is a major factor dictating the amount of energy emitted. Chlorophyll content is associated with the photosynthetic ability of the plant (Tuberosa 2012), which consequently determines the ability of the plant to stay green. Leaf light reflectance is associated with increased hairiness. The hairiness minimises water loss by increasing the boundary layer resistance to water vapour movement away from the leaf surface (Farooq et al. 2009), which consequently helps the plant to stay green. A positive correlation between grain yield and stay green at maturity under drought stress has been reported in sorghum (Burke et al. 2010).

Dehydration tolerance is the ability of the plant to grow, reproduce and even repair injury to a marked degree in spite of its exposure to drought stress; equal to that damaging a susceptible genotype (Tuberosa 2012). Although the plant is damaged, yield loss or lowering of quality are minimal. Denny (2007) described tolerance as offering protection from direct strain or damage. The plant tends to lose proteins, nucleic acids and cell membranes because of the accumulation of toxic ions in order to protect itself from direct damage (Abid et al. 2018). Protein breakdown is one of the important mechanisms for the adaptation of plants to drought stress (Tuberosa 2012). Plants tend to respond to drought stress by synthesis of protective proteins such as dehydrins and chaperones (Arumingtyas et al. 2013). The dehydrins have high flexibility, structural adaptability and extended conformational states, which contribute to continued plant desiccation stress tolerance (Feller and Vaseva 2014). Molecular chaperones participate in adenosine triphosphate-dependent protein unfolding or assembly/disassembly reactions and prevent protein denaturation during drought stress (Farooq et al. 2009).

Tolerance is often confused with a low level of resistance or moderate resistance. The plant may be regarded as susceptible based on the extent of damage. A combination of morphological, biochemical and physiological responses to drought helps to prevent membrane disintegration and provides tolerance against drought and cellular dehydration (Talebi et al. 2013). Soybean has shown a wide variation in drought tolerance mechanisms ranging from morphological in terms of root length density for nutrient and water uptake (Joris et al. 2013) to biochemical in terms of soluble sugars, protein, proline changes to cope with osmotic changes in their tissue (Chowdhury et al. 2016; Mwenye 2018b). The soybean has also shown variable drought tolerance physiologically by closing of stomata or leaf rolling to reduce transpiration rate and enhance photosynthetic capacity (Hossaina et al. 2014; Fuganti-Pagliarini et al. 2017).

2.6 Breeding approaches for drought tolerance

2.6.1 Field selection

Screening under natural drought stress conditions in the target environments is difficult because of the irregular and erratic drought response (Farooq et al. 2009) and also because water requirement is variable from year to year (Manavalan et al. 2009). Within a season drought can occur at different growth stages. Hence, a genotype which is successful in one year, might fail in another year (Makanginya 2012). Plant performance in terms of growth, development, biomass accumulation and yield under field conditions, therefore, depends on acclimation ability to environmental changes and stresses (DaMatta and Ramalho 2006). Unfortunately, a genotype may not have the ability to exercise specific tolerance mechanisms that involve a complex network of biochemical and molecular processes. Inconsistencies in the field may result in inconsistent selection pressure from one cycle to the next. As such, field trials need be large enough and replicated in order to eliminate micro-environmental errors (Kivuva 2013).

2.6.2 Selection under managed-stress environments

Different selection techniques to screen for drought stress have been developed under managed-stressed environments and include pot experiments, chamber house experiments, rainout shelters (Kundel et al. 2018) and rhizotron experiments (Neves-Borges et al. 2012; Kivuva 2013). Selection under controlled stress environments has been reported to be more manageable (Farooq et al. 2009) because water can be precisely controlled. In addition, selection under a controlled environment allows precise quantification of plant traits that influence drought adaptation (Manavalan et al. 2009). It also allows destructive harvests to quantify trait productivity (Kawaletz et al. 2014). However, selection under controlled environments has its own disadvantages, which can include labour intensiveness, a limiting number of treatments that can be planted due to limited floor space, and restriction of root growth due to pot size (Kawaletz et al. 2014; Mwenye 2018b). In addition, there are other challenges such as increased temperatures in periods of high ambient temperature (Kundel et al. 2018) and capital expenses (Jensen 2010) because special equipment to control the environment is required, as well as failure to reflect the reality in the field (Kivuva 2013; Kawaletz et al. 2014; Osmolovskaya et al. 2018).

2.6.3 Selection based on yield per se

The yield per se selection method conceptualises that a genotype, with minimal genotype by environment interaction (GEI) and a stable yield in its target environment and across environments, is desirable (Makanginya 2012; Fikere et al. 2014). However, increasing yield through selection for yield per se has been difficult to achieve since yield is quantitatively inherited, with low heritability due to small genotypic variance or large GEI variances (Makanginya 2012). The problem of large GEI variances in selection for yield per se can be minimised by considering genotypic adaptation across environments rather than selections from one environment (Makanginya 2012). There is also need to better understand the causes and effects of GEI in order to effectively select for yield per se. This can be well understood through the use of regression analysis methods (Karasu et al. 2009) and multivariate statistical methods such as additive main effect and multiplicative interaction (AMMI) analysis (Fikere et al. 2014) and genotype main effects and genotype by environment interaction (GGE) biplot analysis (Zhe et al. 2010; Crossa 2012; De Leon et al. 2016).

2.6.4 Selection based on developmental traits

Major developmental traits that can be used to select for drought tolerance include root size, root development and root-to-shoot ratios. Root characters such as biomass, length, density and depth are the main drought avoidance traits that contribute to final yield under drought stress environments (Farooq et al. 2009; Fenta et al. 2014). Regarding the root size, progressive water restriction was shown to significantly reduce root fresh and dry mass of plant species and susceptible genotypes respond even more sharply. Extensive root systems have proved to assist plants in maintaining higher water potential under drought stress conditions (Chaichi et al. 2019). The increase in root growth (development) is an indicator of the ability of a genotype to withstand WLS (Boutraa et al. 2010). Fried et al. (2019) reported variability in root development among soybean genotypes, citing that genotypes with increased root development under drought stress yielded more than those with reduced root development. In addition, drought tolerant soybean genotypes tend to partition shoot biomass to roots in order to tap water from deep horizons for their survival under severe drought stress environments, rendering larger root-to-shoot ratios (Hossaina et al. 2014; Mwenye et al. 2018a). This reduced shoot biomass, to compensate for root size (length) in drought tolerant genotypes under drought stress, has also been observed in wheat (Chaichi et al. 2019).

2.6.5 Selection based on assessment of plant water status and plant function

Selection based on assessment of plant water status involves assessing symptoms such as leaf rolling, leaf desiccation, leaf tip burning, canopy temperature and spectral reflection from leaves (Saglam et al. 2014; Blomstedt et al. 2018). Drought tolerant plants tend to keep a higher relative water content (Silim et al. 2009; Fandika et al. 2014) than drought sensitive plants (Boutraa et al. 2010). To assess the ability of water retention of excised leaves on soybean genotypes, Hossaina et al. (2014) observed that drought tolerant genotypes exhibited a significantly slower percentage weight loss reduction, percentage relative water content, osmotic potential and turgor leaf potential than susceptible genotypes. Selection based on plant function involves assessment of cell membrane stability and chlorophyll florescence. Water-limited stress negatively affects cellular membranes and organelles such as mitochondria and chloroplasts by causing cellular content leakage outside the cell (Boutraa et al. 2010). Although WLS affects most of the functions of plant growth, this effect depends on the level of water stress, the length of time to which the plant is subjected to water stress and the genotype (Hossaina et al. 2014; Fandika et al. 2014) of plant species.

Shoots have also been widely used in the selection of soybean genotypes to enhance drought tolerance (Fenta et al. 2011). Plant shoots have been used as the first step to understanding the genetic control of tolerance in order to enhance the efficiency of molecular breeding strategies. It also helps to improve the understanding of the complex network of drought tolerance-related traits thus improving selection efficiency (Mir et al. 2012). Just like dry matter yield per plant, photosynthetic capacity and WUE, shoots can easily be measured in soybean at different intervals during the growing cycle.

2.6.6 Selection based on indices

Indices, as selection criteria for screening and identifying high yielding drought tolerant genotypes, have successfully and extensively been used in several crops such as wheat (Abd El-Mohsen et al. 2015), sweetpotato (Kivuva 2013) and soybean (Bayoumi et al. 2008; Farshadfar et al. 2012; Kargar et al. 2014; Ali and El-Sadek 2016). They provide a measure of drought tolerance, based on yield loss under drought conditions in comparison to normal conditions. Principal component analysis (PCA) has been successfully used as a statistical technique to determine the effectiveness of several drought tolerance indices in discriminating between tolerant and susceptible genotypes in soybean (Kargar et al. 2014; Mwenye 2018b) and other crops such as sweetpotato (Makanginya 2012) and sunflower (Gholinezhad et al. 2014).

2.7 Breeding methods and efforts for tolerance in soybean

Genotype development in soybean tends to be difficult because traits are complex and quantitative in nature (Hu et al. 2011; Kumawat et al. 2016). However, efforts have been made to produce drought tolerant genotypes using knowledge of responses of plants to drought stress (Wu et al. 2011; Hossaina et al. 2014) and the coping mechanisms involved (Farooq et al. 2009; Kivuva 2013). The two most important breeding strategies in crop plants are conventional breeding and biotechnology (Kole et al. 2015). These strategies have been used to identify drought tolerant plants in many plant species through natural selection, conventional crosses, marker-assisted selection (Kumawat et al. 2016) using quantitative trait loci (QTL) (Hu et al. 2011; Chaudhary et al. 2015), and transgenic approaches (Yooyongwech et al. 2013).

2.7.1 Conventional breeding

Conventional breeding uses selection, hybridisation and polyploidy as main basic procedures to manipulate plant chromosome combinations. This method has helped a large number of genotypes to be successfully developed (Kole et al. 2015; Miladinović et al. 2015). While selection helps in identifying lines with desired traits, hybridisation brings together desired traits found in different plant genotypes into one plant genotype via cross-pollination. Polyploidy represents an increase in the chromosome number and genetic variability (Walling et al. 2006). With polyploidy, breeding takes advantage of an individual with more than two sets of homologous chromosomes or genomes, known as polyploidy (Gill et al. 2009). Changes in chromosome number may involve loss or gain of one or a few chromosomes or the whole genome (Shultz et al. 2006).

Conventional plant breeding procedures such as backcrossing, single pod descent, pedigree breeding and bulk population breeding are some of the more common procedures used to develop drought tolerant soybean genotypes (Miladinović et al. 2015). However, conventional breeding has been compounded by a number of factors such as annual breeding cycles, biological as well as genetic constraints, which all render the method insufficient (Hickey et al. 2017).

Induced mutation has also been used as one of the conventional breeding methods to induce genetic variability (Kole et al. 2015) instead of relying only on the introduction (Farooq et al. 2009) from the wild species' gene pool or from other genotypes (Van Oosten et al. 2016). Mutation breeding is one of the breeding tools that can assist crop improvement (Rajcan et al. 2005). Mutants have been used to validate the drought assay in abscisic acid (ABA) biosynthesis in Arabidopsis (Harb et al. 2010).

2.7.2 Use of biotechnology

Biotechnology can be defined as method that utilizes biological systems to develop products and forms the interface between biology and engineering (Sadiku et al. 2018). The application of biotechnology is a key to reducing time consuming and labour intensiveness of conventional breeding methods (Hickey et al. 2017). Such biotechnology techniques would include *in vitro* and genetic engineering. *In vitro* techniques involve the cultivation of plant organs, tissue or cells in a test tube on artificial media (Anithakumari 2011) and these have been used as a supplement to increase breeding efficiency of crops (Makanginya 2012) in situations where conventional breeding methods are not efficient. Biotechnological advances in molecular breeding have allowed for alteration of the soybean genome and transcriptome and have eventually promised significant yield gains (Ainsworth et al. 2012).

Genetic engineering methods greatly facilitate conventional plant breeding, because it is much faster, with smaller populations. It also facilitates the identification of climate resilient genes (Kole et al. 2015), thereby opening new possibilities in plant breeding.

The use of marker assisted techniques have also greatly helped in understanding the molecular mechanisms of abiotic stress responses (Thomson et al. 2010). In order to identify QTL for drought tolerance and recovery potential, molecular markers have been used (Anithakumari 2011). For instance, the identification and use of yield QTL is a significant and practical achievement that helps to clarify the contributors to this highly polygenic trait (Hu et al. 2011; Ainsworth et al. 2012). The use of QTL has also made it easy and effective to identify homologues (Kumawat et al. 2016). Genetic engineering has also helped to identify strategies for altering soybean metabolism (Chaudhary et al. 2015). This includes improving photosynthetic efficiency, and altering sink strength and metabolism of soybean in order to enhance yield (Ainsworth et al. 2012; Le et al. 2012).

Transcriptome analysis is also used in identifying common and specific drought stress-responsive genes and their promoter *cis*-regulatory elements (Harb et al. 2010; Weber et al. 2014). Although genetic engineering can facilitate the transgenic expression of some of the stress-regulated genes, resulting in increased tolerance to drought, enhanced expression of these genes is frequently associated with retarded growth (Farooq et al. 2009), which in turn may limit its practical use. In addition, molecular techniques are more reliant on efficient phenotyping of lines, hybrids or genotypes and identification of genotype-phenotype relationships (Hickey et al. 2017). Genetic engineering methods are more expensive and requires skilled labour compared to conventional breeding methods. These methods are further hampered by the complexity of genetic inheritance (Kumawat et al. 2016), metabolic partitioning as well as developmental regulations (Chaudhary et al. 2015).

Although biotechnology has resulted in discovery of traits that control WUE and improved drought tolerance, only a few of these traits have been implemented in the field (Van Oosten et al. 2016). As such, simultaneous use of conventional breeding strategies in combination with biotechnology can provide the possibility of broadening genetic variability of cultivated soybean (Ainsworth et al. 2012). The simultaneous strategy has the possibility of new germplasm with good quality traits.

2.8 Concluding remarks

Constraints to crop production are attributed to a number of factors, both physiological and environmental stress factors, and drought is one of the most critical environmental factors affecting soybean crop production. Drought stress occurrence, intensity and duration is unpredictable. Drought stress affects soybean morphologically, biochemically as well as physiologically. Drought stress reduces all morphological traits, impairs biochemical processes, and causes physiological process malfunctions. It is, therefore, evitable to characterise the drought factor in order to have the most effective combination of adaptation features in a genotype. In order to effectively develop a drought tolerant genotype, there is a need to understand the nature of the trait to be manipulated. Although the use of conventional breeding alone is no longer sufficient, it still remains the most used method. Biotechnology techniques assist conventional breeding and no genotype development is possible without conventional breeding. Therefore, integration of all breeding methods will be paramount in a successful breeding programme for soybean improvement.

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CHAPTER 3

Morphological and physiological evaluation of soybean under water-limited stress conditions

3.1 Abstract

Water-limited stress (WLS) can have a large negative effect on soybean yield as it shortens the seed-filling period and reduces seed weight, which is an important yield component of soybean. This study aimed at identifying genotypes which perform well under water-limited stress (WLS) and to determine traits' response to WLS. In this study, 12 soybean genotypes were evaluated at Kasinthula Research Station in the 2016 and 2017 seasons, respectively in a field trial subjected to three water levels namely; a control (non-WL), 50% WLS and 70% WLS. The experiment was a factorial laid out in a randomised complete block design (RCBD) with three replications. The morphological and physiological traits measured included plant height, number of nodes per plant, number of pods per plant, biomass, number of seeds per plant, 100-seed weight, grain yield, WUE, relative chlorophyll content (SPAD), quantum yields of efficiency for photosystem II photochemistry (ΦII), the quantum yields of non-photochemical exciton quenching (ΦNPQ) and non-regulatory energy dissipation (ΦNO). Data collected were subjected to combined analysis of variance across seasons and for all WLS levels. All traits were highly significantly ($P<0.001$) associated with WLS except number of nodes per plant and SPAD while ΦNO was significant at ($P<0.01$). Genotype effect was highly significant for all traits except ΦII. Different WLS levels negatively affected all soybean genotypes. Genotypes TGX1990-137F and TGX1988-70F were better performing under WLS conditions compared to the rest of the genotypes and were regarded more tolerant. However, the mechanism of tolerance was not clear. Plant height, number of nodes per plant, 100-seed weight, WUE and SPAD exhibited less reaction to WLS and were thus regarded as less sensitive traits. Determining the response of plants to WLS using both morphological and physiological traits can help to identify good performing genotypes for cultivation under WLS conditions. However, the interaction observed between WLS and genotype calls for additional studies in multiple locations across seasons to determine stability of these genotypes under WLS conditions.

Keywords: Genotypes, photosynthetic capacity, grain yield, stress, water use efficiency

3.2 Introduction

Soybean, together with beans and groundnuts, are the most important leguminous crops in Malawi since they provide sources of protein for both humans and animals (Monyo 2013;

Kananji et al. 2013; Nzima and Dzanja 2015). Soybean is further used as a rotational crop with maize, thereby helping to halt the precipitous decline in soil fertility by fixing nitrogen (Manavalan et al. 2009) in the soils of Malawi (Kananji et al. 2013).

In Malawi, the soybean industry has potential to grow due to the increase in both local and international demand. Malawi was expected to contribute 170000 MT per year towards the export market in Southern Africa Developing Countries (SADC) countries, yet its average production is only 77175 MT per year (AICC 2016). This shows that there is an export market for soybean from Malawi. As such, the private sector, especially processors, has shown interest to support and enhance soybean production in order to meet both local and international demand. In addition, with the urge to combat climate change effects and improve soil fertility of Malawi, there is also a political will and government has put initiatives in place to enhance soybean production (Sopo and Mulekano 2014; Mubichi 2017).

Soybean morphological traits such as plant height, number of nodes per plant, number of pods per plant, biomass, number of seeds per plant, 100-seed weight and WUE have been reported as primary yield components that are directly linked to overall grain yield (Kobraei et al. 2011; Mwenye 2018). However, drought stress, one of the most important abiotic stress factors in soybean (Chowdhury et al. 2016), negatively affects these morphological traits, which may translate to a large effect on total grain yield. Water-limited stress reduces plant growth such as plant height, which limits the number of nodes and pods produced per plant (Ku et al. 2013; K-State Research and Extension 2016). Purcell et al. (2014) observed a shortened seed-filling period, which eventually resulted in reduced biomass accumulation, number of seeds per plant and seed weight per plant. Characterisation of these traits, when selecting high yielding and tolerant genotypes to be cultivated under WLS, would be important. In order to contribute to improvement of income, food and nutritional security levels of smallholder farmers and expanding the domestic and international soybean market in Malawi, development of appropriate soybean genotypes that can withstand the effect of drought stress is crucial.

Leaf photosynthetic capacity is one of the most important factors for obtaining higher grain yield in crop plants (Sayed 2003; Shao et al. 2013; Li et al. 2016). Chlorophyll fluorescence has efficiently been used as a tool to project the extent of damage drought stress causes in photosystems (Feller and Vaseva 2014; Narina et al. 2014; Guidi et al. 2019). The most commonly used chlorophyll fluorescence-based photosynthetic parameters include the quantum efficiency for photosystem II photochemistry (ΦII), the quantum yields of non-photochemical exciton quenching (ΦNPQ) and non-regulatory energy dissipation (ΦNO) (Baker 2008). These parameters are good indicators of plant status as they detect photosynthetic processes, onset of photo-inhibition, as well as photodamage in the plant (Kuhlgert et al. 2016). For instance, ΦII indicates the amount of light that has been absorbed and used by photochemistry (Kramer et al. 2004). During photochemistry, there is over-excitation captured light that is normally dissipated for other regulated processes such as ΦNPQ, which help to detect the capacity of a genotype to protect itself from photodamage (Ghotbi-Ravandi et al. 2014; Guidi et al. 2019). However, due to competition within photosystem II between ΦII, ΦNPQ and ΦNO (Baker 2008) for excitation energy (Kramer et al. 2004), the over-excitation light may be captured by non-regulatory energy processes such as ΦNO. Such non-regulatory energy dissipation (ΦNO) may cause acid photodamage of photosystem II when in a high amount (Kramer and Evans 2011). As such, ΦNO detects the amount of captured light that is used for unregulated processes (Baker 2008), which are lethal to plant life. Considering that the effects of WLS on photosynthesis processes are directly linked to overall food production (Chen et al. 2015; Chowdhury et al. 2016), chlorophyll fluorescence probes-based photosynthetic parameters would be highly useful in improving yield estimation by combining chlorophyll fluorescence with actual yield and yield component data.

Currently, there are no soybean genotypes in Malawi that are recommended for cultivation under WLS conditions. Knowledge on the effects of WLS on morphological and physiological characteristics will help to efficiently characterise Malawi soybean genotypes and select genotypes that can perform well in WLS environments. The aim of this study was to evaluate 12 soybean genotypes for grain yield, yield components and physiological traits under different WLS levels under field conditions. The specific objectives were to (1) identify genotypes with superior performance under optimum and WLS conditions and (2) identify traits that are sensitive to WLS by determining traits' response to different WLS levels.

3.3 Materials and methods

3.3.1 Plant material

The experimental plant material consisted of 12 soybean genotypes, of which four were released genotypes (Monyo 2013) and eight were promising lines from the International Institute for Tropical Agriculture (IITA) germplasm (Table 3.1).

Table 3.1: Characteristics of soybean genotypes used as experimental material

Name of genotype	Description of genotype	Source
Makwacha	It was recommended for production in Malawi in 2003. Medium to late maturity (120 to 140 days), medium to high altitude, large-seeded with light cream colour, white helium, produce white flowers and grey hairs, exhibits indeterminate growth and yields up to 3000 kg ha^{-1}	Zimbabwe
Nasoko	It was recommended for production in Malawi in 2002. Medium to late maturity (120 to 140 days), medium to high altitude, large-seeded with cream colour, white hilum, produce white flower and grey hairs, exhibit indeterminate growth and yields up to 3000 kg ha^{-1}	Zimbabwe
TGX1987-10F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
TGX1987-23F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
TGX1987-62F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
TGX1988-18F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
TGX1988-70F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
Magoye	It is the widely grown promiscuous genotype in Malawi	Malawi
TGX1988-9F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
TGX1989-46	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
TGX1990-137F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
Tikolore	It was recommended for production in Malawi in 2011. Medium to late maturity (120 to 140 days), low, medium to high altitude, small-seeded, brown hilum, promiscuous (may not require inoculation), tolerant to frogeye disease, susceptible to rust and yields up to 2500 kg ha^{-1}	IITA

3.3.2 Experiment location, design and water regime

The field experiment was planted at Kasinthula Research Station (Department of Agricultural Research Services) in Malawi during the 2016 and 2017 dry periods from July to October. Kasinthula Research Station is located 10 km south of the Chikwawa District, at 60 m above sea level in the Shire Valley of Malawi (160 00' S 340 05' E). The station has a mean annual rainfall of 700 mm, most of which comes within the short period from November/December to February/March. This low rainfall necessitates the use of irrigation for crop production. Maximum temperatures range from 25ºC to 49ºC, while the minimum temperature ranges from 10ºC to 20ºC. Kasinthula soils are loamy sand, comprising of 80% sand, 12% clay and 8% silt with a pH level of 5.0.

The experiment was laid out in in a split plot design with three replications. Factor A which was a main factor included three water regimes, while factor B which was a sub-factor included 12 soybean genotypes. Seeds were planted by hand and spaced 5 cm within rows and 50 cm between the rows. Each entry in a replication in the field was represented by four rows, containing 100 plants per row of 5 m. The two outer rows were guard rows and data were collected from the two inner rows (net plot) with 200 plants per treatment. Fertilizer (NPK 8:18:15) was incorporated in the soil at the rate of 18 g fertiliser per meter length of the ridge by making a groove in the middle of the ridge (Kananji et al. 2013). Soybean seed was not inoculated with *Rhizobium* (in order to mimic farmers' field conditions) because most farmers in Malawi do not use inoculants.

To determine when and how much water to apply, soil moisture storage was estimated from the available water holding capacity of 100 mm m^{-1} in the crop root zone (Fandika et al. 2011). Crop water consumptive use (ETc) was computed from climatic data using the soil water balance schedule as described by Fandika et al. (2014). The Class A Pan evaporation method was used for estimating reference crop evapotranspiration on a daily basis (Fandika et al. 2011). The available soil water was determined by using field capacity, wilting point and the soybean maximum root zone of 0.6 m (FAO 2015). Irrigation in each whole treatment plot was done based on the maximum allowable depletion (MAD) of soybeans, which is at 35% (FAO 2015).

A siphon and a stopwatch were used to measure the amount of water applied to the main treatments and to determine the water application duration, respectively. Before use, the siphon was calibrated and was found to discharge 2.7 L of water per second. Amount of water per irrigation event in non-stress (T1) was derived from the formula: total irrigation (T1) = siphon discharge (L/s) x time (s). For T1, total irrigation was a sum of irrigation at each and every irrigation event, calculated from siphon and time taken to irrigate each whole treatment. For the 50% WLS level (T2), irrigation was done in 50% of the time taken to irrigate T1. Therefore, the amount of water per irrigation event in T2 was derived from the formula: total irrigation (T2) = siphon discharge (L/s) x 50% of T1 time (s). For the 70% WLS level (T3), irrigation was done in 30% of time taken to irrigate T1. Therefore, the amount of water per irrigation event in T3 was derived from the formula: total irrigation (T3) = siphon discharge (L/s) x 30% of T1 time (s).

3.3.3 Data collection

At maturity, plants were harvested to determine plant height (cm), number of nodes per plant, number of pods per plant, biomass, number of seeds per plant, 100-seed weight, grain yield, and WUE. Plants from the middle two rows per plot were harvested. Five plants were randomly picked from which plant height was measured in cm from the base of the plant to the top of the plant using a measuring tape. The numbers of nodes and pods per plant were respectively counted. Plant biomass was determined by weighing the above ground biomass (in g) using a weighing balance. Weight (in g) of 100 seeds was also determined by weighing 100 seeds in a beam balance weighing scale. Seeds from plants of the two middle rows harvested were manually threshed to calculate grain yield (kg ha^{-1}). Water use efficiency was calculated as the ratio of the total yield (kg ha^{-1}) to the total amount of water (m^3) used throughout the growing period.

Data collection on chlorophyll florescence-based photosynthetic parameters commenced four weeks after planting and was measured biweekly from 10:00 am to 12:00 pm using a hand-held MultispeQ instrument (Mwale et al. 2017; Putranto 2018). Four plants per treatment were randomly sampled and chlorophyll florescence-based photosynthetic parameters determined from one middle leaf and one upper canopy leaf. The MultispeQ instrument uses measurement protocols. As such, data were collected using two measurement protocols and these are (1) “The One Protocol (ΦII, PSII, NPQ) II” and (2) the “Chlorophyll content (SPAD) III”. The One Protocol measures ΦII, ΦNPQ and ΦNO. The “Chlorophyll content (SPAD) III” protocol measures SPAD. It takes ~12 seconds per measurement. The MultispeQ instrument is comprised of four light-emitting diodes that have peak emission wavelengths at 530 nm, 605 nm, 650 nm and 940 nm, and a photodiode detector that is sensitive to 700 to 1160 nm light for detection of both chlorophyll fluorescence and absorbance changes in the near infrared light spectrum (Kuhlgert et al. 2016; Bergström 2017).

3.3.4 Data analysis

Data collected were subjected to analysis of variance (ANOVA) using Genstat 64-bit Release 19.1, 19th Edition (VSN International 2018). The trait mean values of genotypes for each experiment were used to analyse relationships among traits under well-watered (non-WL) and WLS conditions. The least significant difference (LSD) test was used to separate the means at 5% level of significance.

3.4 Results

3.4.1 Response of morphological traits and water use efficiency of genotypes to different water-limited stress levels

Combined ANOVA on 12 soybean genotypes was performed to investigate the response of different genotypes to different WLS levels (Table 3.2). The WLS effect was the highest contributor of the variation that was observed across all traits except for number of nodes and WUE which was explained by the high mean squares. Genotype effect contributed most to the variation observed for number of nodes and WUE. Highly significant WLS effects were observed for plant height, number of pods per plant, biomass, number of seeds per plant, 100-seed weight, soybean grain yield and WUE. Non-significant WLS effects were observed for number of nodes per plant. Genotype effects were highly significant for all traits. Highly significant ($P<0.0001$) WLS level by genotype (WLSxG) interaction effects were observed for biomass and number of seeds per plant, while significant ($P<0.01$) WLSxG effects were observed for number of pods per plant and grain yield.

Significant WLS and genotype effects observed for plant height, number of nodes per plant, number of pods per plant, biomass accumulation, number of seeds plant per plant and 100-seed weight indicated that these traits were highly influenced by both the level WLS and the genotype of the plant. Non-significant WLS and WLSxG interaction effects for number of nodes per plant indicated that this trait is genetically determined and that environment does not have a significant influence on the trait. The significant WLSxG effects observed for number of pods per plant, biomass accumulation and number of seeds per plant signified the difference in response of genotypes to various levels of WLS for the traits.

The WLS contributed most to the total variation in plant height, number of pods per plant, biomass, number of seeds per plant, 100-seed weight and soybean grain yield, which was confirmed by highest mean square values. For number of nodes per plant and WUE, most of the total variation was contributed by genetic differences.

Table 3.2: Combined analysis of variance showing mean square values of morphological traits and water use efficiency of 12 soybean genotypes subjected to three water-limited stress levels

Variable	Sources of variation					
	Replication	**WLS**	**Genotype (G)**	**WLSxG**	**Residual**	**CV (%)**
Plant height (cm)	28.92	800.85***	209.11***	34.40	44.28	15.70
Number of nodes per plant	0.09	1.95	22.60***	1.83	1.54	12.00
Number of pods per plant	31.26	2790.91***	376.37***	96.74**	46.77	31.90
Biomass (g)	12.880	1108.02***	46.32***	18.96***	5.92	23.30
Number of seeds per plant	11.07	10601.33***	1179.36***	322.57***	74.33	22.70
100-seed weight (g)	1.48	107.01***	69.25***	4.83	6.16	18.80
Grain yield (kg ha^{-1})	37606.00	25375006.00***	3528617.00***	652176.00**	324509.00	33.90
WUE (kg ha^{-1} m^{-3})	0.00	0.47***	0.51***	0.06	0.05	32.40

***, **, * Significant at $P<0.001$, $P<0.01$, $P<0.05$, respectively; WUE = water use efficiency; WLS = water-limited stress; CV = coefficient of variation

Results have demonstrated that morphological traits were significantly (P<0.001; <0.01) reduced in plants exposed to WLS (Table 3.3) but the percentage reduction differs from trait to trait. Plant height, number of pods per plant, biomass, number of seeds per plant, 100-seed weight, grain yield and WUE were all significantly reduced by WLS.

Morphological traits have shown a reduction in both the 50% WLS level and 70% WLS level compared to non-WL (Table 3.3). The reduction effect of WLS was more severe on plants subjected to 70% WLS compared to 50% WLS. The reduction rate varied from one trait to the next with grain yield (51.95%), biomass (51.45%), number of seeds per plant (45.54%) and number of pods per plant (43.71%) recording the highest reduction percentage at 70% WLS. Grain yield loss at 70% WLS was almost twice the grain yield loss at 50% WLS. Plant height (14.58%), number of nodes per plant (3.08%), 100-seed weight (16.92%) and WUE (21.61%) were less affected with reductions of less than 25% at 70% WLS.

Genotype TGX1988-9F (48.29 cm), followed by TGX1990-137F (47.39 cm) had the tallest plants, while Makwacha (36.44 cm) had the shortest plants across WLS levels (Table 3.4). Although non-significant WLS effects were observed for the number of nodes per plant, genetic main effects caused differences in number of nodes found per genotype. Genotype TGX1987-10F (12.17), followed by TGX1988-9F (11.94) produced the highest number of nodes per plant, while Makwacha (8.47) had the least number of nodes per plant. For number of pods per plant, significant WLS effects, genotype effects and WLSxG effects with observed with Magoye (29.75), followed by TGX1988-9F (27.51) and TGX1987-10F (26.42) producing the largest number of pods per plant, while Tikolore (14.96) produced the least pods per plant. Biomass production was highest in genotype Magoye (14.61 g), while TGX1988-18F (8.99 g) and TGX1990-137F (8.98 g) produced the least biomass. Magoye (59.60) produced the highest number of seeds per plant and was followed by TGX1987-10F (42.40). Nasoko (16.91 g), Makwacha (15.60 g) and TGX1987-23F (14.25 g) produced the largest 100-seed weight values and thus the largest seeds compared to the rest of the genotypes.

The genotype mean WUE values varied between 0.34 kg ha^{-1} m^{-3} (TGX1987-10F) and 0.83 kg ha^{-1} m^{-3} (Magoye) with a mean value of 0.62 kg ha^{-1} m^{-3} (Table 3.4). Magoye, followed by TGX1990-137F (0.78 kg ha^{-1} m^{-3}) used the available water most efficiently to produce optimum grain yield compared to other tested genotypes across WLS levels, while TGX1987-10F was the least efficient.

Table 3.3: Water-limited stress level mean values of morphological traits and water use efficiency of 12 soybean genotypes

Morphological traits	Non-WL	50% WLS	70% WLS	Mean	$LSD_{0.05}$	Reduction % at 50% WLS	Reduction % at 70% WLS
Plant height (cm)	45.27	42.83	38.67	42.26	2.19	5.39	14.58
Number of nodes per plant	10.45	10.34	10.13	10.31	0.41	1.09	3.08
Number of pods per plant	28.12	20.27	15.83	21.41	2.25	27.92	43.71
Biomass (g)	14.79	9.34	7.18	10.44	0.80	36.85	51.45
Number of seeds per plant	51.49	34.37	28.04	37.96	2.84	33.25	45.54
100-seed weight (g)	14.36	13.28	11.93	13.19	0.82	7.52	16.92
Grain yield (kg ha^{-1})	2285.00	1665.00	1098.00	1682.00	187.40	27.13	51.95
WUE (kg ha^{-1} m^{-3})	0.72	0.68	0.56	0.65	0.04	5.97	21.61

LSD = Least significant difference; WUE = water use efficiency; WLS = water-limited stress

Table 3.4: Water-limited stress level mean values and ranking of morphological traits and water use efficiency of 12 soybean genotypes

Genotype	Plant height (cm)	Number of nodes per plant	Number of pods per plant	Biomass (g)	Number of seeds per plant	100-seed weight (g)	WUE (kg ha^{-1} m^{-3})
Magoye	39.39(10)	10.01 (8)	29.75 (1)	14.61 (1)	59.60 (1)	11.07 (10)	0.83 (1)
TGX1987-23F	41.63 (7)	10.43 (5)	19.53 (8)	12.03 (2)	41.63 (3)	14.25 (3)	0.43 (9)
TGX1987-62F	43.67 (4)	10.77 (4)	24.00 (4)	11.27 (3)	39.53 (5)	12.75 (9)	0.72 (3)
TGX1988-9F	48.29 (1)	11.94 (2)	27.51 (2)	10.82 (4)	41.14 (4)	10.40 (11)	0.42 (10)
TGX1987-10F	44.79 (3)	12.17 (1)	26.42 (3)	10.13 (5)	42.40 (2)	10.39 (12)	0.34 (11)
TGX1988-70F	39.11 (11)	9.01 (11)	19.87 (7)	10.09 (6)	33.49 (9)	14.14 (4)	0.69 (5)
Nasoko	41.00 (8)	9.32 (10)	16.33 (11)	9.96 (7)	31.74 (10)	16.91 (1)	0.71 (4)
TGX1989-46	42.18 (6)	11.32 (3)	20.84 (5)	9.51 (8)	31.49 (11)	13.03 (7)	0.63 (7)
Tikolore	42.58 (5)	10.18 (7)	14.96 (12)	9.49 (9)	34.74 (7)	13.36 (6)	0.61 (8)
Makwacha	36.44 (12)	8.47 (12)	18.82 (9)	9.37 (10)	29.08 (12)	15.60 (2)	0.68 (6)
TGX1988-18F	40.64 (9)	9.79 (9)	20.70 (6)	8.99 (11)	34.05 (8)	13.61 (5)	0.63 (7)
TGX1990-137F	47.39 (2)	10.26 (6)	18.15 (10)	8.98 (12)	36.69 (6)	12.79 (8)	0.78 (2)
Mean	42.26	10.31	21.41	10.44	37.96	13.19	0.62
$LSD_{0.05}$	7.58	1.14	7.79	2.77	9.82	2.83	0.23

LSD = Least significant difference; WUE = water use efficiency; Number in bracket is ranking based on genotype performance running downwards from 1 as the best

3.4.2 Grain yield response of genotypes to different water-limited stress levels

Mean grain yield values for each WLS level and genotype are presented in Table 3.5. The 50% WLS and 70% WLS resulted in a significant reduction of grain yield compared to non-WL. The reduction rate increased with an increasing WLS level. The non-WL treatment produced the highest mean grain yield of 2285 kg ha^{-1} across the 12 genotypes and was followed by 50% WLS (1665 kg ha^{-1}) and 70% WLS (1098 kg ha^{-1}). Compared to non-WL, the effect of 70% WLS was most severe, resulting in a grain yield reduction of 49.48% compared to 50% WLS with a yield reduction of 25.05%.

Some genotypes showed higher vulnerability to WLS than other genotypes. Based on the genotype mean across the WLS levels, genotype Magoye (2329 kg ha^{-1}) followed by TGX1987-62F (2145 kg ha^{-1}) and TGX1990-137F (2046 kg ha^{-1}) were the highest yielding genotypes compared to other tested genotypes. However, these genotypes have shown large yield reductions of 57.97%, 52.54% and 43.64% at 70% WLS, respectively. Genotypes TGX1988-9F (1015 kg ha^{-1}), TGX1987-10F (1028 kg ha^{-1}) and TGX1987-23F (1055 kg ha^{-1}) were the lowest yielding genotypes across WLS levels. However, from these three genotypes, TGX1988-9F (20.97%), followed by TGX1987-23F (31.21%) showed the lowest yield reductions of all genotypes at 70% WLS. Genotypes TGX1989-46 (70.08%) and TGX1987-10F (59.72%) were the most sensitive to 70% WLS and showed the highest reduction percentages across all genotypes.

3.4.3 Physiological trait response of genotypes to different water-limited stress levels

Combined ANOVA was performed to investigate the response of physiological traits of 12 soybean genotypes to different WLS levels (Table 3.6). The WLS effect contributed most to the variation observed on ΦII and ΦNPQ traits while genotype effects contributed most to SPAD and ΦNO which was explained by the high mean squares. Highly significant ($P<0.0001$) WLS effects were observed for ΦII, ΦNPQ and ΦNO, while WLS effects were non-significant for SPAD. Genotype effects were highly significant for SPAD, ΦNPQ and ΦNO. Non-significant WLSxG interaction effects were observed for all physiological traits under study. This indicated that ranking of genotypes for measured traits remained the same at different WLS levels. The level of WLS contributed most to the total variation in ΦII and ΦNPQ, while for SPAD and ΦNO most of the total variation was contributed by genotypes. Water-limited stress conditions resulted in a significant reduction of ΦII (Table 3.7). Both 50% WLS (0.40) and 70% WLS (0.39) reduced ΦII compared to non-WL (0.44).

Table 3.5: Mean grain yield value and ranking of 12 soybean genotypes under different water-limited stress levels

Genotype	Grain yield (kg ha^{-1})				Reduction % at 50% WLS	Reduction % at 70% WLS
	Non-WL	50% WLS	70% WLS	Genotype mean		
Magoye	3300.00 (1)	2299.00 (2)	1387.00 (2)	2329.00 (1)	30.33	57.97
Makwacha	2517.00 (6)	1682.00 (7)	1177.00 (6)	1792.00 (7)	33.17	53.24
Nasoko	2755.00 (3)	1766.00 (6)	1182.00 (5)	1901.00 (4)	35.90	57.10
TGX1987-10F	1363.00 (10)	1172.00 (10)	549.00 (12)	1028.00 (11)	14.01	59.72
TGX1987-23F	1272.00 (11)	1018.00 (11)	875.00 (11)	1055.00 (10)	19.97	31.21
TGX1987-62F	2634.00 (4)	2552.00 (1)	1250.00 (4)	2145.00 (2)	3.11	52.54
TGX1988-18F	2281.00 (8)	2013.00 (4)	1127.00 (7)	1807.00 (6)	11.75	50.59
TGX1988-70F	2446.00 (7)	1868.00 (5)	1255.00 (3)	1856.00 (5)	23.63	48.69
TGX1988-9F	1135.00 (12)	1013.00 (12)	897.00 (9)	1015.00 (12)	10.75	20.97
TGX1989-46	2951.00 (2)	1181.00 (9)	883.00 (10)	1672.00 (8)	59.98	70.08
TGX1990-137F	2626.00 (5)	2031.00 (3)	1480.00 (1)	2046.00 (3)	22.66	43.64
Tikolore	2137.00 (9)	1381.00 (8)	1110.00 (8)	1542.00 (9)	35.38	48.06
Treatment mean	2285.00	1665.00	1098.00	1682.00	25.05	49.48
$LSD_{0.05\ (WLS)}$				187.40		
$LSD_{0.05\ (G)}$				374.70		
$LSD_{0.05\ (WLSxG)}$				649.00		

LSD = Least significant difference; WLS = water-limited stress; G = genotype; WLSxG = interaction between WLS and G; Number in bracket is ranking based on genotype performance running downwards from 1 as the best

ΦNPQ significantly increased with an increase in WLS levels from non-WL (0.19), to 50% WLS (0.23) to 70% WLS (0.25). However, ΦNO was significantly higher under non-WL (0.37) compared to both the 50% WLS (0.36) and the 70% WLS (0.36).

Genotype mean values of the four physiological traits are presented in Table 3.8. For SPAD, the top three genotypes were TGX1990-137F (39.32), Makwacha (38.76) and Nasoko (38.07), while TGX-1987-10F produced the lowest SPAD value (33.96). For ΦNPQ, the top two genotypes were TGX-1987-10F (0.24) and TGX-1987-23F (0.24), while TGX1990-137F produced significantly the lowest ΦNPQ value (0.19). For ΦNO, the top three genotypes were Nasoko, TGX1990-137F and Tikolore, all three with a ΦNO value of 0.38.

Table 3.6: Combined analysis of variance showing mean square values of physiological traits of 12 soybean genotypes subjected to water-limited stress

Variable	Source of variation					
	Replication	WLS	Genotype (G)	WLSxG	Residual	CV (%)
SPAD	187.35	210.28	1161.03***	82.73	82.58	24.38
ΦII	0.04	1.19***	0.02	0.02	0.01	28.28
ΦNPQ	0.01	1.67***	0.08***	0.02	0.02	10.94
ΦNO	0.00	0.04*	0.05***	0.01	0.01	31.61

***, **, * Significant at P<0.001, P<0.01, P<0.05, respectively; WLS = Water-limited stress; CV = coefficient of variation; SPAD = relative chlorophyll content, ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching, ΦNO = non-regulatory energy dissipation

Table 3.7: Water-limited stress level mean values and ranking of physiological traits measured across 12 soybean genotypes

Physiological traits	Non-WL	50% WLS	70% WLS	Mean	$LSD_{0.05}$
SPAD	36.49	37.18	36.74	36.80	0.61
ΦII	0.44 (1)	0.40 (2)	0.39 (3)	0.41	0.01
ΦNPQ	0.19 (3)	0.23 (2)	0.25 (1)	0.22	0.01
ΦNO	0.37(1)	0.36 (2)	0.36 (2)	0.37	0.01

LSD = Least significant difference; WLS = water-limited stress; SPAD = relative chlorophyll content, ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching, ΦNO = non-regulatory energy dissipation; Number in bracket is ranking based on performance running downwards from 1 as the best but for ΦNO it is the worse genotype

Table 3.8: Water-limited stress level mean values and ranking of physiological traits and water use efficiency of 12 soybean genotypes

Genotype	SPAD	ΦII	ΦNPQ	ΦNO
Magoye	37.09 (7)	0.42	0.23 (2)	0.36 (3)
Makwacha	38.76 (2)	0.41	0.22 (3)	0.37 (2)
Nasoko	38.07 (3)	0.41	0.21 (4)	0.38 (1)
TGX1987-10F	33.96 (12)	0.41	0.24 (1)	0.35 (4)
TGX1987-23F	35.56 (9)	0.40	0.24 (1)	0.36 (3)
TGX1987-62F	37.57 (5)	0.41	0.23 (2)	0.36 (3)
TGX1988-18F	36.63 (8)	0.41	0.23 (2)	0.36 (3)
TGX1988-70F	37.64 (4)	0.41	0.23 (2)	0.37 (2)
TGX1988-9F	34.85 (11)	0.41	0.23 (2)	0.36 (3)
TGX1989-46	34.92 (10)	0.41	0.23 (2)	0.36 (3)
TGX1990-137F	39.32 (1)	0.43	0.19 (5)	0.38 (1)
Tikolore	37.18 (6)	0.40	0.21 (4)	0.38 (1)
Mean	36.80	0.41	0.22	0.37
$LSD_{0.05}$	2.14	0.03	0.03	0.03

LSD = Least significant difference; SPAD = relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; WLS = water-limited stress; Number in bracket is ranking based on genotype performance in descending order 1 as the best but for ΦNO it is the worse genotype

3.5 Discussion

Morphological traits and WUE have consistently been used to select good performing genotypes under WLS conditions (Kobraei et al. 2011; Mabulwana 2013; Fried et al. 2019; Nadeem et al. 2019). Similarly, response of morphological traits and WUE of soybean genotypes to different water-limited stress levels were investigated in order to identify genotypes that perform well under WLS as well as determine the traits' response to WLS the current study. Significant WLS effects were observed for all morphological traits, except for number of nodes per plant. This was in accordance with Kobraei et al. (2011), Souza et al. (2013) and Fenta et al. (2014) who observed significant reduction in morphological traits of soybean under different WLS levels. Morphological traits that were sensitive and thus showed large reductions of over 40% under 70% WLS level included number of pods per plant, biomass, number of seeds per plant and grain yield. This was in accordance with Manavalan et al. (2009), Fenta et al. (2014) and Mwenye (2018) who observed a large reduction in biomass, number of pods per plant, number of seeds per plant per plant and grain yield under WLS conditions.

A reduction in plant height under WLS conditions is associated with plant growth inhibition. In a previous study by Mabulwana (2013), a plant height reduction of up to 22.30% in soybean under severe WLS was reported. Similar results were observed in the current study where 70% WLS reduced plant growth with 14.58%. Farooq et al. (2009) reported the cause of reduced plant growth to be a result of impaired mitosis under WLS, leading to limitation of cell division which finally obstructed cell elongation. Mabulwana (2013) further concluded that a reduction in plant height has a direct negative effects on the numbers of nodes and pods, and eventually grain yield because reduced plant height, as a result of growth inhibition, translates to fewer nodes available where pods can be formed. However, in the current study the number of nodes per plant were not significantly reduced under WLS conditions. In addition, the tallest genotypes did not produce the highest yield across WLS levels, which showed that plant height might not directly contribute to overall grain yield. The results corroborate findings of Mwenye (2018) who did not find a distinct relationship between grain yield and plant height in soybean.

A reduction in number of pods per plant is associated with a reduction in number of flowers per plant and pod abortion. Under WLS conditions, the flowering stage is shortened, which results in the production of fewer flowers (Su et al. 2013). These fewer flowers may also be prone to abortion when drought stress persists, leaving only a few flowers to form pods (Nadeem et al. 2019). Pod abortion has been reported under WLS, which consequently led to an overall reduced number of pods in soybean (Zhao et al. 2018). Nadeem et al. (2019) attributed the decreased rate of pod filling in soybean to a decrease in net photosynthesis under WLS conditions.

The decrease in biomass production under WLS conditions is a result of foliage reduction, leading to a decrease in transpiration area and lower intercepted radiation (Pinheiro and Chaves 2011; Wang et al. 2016). Plants tend to reduce leaf area, leaf elongation and leaf number, respectively as avoidance mechanisms that minimises water loss when the stomata are closed (Farooq et al. 2009; Manavalan et al. (2009). This leads to reduced total canopy area and consequently plant biomass (Souza et al. 2013; Toscano et al. 2019). In addition, reduced biomass is linked to plants' tendency to increase the proportion of assimilated carbohydrates that are incorporated in roots relative to shoots under WLS conditions (Farooq et al. 2009; Hofer et al. 2017; Mwenye 2018).

Number of seeds per plant was also affected by WLS in the present study with a reduction rate of 45.54% under 70% WLS. Ku et al. (2013) reported similar findings in soybean. A reduction in number of seeds per plant under WLS conditions is linked to developmental stages of which flowering and pod-filing are regarded as most critical (Kobraei et al. 2011; Zhao et al. 2018; Nadeem et al. 2019). Manavalan et al. (2009) attributed reduced number of seeds per plant under WLS during flowering and pod-filing stages to shortened flowering period coupled with reduced pod set, which was translated to fewer flowers followed by fewer pods, increased pod abortion and consequently fewer seeds per unit area in soybean. Mabulwana (2013) suggested that the reduced number of seed could be due to production of fewer seeds per pod under WLS.

The WLS reduces seed size (100-seed weight) and overall seed quality of soybean (Kananji et al. 2013). Under WLS conditions, the proportion of large diameter seeds to small diameter seeds tends to decrease, resulting in a reduced 100-seed weight (Ku et al. 2013). Zhao et al. (2018) has reported a significant reduction in seed weight and seed size in soybean under WLS, which was attributed to seed weight distribution. Wijewardana et al. (2019) attributed the reduction in seed weight and seed size in soybean to increased production of small, shrivelled, and wrinkled seed under WLS. Manavalan et al. (2009) reported a seed size reduction of up to 32%, which was attributed to a shortened seed-filling period, resulting in smaller seeds in soybean. Kobraei et al. (2011) suggested that the reduction of 100-seed weight could be a result of disruption in photosynthesis and remobilisation in soybean plants due to WLS.

Grain yield loss due to WLS has been reported in soybean (Farooq et al. 2009; Pathan et al. 2010; Fried et al. 2019). The results in the current study are in line with the findings of Nadeem et al. (2019) who indicated that the extent of reduction in grain yield is dependent on the intensity and duration of drought stress, crop developmental stage and genotypic variability. Farooq et al. (2009) reported a yield reduction of between 46-71% in soybean when WLS occurred at the reproductive stage. In addition, a reduction in grain yield under WLS conditions has been associated with a decrease in yield components such as the number of pods per plant, the number of seeds per plant and plant biomass (Manavalan et al. 2009; Mwale et al. 2017). This was confirmed in the current study by the highly significant reduction in these yield components, which consequently might have reduced grain yield in all genotypes.

In terms of genotype mean values, Magoye ranked in the top position across WLS levels for yield components such as number of pods per plant, biomass accumulation and number of seeds per plant. In addition, this genotype ranked in the top position for grain yield under non-WL conditions and in the second position for both the 50% WLS and 70% WLS levels. However, it exhibited a large grain yield loss of up to 58% when exposed to 70% WLS. This large reduction in grain yield could be attributed to the large reductions observed in plant biomass (51.45%) and number of pods per plant (45.54%). Therefore, Magoye was regarded as a high yield potential genotype but is sensitive to WLS. Genotypes that ranked in the top five positions for plant height, number of nodes per plant, number of pods per plant, biomass and number of seeds per plant included TGX1987-62F, TGX1988-9F and TGX1987-10F. From these genotypes only TGX1987-62F produced a high mean grain yield across the WLS levels and ranked second (2145.00 kg ha^{-1}), however, it exhibited a large yield loss of up to 52% under the 70% WLS.

Desirable genotypes are those that exhibit a low yield reduction percentage under WLS conditions (Makanginya 2012; Mabulwana 2013; Mwenye 2018). Such genotypes, with grain yield reductions of less than 45%, would include TGX1988-9F, TGX1987-23F and TGX1990-137F with grain yield reductions of 21%, 31% and 44% respectively. Of these three genotypes, TGX1990-137F was one of the promising genotypes for cultivation under WLS conditions. Although TGX1990-137F ranked fifth under Non-WL conditions, it ranked first for the 70% WLS, third for the 50% WLS and third across the WLS levels.

Variation in grain yield among genotypes in response to WLS has been reported in soybean (Kobraei et al. 2011; Mabulwana 2013; Hossaina et al. 2014) and other crops such as cowpea

(Mwale et al. 2017). The significant WLSxG interaction effect observed for grain yield confirmed that grain yield is both genetically and environmentally determined and that is has a small genotypic variance coupled with large GEI variances (Karasu et al. 2009; Fikere et al. 2014). Thus, there is a need for further characterisation of the genotypes for grain yield and its components across multiple environments and seasons.

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Results from the current study confirmed previous reports (Kobraei et al. 2011; Ku et al. 2013; Mabulwana 2013; Mwenye 2018), which showed that WLS reduces morphological traits values, which could ultimately lead to a reduced grain yield. Water-limited stress reduced plant height, number of nodes per plant, number of pods per plant, biomass, number of seeds per plant and 100-seed weight. It was shown that WLS impairs several activities ranging from carbon assimilation, increased oxidative damage and enzymes activity to ion balance (Nadeem et al. 2019). Reduction in morphological traits under WLS is an indication of abscission in reproductive structures (Mwale et al. 2017) and failure to partition photo-assimilates to reproductive sink (Pinheiro and Chaves 2011).

Water use efficiency has been used as a tool to measure a genotype's tolerance and to determine crop yield under WLS conditions (Manavalan et al. 2009). A high WUE has been associated with high yielding WLS tolerant genotypes (Fried et al. 2019). In the current study, the mean WUE was significantly reduced under WLS conditions, with the largest reduction (21.61%) under 70% WLS.

Highly significant differences were observed between genotypes in terms of WUE. Across the different WLS levels, genotype Magoye ranked in the top position with the highest WUE value (0.83 kg ha^{-1} m^{-3}) and this genotype was previously identified as the top performer in terms of grain yield, number of pods per plant, biomass and number of seeds per plant. The next three genotypes, which ranked in the top three positions for WUE were TGX1990-137F (0.78 kg ha^{-1} m^{-3}), TGX1987-62F (0.72 kg ha^{-1} m^{-3}) and Nasoko (0.71 kg ha^{-1} m^{-3}). However, of these genotypes only TGX1987-62F ranked in the top five positions for grain yield components (plant height, number of nodes per plant, number of pods per plant, biomass and number of seeds per plant) and it ranked in the top four positions for grain yield under non-WL and WLS conditions. Although TGX1990-137F also ranked in the top five positions for grain yield under non-WL and WLS conditions, this genotype performed poorly (below the genotype average) in terms of number of nodes per plant, number of pods per plant, biomass, number of seeds per plant and 100-seed weight.

Although two genotypes (Magoye and TGX1987-62F) were identified as top performing in terms of grain yield, its components and WUE, these genotypes showed a grain yield reduction of above 50% under 70% WLS. In addition, genotypes TGX1987-23F and TGX1988-9F, which showed grain yield reductions of 31% and 21% under 70% WLS, ranked in the bottom positions (rank nine and 10, respectively) for WUE. Thus, genotypes that exhibit high WUE do not show the lowest yield reduction under severe WLS. Although variability was present among genotypes, values for WUE obtained were within the reported range for soybean (Visser 2014; Marrs 2017).

Lei et al. (2006) linked WUE to the relationship between photosynthesis and respiration as it provides information about the function of water metabolism. Genotypes that do not have the ability to adjust their organ size to reduce water loss during drought stress have shown substantial reduction in WUE in previous reports (Fandika et al. 2011; Fuganti-Pagliarini et al. 2017). Stomatal control has been used by many crops as an important mechanism of improving crop WUE (Farooq et al. 2009; Manavalan et al. 2009; Ku et al. 2013; Zhao et al. 2018). Hossaina et al. (2014) observed that soybean genotypes that had the ability to induce stomatal closure during drought stress showed an increase in leaf instantaneous WUE, which remained elevated during the entire period of dehydration. Wang et al. (2016) similarly observed a larger increase in instantaneous WUE with an increase in WLS level when they investigated leaf gas exchange and fluorescence response to drought in wheat. They attributed it to reduction in transpiration loss due to stomatal limitation.

In the current study, chlorophyll florescence-based photosynthetic parameters (physiological traits) were evaluated in order to determine changes that might occur in photosystem II operating efficiency under different WLS levels. Chlorophyll florescence-based photosynthetic parameters are responsible for photosynthetic processes (Baker 2008; Guidi et al. 2019). Water-limited stress has been reported to down-regulate the photosynthesis process, impair photosynthetic activities (Farooq et al. 2009; Chowdhury et al. 2016; Fuganti-Pagliarini et al. 2017), inhibit the electron transport and reduce PSII photochemical activities (Guidi et al. 2019), which result in reduced crop productivity (Yooyongwech et al. 2013; Meng et al. 2016). As such, it is important to include physiological traits, when identifying good performing genotypes to be grown under WLS conditions, as well as determining traits' response.

Significant WLS effects have been observed for physiological traits such as ΦII, ΦNPQ and ΦNO. This was in accordance with Ghassemi-Golezani and Lotfi (2012), Jumrani and Bhatia (2019) in soybean, Mwale et al. (2017) in cowpea and Arabzadeh (2013) in saxaul plants who observed a reduction in ΦII, and increased ΦNPQ when genotypes were exposed to increased WLS levels. However, for relative chlorophyll content (SPAD), non-significant WLS effects were observed. Thus, the different WLS levels did not result in significantly different SPAD values. Highly significant genotype effects were observed for SPAD, ΦNPQ and ΦNO. Although significant WLS and genotype effects were observed for most physiological traits, no significant WLSxG effects were observed for any of the physiological traits. This indicated the genotypes responded similarly for each WLS level and no cross-over of genotype rankings occurred. This was in accordance with findings of Fuganti-Pagliarini et al. (2017).

Genotypes that show an ability to efficiently transport photosynthate to sink under severe WLS conditions (to obtain optimum yield) need to be identified for cultivation under WLS conditions. These genotypes should have high levels of SPAD (Makbul et al. 2011) and ΦII with low levels of ΦNO, and an increased level of ΦNPQ (Mwale et al. 2017) under WLS conditions.

In the current study, different WLS levels did not result in a significant change or reduction in SPAD values and all genotypes generally maintained high relative chlorophyll content under different WLS levels. This was not in accordance with findings from literature. A reduction of up to 31% in SPAD under WLS conditions has been reported in soybean (Nadeem et al. 2019). Degradation in photosynthetic pigment (SPAD) when plants are exposed to soil WLS is one of the most sensitive responses to drought stress (Yooyongwech et al. 2013). Contrasting results were also found by Ku et al. (2013) in soybean, Chen et al. (2015) in sorghum and Mwale et al. (2017) in cowpea who observed reduction in SPAD when genotypes were subjected to WLS, which was attributed to the reduction in chloroplast damage that is caused by active oxygen species. Farooq et al. (2009), Pinheiro and Chaves (2011) and Ghassemi-Golezani and Lotfi (2012) reported that active oxygen species reduce carbon dioxide diffusion to the chloroplast for carboxylation, which leads to chlorophyll degradation. The non-reduction in SPAD under WLS conditions could be attributed to the capacity of the genotypes under study to protect themselves against the active oxygen species thereby maintain high SPAD even under high WLS. Yooyongwech et al. (2013) found similar stabilisation of relative chlorophyll content under severe soil water stress in some of sweet potato genotypes.

In the current study, significant genotype effects were observed for SPAD. The top three ranking genotypes were TGX1990-137F (39.32), Makwacha (38.76) and Nasoko (38.07), while TGX1987-10F (33.96), TGX1988-9F (34.85) and TGX1989-46 (34.92) recorded the lowest SPAD values. This is due to genetic differences. Similar results in terms of genetic differences have been observed for SPAD in soybean by Ghassemi-Golezani and Lotfi (2012), Mabulwana (2013) and Fenta et al. (2014).

Genotypes that maintain high quantum yield of efficiency for photosystem II (ΦII) under WLS show that they have the ability to efficiently partition more assimilates for reproductive processes (Narina et al. 2014) in the face of WLS. Such genotypes also ensure that most of the captured light is being absorbed and used by photochemistry (Kramer et al. 2004) rather than dissipated for other regulated and non-regulated processes (Ghotbi-Ravandi et al. 2014). The photosynthetic parameters such as maximum quantum yield of photosystem II, quantum yield of efficiency and net photosynthetic rate tend to significantly decline when crops are subjected to severe soil WLS (Yooyongwech et al. 2013). Similar observations were made in the current study since the treatment mean ΦII values (across genotypes) were significantly reduced with an increase in WLS level. The reduction in quantum yield might be the result of a number of factors including decline in stomatal aperture (Pinheiro and Chaves 2011), damage to photosystem II (Narina et al. 2014), reduced electron transport (Wang et al. 2016) and carboxylation efficiencies (Ghotbi-Ravandi et al. 2014), which ultimately reduce photosynthesis efficiency. In addition, the reduction of quantum yield under WLS conditions has been associated with the degradation of D1 protein (Ghassemi-Golezani and Lotfi 2012), resulting in photo-inhibition.

In the current study, both genotype and their WLSxG interaction effects were not significant for ΦII. Thus, there were no significant genotypic differences nor did the genotypes respond differently to the different WLS levels. This, was not in accordance with a previous observation that was made in wheat where ΦII decreased when plants were subjected to WLS (Wang et al. 2016). Results from the current study are in line with findings of Ghassemi-Golezani and Lotfi (2012) who reported non-significant variability in ΦII among soybean genotypes subjected to different WLS. Since genotypes did not show a reduction in ΦII values under different WLS levels in the currents study, it is an indication that they have some tolerance to photo-oxidative stress (Arena and Vitale 2012; Narina et al. 2014).

Wang et al. (2018) reported that WLS has an effect on photosynthetic parameters such as the quantum yields of non-photochemical exciton quenching (ΦNPQ) as well as non-regulatory energy dissipation (ΦNO) (Arena and Vitale 2012; Wang et al. 2016). In the current study, the treatment mean values of ΦNPQ were significantly increased as the WLS level increased. On the other hand, the treatment mean values of ΦNO showed a significant reduction under WLS conditions compared to non-WL conditions. Genotypes with increased quantum yields of non-photochemical exciton quenching (ΦNPQ) and low non-regulatory energy dissipation (ΦNO) under WLS are preferred because they show the ability to protect themselves against over-excitation by down-regulating non-photochemical quenching mechanisms (Pinheiro and Chaves 2011; Arena and Vitale 2012; Wang et al. 2018) that dissipate excess light energy as heat (Wang et al. 2016; Tietz et al. 2017) in the light harvesting complexes.

Genotypes TGX1987-10F, Magoye, TGX1987-23F, TGX1987-62F, TGX1988-18F and Makwacha with high ΦNPQ and low ΦNO can be considered as genotypes with the ability to let the flow of protons, from the thylakoid lumen to the chloroplast stroma that cause lumen acidification (Carstensen et al. 2018), trigger more energy dissipation via the quantum yields of non-photochemical exciton quenching than loss by non-regulated processes (Guidi et al. 2019). This down-regulation process ultimately prevents the accumulation of reactive intermediates, which are damaging to plant life (Mwale et al. 2017; Wang et al. 2018). This showed that physiological traits of soybean are equally important in determining traits' response to WLS in characterising genotypes for cultivation under WLS.

3.6 Conclusions and recommendations

Genotype Magoye was the top grain yielding genotype under non-WL and across WLS conditions and it produced the highest number of pods per plant, biomass, number of seeds per plant and was most effective in terms of WUE. However, this genotype was not regarded as drought tolerant as it showed a large grain yield reduction under severe WLS conditions. Genotype TGX1990-137F can be classified as a good performing under WLS because they produced high grain yields across the different WLS conditions, showed relatively lower yield reduction percentage, higher SPAD and WUE under severe WLS. TGX1988-70F was a better performer especially under moderate WLS condition. Genotypes TGX1987-23F and TGX1988-9F had low grain yields, but can also be classified as good performing under WLS as they had the lowest yield reduction percentage, high ΦNPQ and low ΦNO. Although genotype TGX1987-10F exhibited higher ΦNPQ and low ΦNO, it had the lowest SPAD, a high yield reduction percentage and the lowest grain yield under both non-WL and WLS conditions. Traits that were sensitive to WLS included grain yield, biomass, number of seeds per plant and number of pods per plant since these exhibited a large reduction percentage when exposed to severe WLS. Traits that were less sensitive to WLS were plant height, number of nodes per plant, 100-seed weight, WUE and SPAD. Therefore, SPAD can be considered as less sensitive to WLS compared to other tested physiological traits. In order to discriminate drought tolerant from sensitive genotypes, further classification using multiple analytical tools such as drought tolerance indices, regression, principal component analysis and clustering methods need to be employed. In addition, correlation of morphological traits with physiological traits to determine if physiological traits could have an indirect positive or negative effect on grain yield and its related traits would be important to identify traits that can be selected for improving yield under different WLS regimes.

3.7 References

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CHAPTER 4

Relationships among morphological and physiological traits of soybean under optimum and water-limited conditions

4.1 Abstract

Abiotic stresses, particularly water-limited stress (WLS), severely affect soybean grain yield. Due to the complexity of drought tolerance, one of the difficulties in selecting genotypes is the identification of traits contributing to improved stress tolerance. This study aimed at identifying traits that can be selected to improve yield and to identify genotypes that can be incorporated into the breeding programme under WLS. The experiment was carried out on 12 soybean genotypes, planted under field conditions over two consecutive crop seasons (2016 and 2017) in Malawi. The WLS was applied at two levels, 50% WLS and 70% WLS, with a non-WL as control in a split plot experiment with three replications. The traits measured included plant height, number of pods per plant, biomass, number of seeds per plant, 100-seed weight, grain yield, water use efficiency, relative chlorophyll content (SPAD), quantum yields of efficiency for photosystem II photochemistry (ΦII), the quantum yields of non-photochemical exciton quenching (ΦNPQ) and non-regulatory energy dissipation (ΦNO). The data was subjected to correlation, principal component and multiple regression analysis to identify traits that make a contribution to better yield performance under optimum and WLS conditions. Results showed that selection criteria differed from one WLS level to the next for both traits and genotypes. Results revealed that WUE, SPAD, number of pods per plant and 100-seed weight had the strongest correlations with grain yield. Multiple regression was utilised to examine soybean grain yield and results indicated that WUE explained 99% of variation in grain yield across WLS regimes. Therefore, WUE, SPAD, number of pods per plant and 100-seed weight can be used in soybean breeding programmes for WLS genotype improvement. Genotypes TGX1988-70F and TGX1990-137F were strongly positively associated with good grain yield and WUE under 70% WLS while Magoye and TGX1987-62F were strongly positively associated with grain yield under non-WL and 50% WLS conditions, respectively. TGX1988-70F and TGX1990-137F can be used in breeding for high yield performance under drought stress conditions to obtain higher genetic gains. A combination of statistical analytical tools effectively identified correlation among traits, traits contributing to yield improvement and genotypes to be incorporated in the breeding programme.

Keywords: Morphological traits, physiological traits, grain yield, water-limited stress

4.2 Introduction

Soybean is one of the most important crops worldwide, producing twice as much protein per hectare as any other major vegetable crop (FAO 2005). Soybean provides 70% of the total meal for the animal feed industry (Chaudhary et al. 2015). Despite the crop's importance, soybean yield is severely limited by biotic and abiotic factors to which plants are subjected. Among abiotic factors, water-limiter stress (WLS) is one of the most important economic factors responsible for soybean crop failure, which lead to severe reductions in grain yield and seed quality (Kobraei et al. 2011; Ku et al. 2013; Mwenye 2018). Its negative effects on morphological and physiological traits has resulted in grain yield reduction ((Hossaina et al. 2014); Edugbo et al. 2015; Giordani et al. 2019; Fried et al. 2019). Although soybean is regarded a short season crop (early-maturing), with an average of 85 days to maturity in tropical areas (FAO 2015) and flowering that occurs over a long time window, not all genotypes are early-maturing and have the ability to escape drought effects (Ku et al. 2013; Adhikari et al. 2015).

Identification and development of drought tolerant plants become more important when considering the impact of climate change, which is attributed to the increase in levels of greenhouse gases in the atmosphere, on plant growth and development (Adhikari et al. 2015; Akaogu et al. 2017; Nadeem et al. 2019). The effects of WLS varies from one genotype to the next and depends on intensity, duration and period of its occurrence (Manavalan et al. 2009; Kobraei et al. 2011; Nadeem et al. 2019). With a low intensity of WLS, plants have the capacity to recover and may tolerate shorter levels of WLS than a higher intensity level (Mwale et al. 2017). This makes it complex to quantify yield and yield traits in simple linear terms under WLS.

Due to the complexity of plant responses to WLS, identification of more drought tolerant genotypes also becomes difficult. Phenotyping and identification of traits contributing to yield performance under WLS conditions, using several analytical methods, might be a possible way of dissecting the complexity. Morphological traits have been consistently linked with overall grain yield in various crops (Kamrani 2015; Akaogu et al. 2017; Mwale 2017) including soybean (Kobraei et al. 2011; Ku et al. 2013; Ghanbari et al. 2018). In addition, physiological traits such ΦII and SPAD (Liu et al. 2012), as well as WUE (Manavalan et al. 2009; Fried et al. 2019) have been used as effective traits in selection of genotypes for drought tolerance. As such, a comprehensive characterisation of soybean genotypes under WLS conditions may unveil traits contributing to grain yield, which will ultimately help in the identification of tolerant genotypes. The aim of the present study was to determine interrelationships among morphological and physiological traits through correlations, multiple regression and principal component analysis under three different water regimes. The specific objectives were to (1) determine how breeding selection criteria might differ across the different water regimes, (2) identify traits that can be selected to improve yield under different water regimes and (3) identify genotypes that can be used in breeding for high yield performance under drought stress conditions.

4.3 Materials and methods

4.3.1 Plant material

The soybean genotypes used as planting material were described in Chapter 3, section 3.3.1 and shown in Table 3.1.

4.3.2 Experimental location, design and water regime

The experiment was conducted in the experimental site of DARS, specifically the Kasinthula Research Station during the 2016 and 2017 dry periods from July to October as described in Chapter 3, section 3.3.2. The same planting and trial management practices as described in Chapter 3, section 3.3.2, were followed. The experiment was laid out in a factorial design with three replications. Factor A included three water regimes, while factor B included 12 soybean genotypes. The water regimes were determined and applied as described in section 3.3.2 to obtain data on non-WL, 50% WLS and 70% WLS levels.

4.3.3 Data collection

Data on morphological traits, physiological traits and WUE were collected based on measurements as described in Chapter 3, section 3.3.3.

4.3.4 Data analysis

In order to identify suitable trait combinations for screening soybean genotypes under drought stress conditions, correlation analysis, multiple regression and principal component analysis (PCA) were performed using GenStat 19th edition statistical package (VSN International 2018). The multiple regression analysis in soybean under different water regime conditions was done by considering the grain yield (kg ha^{-1}) as the dependent variable and the other characters as independent variables. Direct path analysis (using standardised values) was performed to identify traits that contribute directly towards yield. The PCA was performed to identify traits that positively contribute to total variation among soybean genotypes. The PCA biplot was constructed to identify positive associations among groups of traits, their association with grain yield and to identify genotypes that can be included in the breeding programme.

4.4 Results

4.4.1 Identification of interrelationships among morphological and physiological traits using correlation analysis under different water-limited stress levels

A total of seven morphological traits, four physiological traits and WUE were subjected to correlation analysis under non-WL (Table 4.1), 50% WLS (Table 4.2) and 70% WLS (Table 4.3), respectively. Morphological traits that showed highly significant and positive correlations under non-WL included biomass with number of pods per plant (r = 0.75) and number of seeds per plant (r = 0.79); number of nodes per plant with plant height (0.63); number of pods per plant with number of seeds per plant (0.72) and 100-seed weight with grain yield (r = 0.41) (Table 4.1).

The physiological traits generally did not show significant positive correlations amoung each other, except for ΦII and SPAD (r = 0.34). However, significant to highly significant negative correlations were observed between ΦII with ΦNO (r = -0.25) and ΦNPQ (r = -0.62); ΦNPQ with SPAD (r = -0.47), as well as ΦNO with ΦNPQ (r = -0.61). Significant to highly significant correlations were observed between morphological and physiological traits and included a positive correlation between 100-seed weight and SPAD (r = 0.41); a positive correlation between number of pods per plant with ΦNPQ (r = 0.36); and a negative correlation between number of pods plant with ΦNO (r = -0.50). Grain yield showed highly significant positive correlations with both morphological and physiological traits and included grain yield with 100-seed weight (r = 0.41), SPAD (r = 0.46) and WUE (r = 1.00), respectively. However, grain yield showed a significant negative correlation with number of nodes per plant (r = -0.29).

Under 50% WLS, significant ($P<0.05$) to highly significant ($P<0.01$) positive correlations were evident (Table 4.2) for biomass with number of pods per plant (r = 0.59) and number of seeds per plant (r = 0.72), respectively; number of nodes per plant with plant height (r = 0.54); and number of seeds per plant with number of pods per plant (r = 0.67). The physiological traits generally did not show significant positive correlations. However, highly significant and negative correlations were observed between ΦII and ΦNPQ (r = -0.52), as well as between ΦNO and ΦNPQ (r = -0.86).

Table 4.1: Correlation coefficients between morphological and physiological traits of 12 soybean genotypes under non-water limited conditions

	Biomass	**100-seed**	**Nodes**	**ΦII**	**ΦNO**	**ΦNPQ**	**Pods**	**SPAD**	**WUE**	**Yield**	**Height**	**Seeds**
Biomass	-											
100-seed	-0.06	-										
Nodes	0.22	-0.13	-									
ΦII	-0.01	0.18	-0.04	-								
ΦNO	-0.26*	0.01	0.02	-0.25*	-							
ΦNPQ	0.21	-0.16	0.02	-0.62***	-0.61***	-						
Pods	0.75***	-0.17	0.24	0.05	-0.50***	0.36*	-					
SPAD	0.04	0.41***	-0.08	0.34*	0.23	-0.47***	-0.13	-				
WUE	0.13	0.43***	-0.30*	0.10	0.07	-0.13	-0.01	0.47***	-			
Yield	0.11	0.41***	-0.29*	0.09	0.10	-0.16	-0.04	0.46***	1.00***	-		
Height	0.08	0.00	0.63***	0.11	0.09	-0.16	0.06	0.18	-0.14	-0.13	-	
Seeds	0.79***	-0.24	0.31*	0.02	-0.20	0.14	0.72***	-0.04	0.06	0.05	0.19	-

***, **, * Significant at $P<0.001$, $P<0.01$, $P<0.05$, respectively; SPAD = Relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; Height = plant height; nodes = number of nodes per plant; pods = number of pods per plant; seeds = number of seeds per plant; 100-seed = 100-seed weight; WUE = water use efficiency

Table 4.2: Correlation coefficients between morphological and physiological traits of 12 soybean genotypes under 50% water-limited stress conditions

	Biomass	100-seed	Nodes	ΦII	ΦNO	ΦNPQ	Pods	SPAD	WUE	Yield	Height	Seeds
Biomass	-											
100-seed	0.08	-										
Nodes	0.09	-0.47***	-									
ΦII	0.04	-0.02	0.01	-								
ΦNO	-0.23	0.02	0.01	0.01	-							
ΦNPQ	0.17	-0.01	-0.01	-0.52***	-0.86***	-						
Pods	0.59***	-0.27*	0.16	-0.10	-0.55***	0.53***	-					
SPAD	0.15	0.42***	-0.40***	0.22	-0.20	0.06	0.05	-				
WUE	0.22	0.06	-0.23	0.00	-0.26*	0.22	0.35**	0.35**	-			
Yield	0.23	0.05	-0.22	0.03	-0.22	0.17	0.33**	0.32**	1.00***	-		
Height	-0.05	-0.17	0.54***	0.12	0.51***	-0.50***	-0.30**	-0.08	-0.28**	-0.25	-	
Seeds	0.72***	-0.28**	0.17	0.06	-0.32**	0.24*	0.67***	0.11	0.37**	0.37**	-0.09	-

***, **, * Significant at P<0.001, P<0.01, P<0.05, respectively; SPAD = Relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; Height = plant height; nodes = number of nodes per plant; pods = number of pods per plant; seeds = number of seeds per plant; 100-seed = 100-seed weight; WUE = water use efficiency

Table 4.3: Correlation coefficients between morphological and physiological traits of 12 soybean genotypes under 70% water-limited stress conditions

	Biomass	100-seed	Nodes	ΦII	ΦNO	ΦNPQ	Pods	SPAD	WUE	Yield	Height	Seeds
Biomass	-											
100-seed	0.07	-										
Nodes	0.04	-0.45***	-									
ΦII	0.07	0.03	0.04	-								
ΦNO	-0.02	-0.27	0.28	-0.62***	-							
ΦNPQ	-0.02	0.32**	-0.38**	0.17	-0.88***	-						
Pods	0.56***	-0.14	0.15	0.23	-0.29*	0.22	-					
SPAD	0.03	0.34**	-0.45***	0.14	-0.40***	0.41***	0.08	-				
WUE	0.14	0.19	-0.47***	0.08	-0.24	0.25	0.06	0.42***	-			
Yield	0.14	0.15	-0.44***	0.03	-0.15	0.17	0.03	0.36**	0.99***	-		
Height	-0.07	-0.35**	0.63***	-0.24	0.52***	-0.51***	-0.06	-0.20	-0.13	-0.08	-	
Seeds	0.80***	-0.20	0.12	0.00	0.11	-0.14	0.62***	-0.09	0.16	0.18	-0.01	-

***, **, * Significant at P<0.001, P<0.01, P<0.05, respectively; SPAD = Relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; Height = plant height; nodes = number of nodes per plant; pods = number of pods per plant; seeds = number of seeds per plant; 100-seed = 100-seed weight; WUE = water use efficiency

Significant to highly significant correlations were observed between morphological and physiological traits and included positive correlations between 100-seed weight with SPAD (0.42); ΦNO with plant height (r = 0.51); ΦNPQ with number of pods per plant (r = 0.53) and number of seeds per plant (r = 0.24), respectively. However, significant correlations were observed between number of nodes per plant and SPAD (r = -0.40); ΦNO with number of pods per plant (r = -0.55) and number of seeds per plant (r = -0.32), respectively; and ΦNPQ with plant height (r = -0.50). Grain yield significantly and positively correlated with number of pods per plant (r = 0.33), number of seeds per plant (r = 0.37), SPAD (r = 0.32) and WUE (r = 1.00), respectively. In addition to grain yield, WUE was significantly and positively correlated with number of seeds per plant (r = 0.37), number of pods per plant (r = 0.35) and SPAD (r = 0.35), respectively.

Under 70% WLS, morphological traits that showed significant positive correlations were biomass with number of pods per plant (r = 0.56) and number of seeds per plant (r = 0.80), respectively; number of nodes per plant with plant height (r = 0.63); and number of pods per plant with number of seeds per plant (r = 0.62). The physiological traits generally did not show significant positive correlations, except for ΦNPQ with SPAD (r = 0.41). However, highly significant and negative correlations were observed between ΦNO with ΦII (r = -0.62); ΦNO with ΦNPQ (r = -0.88); and ΦNO with SPAD (r = -0.40).

Significant to highly significant correlations were observed between morphological and physiological traits and included positive correlations between 100 seed weight with SPAD (r = 0.34) and ΦNPQ (r = 0.32), respectively; and plant height with ΦNO (r = 0.52). However, negative correlations were observed between number of nodes per plant respectively with ΦNPQ (r = -0.38) and SPAD (r = -0.45); number of pods per plant with ΦNO (-0.29); and plant height with ΦNPQ (r = -0.51). Grain yield was significantly and positively correlated with SPAD (r = 0.36) and WUE (r = 0.99) but negatively correlated with number of nodes per plant (r = -.044). In addition to grain yield, WUE was positively and significantly correlated with SPAD (r = 0.36) but negatively correlated with number of nodes per plant (r = -0.47).

4.4.2 Identification of morphological and physiological traits that contribute directly to grain yield under different water-limited stress levels

Results from the regression analysis of seven morphological traits, four physiological traits and WUE on grain yield (the dependent variable), indicated that both 100-seed weight and SPAD were significant ($P<0.05$), while WUE was highly significant ($P<0.001$) under non-WL (Table 4.4). Under 50% WLS, number of pods per plant, biomass, SPAD and WUE were all highly significant. Under 70% WLS, only SPAD and WUE were highly significant. Path coefficients, based on standardised values with units of standard deviations of the mean, were obtained in order to compare the relative importance of each trait and identify traits that further contribute directly to grain yield. Results showed that both SPAD and WUE had a significant ($P<0.001$) and thus direct contribution to grain yield under all three WLS levels (Table 4.5). In addition, under non-WL, 100-seed weight also showed a significant ($P<0.05$) and direct effect, while under 50% WLS, number of pods per plant and biomass also had significant and direct effects. The highest contribution of 99% was observed for WUE across the three WLS levels and was followed by SPAD, which contributed 21%, 11% and 13%, respectively to the deviation of grain yield under non-WL, 50% WLS and 70% WLS (Table 4.6).

4.4.3 Association between grain yield, morphological and physiological traits in response to different water-limited stress levels

Associations between grain yield, morphological and physiological traits in response to different WLS levels were investigated using PCA. The 12 traits were grouped into 12 components, which accounted for 100% of the variability among genotypes. The first five principal components (PC) are presented in Tables 4.7, 4.8 and 4.9 showing traits' contribution values, eigenvalues, % variations and the cumulative %. Under non-WL, the first five PCs explained 94.75% of the total variation (Table 4.7). The first PC, which explained 50.99% of the total variation, was positively attributed to number of nodes per plant, ΦNPQ and plant height. The second PC, which explained 20.05% of the total variation was positively attributed to biomass, ΦII, number of pods per plant, number of seeds per plant, SPAD, grain yield and WUE.

Table 4.4: Multiple regression analysis performed on morphological and physiological traits of 12 soybean genotypes under different water-limited stress levels on the dependent variable grain yield

Traits	Non-WL			50% WLS			70%WLS		
	Estimates	SE	t-Stat	Estimates	SE	t Stat	Estimates	SE	t-Stat
Intercept	22150.90	77129.61	0.29	47053.85	42345.52	1.11	-32292.00	17507.80	-1.84
SPAD	-7.66**	2.83	-2.71	-5.43**	1.90	-2.86	-5.14***	1.24	-4.14
ΦII	-22044.48	77143.92	-0.29	-46733.27	42374.28	-1.10	32316.47	17485.94	1.85
ΦNO	-21402.34	77106.03	-0.28	-46669.53	42322.39	-1.10	32768.16	17508.96	1.87
ΦNPQ	-22430.82	77157.71	-0.29	-47304.06	42329.88	-1.12	32174.52	17522.02	1.84
Plant height	1.85	1.23	1.50	-0.48	0.92	-0.52	0.29	0.68	0.43
Number of nodes per plant	4.06	6.10	0.67	4.53	4.09	1.11	0.87	2.87	0.30
Number of pods per plant	-2.14	1.21	-1.77	-3.15***	0.88	-3.59	-0.51	0.84	-0.61
Biomass	0.03	3.12	0.01	9.36***	2.61	3.58	-3.53	2.64	-1.34
Number of seeds per plant	0.06	0.79	0.07	-0.11	0.70	-0.16	0.77	0.62	1.23
100-seed weight	-4.91*	2.22	-2.21	-2.75	2.07	-1.32	0.41	1.56	0.26
WUE	4058.68***	36.12	112.36	2283.30***	15.55	146.81	1652.62***	15.42	107.17
R	1.00			1.00			1.00		
R^2	1.00			1.00			1.00		
SE	56.95			32.82			23.28		

***, **, * Significant at $P<0.001$, $P<0.01$, $P<0.05$, respectively; SPAD = Relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; R = regression coefficient; R^2 = coefficient of determination; SE = standard error; WUE = water use efficiency; WLS = water-limited stress

Table 4.5: Direct path coefficients of morphological and physiological traits as predictors of soybean grain yield under different water-limited stress levels

	Estimate		
Trait	**Non-WL**	**50% WLS**	**70% WLS**
SPAD	-0.03**	-0.02**	-0.04***
ΦII	-0.68	-2.06	2.54
ΦNO	-0.66	-2.74	4.30
ΦNPQ	-0.69	-3.47	3.37
Plant height	0.01	-0.01	0.01
Number of nodes per plant	0.01	0.01	0.00
Number of pods per plant	-0.02	-0.04***	-0.01
Biomass	0.00	0.04***	-0.02
Number of seeds per plant	0.00	0.00	0.02
100-seed weight	-0.02*	-0.01	0.00
WUE	1.04***	1.04***	1.04***
R^2	1.00	1.00	1.00
SE	0.06		0.06

***, **, * Significant at P<0.001, P<0.01, P<0.05, respectively; SPAD = Relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; R^2 = coefficient of determination; SE= standard error; WUE = water use efficiency; WLS = water-limited stress

Table 4.6: Contribution of morphological and physiological traits that directly affect soybean grain yield under different water-limited stress levels

Trait	Estimate	R^2
	non-WL	
SPAD	0.46***	0.21
100-seed weight	0.41***	0.17
WUE	1.00***	0.99
	50% WLS	
SPAD	0.32**	0.10
Number of pods per plant	0.33***	0.11
Biomass	0.23*	0.05
WUE	1.00***	0.99
	70% WLS	
SPAD	0.36***	0.13
WUE	0.99***	0.99

***, **, * Significant at P<0.001, P<0.01, P<0.05, respectively; SPAD = Relative chlorophyll content; WUE = water use efficiency; R^2 = coefficient of determination; WLS = water-limited stress

Table 4.7: Loadings of morphological and physiological soybean traits under non-water-limited stress on the first five principal components

Trait	PC1	PC2	PC3	PC4	PC5
Biomass	0.23635	0.44360	0.10142	0.14518	-0.50910
100-seed weight	-0.32878	-0.17173	0.00387	-0.13204	-0.43645
Number of nodes per plant	0.33075	0.02311	-0.36841	0.09417	0.26284
ΦII	-0.22173	0.31418	-0.20985	-0.69540	0.00951
ΦNO	-0.27947	0.05079	-0.33964	0.62095	-0.01065
ΦNPQ	0.32182	-0.23434	0.35282	0.04780	0.00074
Plant height	0.24812	0.11367	-0.58046	-0.02713	0.17782
Number of pods per plant	0.32623	0.34410	0.08805	-0.15410	0.15908
Number of seeds per plant	0.25984	0.44475	0.09393	0.15113	-0.29719
SPAD	-0.30826	0.21197	-0.27978	0.04898	-0.26076
WUE	-0.28823	0.34503	0.26507	0.12200	0.36622
Grain yield	-0.28673	0.35197	0.26084	0.12874	0.36561
Eigenvalues	6.119	2.406	1.575	0.780	0.491
% Variation	50.99	20.05	13.12	6.50	4.09
% Cumulative	50.99	71.04	84.16	90.66	94.75

SPAD = Relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; WUE = water use efficiency; PC = principal component

Genotype and trait groupings under non-WL conditions are presented in the PCA biplot (Figure 4.1). Traits that contributed most to the total variation in PC1 clustered together in the PCA biplot. Strong positive associations were observed between plant biomass, number of seeds per plant and number of pods per plant as well as between grain yield, WUE and SPAD in the PCA biplot. These associations are verified by the strong and highly significant positive correlations between these traits in the correlations table (Table 4.1). The strong negative association between SPAD and ΦNPQ (PhiNPQ) was confirmed by results from the correlations table. Genotype Magoye that was associated with plant biomass, number of seeds per plant and number of pods per plant in the PCA biplot, was ranked in the top positions for plant biomass, number of seeds per plant, number of pods per plant and grain yield in the ANOVA table (Tables 3.4 and 3.5).

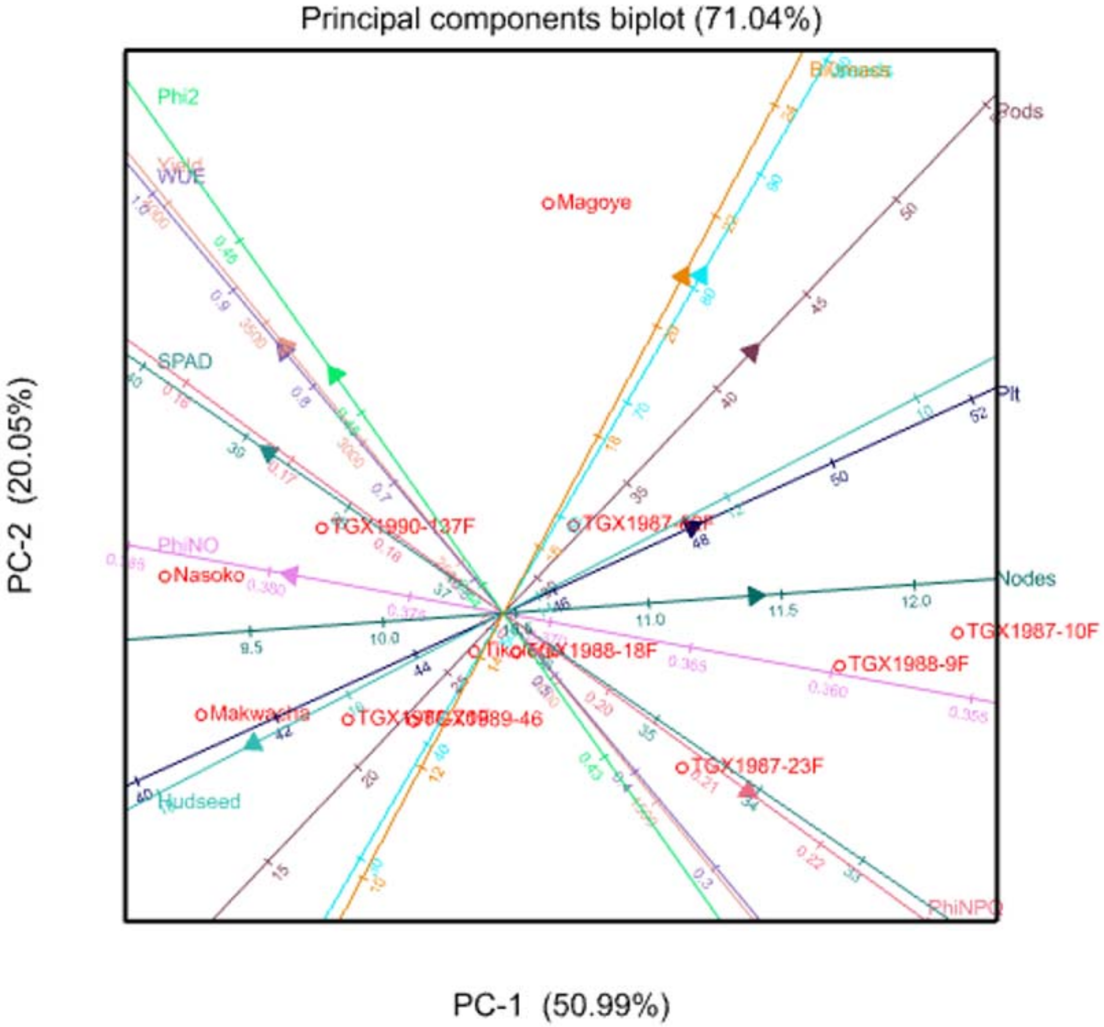

Figure 4.1: Biplot showing the first two principal components presenting the associations between grain yield, morphological and physiological traits under non-water-limited stress conditions. SPAD = Relative chlorophyll content; Phi2 = quantum yield efficiency for photosystem II photochemistry; PhiNPQ = quantum yields of non-photochemical exciton quenching; PhiNO = non-regulatory energy dissipation; Plt = plant height, nodes = number of nodes per plant; pods = number of pods per plant; seeds = number of seeds per plant; Hudseed = 100-seed weight; WUE = water use efficiency

Under 50% WLS, the first five PCs explained 94.07% of the total variation (Table 4.8). The first PC, which explained 32.63% of the total variation was mainly attributed to variation in 100-seed weight, ΦNO and SPAD. The second PC explained 28.83% of the total variation and was attributed to variation in biomass, number of nodes per plant, number of seeds per plant, WUE and grain yield. Results from the PCA biplot (Figure 4.2) indicated strong positive associations between plant biomass and number of seeds per plant as well as between grain yield and WUE. These associations are verified by the strong and highly significant positive correlations between these traits in the correlations table (Table 4.2). The strong negative association between 100-seed weight and number of nodes per plant was confirmed by results from the correlations table.

Table 4.8: Principal component loadings of morphological and physiological traits under 50% water-limited stress conditions

Trait	PC1	PC2	PC3	PC4	PC5
Biomass	-0.02791	0.43513	-0.12712	0.37927	0.11570
100-seed weight	0.36012	-0.19252	-0.31287	0.35084	-0.10981
Number of nodes per plant	0.11318	0.15018	-0.47388	0.19201	0.61939
ΦII	0.18983	0.11343	0.37503	0.63082	-0.11835
ΦNO	0.34500	-0.18224	0.07231	-0.35169	0.46236
ΦNPQ	-0.41999	0.02833	-0.27310	-0.01176	-0.32814
Plant height	-0.19007	-0.14317	0.60721	0.08054	0.33723
Number of pods per plant	-0.25007	0.43215	0.06674	0.00074	0.24761
Number of seeds per plant	-0.11764	0.48894	0.16112	-0.12644	0.03605
SPAD	0.46047	0.06633	0.20338	0.06879	-0.13154
WUE	0.31894	0.35503	0.01791	-0.26998	-0.17345
Grain yield	0.31467	0.35693	0.01146	-0.27265	-0.17812
Eigenvalues	3.92	3.46	1.65	1.29	0.97
% Variation	32.63	28.83	13.73	10.78	8.10
% Cumulative	32.63	61.46	75.19	85.97	94.07

SPAD = Relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; WUE = water use efficiency; PC = principal component

Genotype Magoye that was associated with biomass and number of seeds per plant in the PCA biplot (Figure 4.2), ranked in the top positions for biomass and number of seeds per plant, respectively based on their mean values (Table 3.4). However, genotype TGX1987-62F that was associated with grain yield and WUE (Figure 4.2) ranked in the top and third positions, respectively for grain yield under 50% WLS (Table 3.5) and WUE (Table 3.4).

Under 70% WLS, the first five PCs explained 91.86% of the total variation (Table 4.9). The first PC, which explained 32.30% of the total variation, was positively attributed to variation in 100-seed weight, ΦNO, SPAD, WUE and grain yield. The second PC explained 22.62% of the total variation and was mostly attributed to biomass, number of pods per plant, number of seeds per plant, grain yield and WUE.

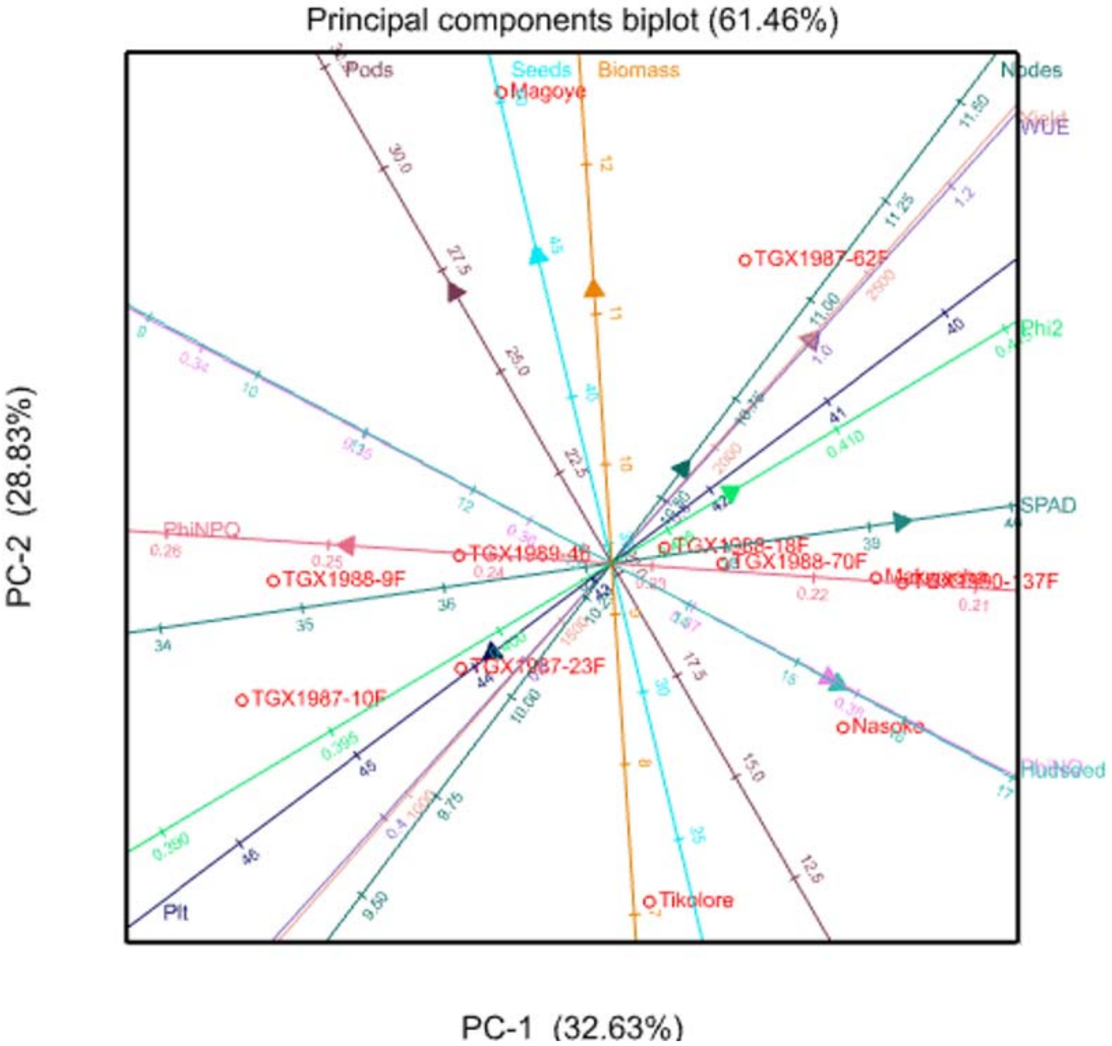

Figure 4.2: Biplot showing the first two principal components presenting the associations between grain yield, morphological and physiological traits under 50% water-limited stress conditions. SPAD = Relative chlorophyll content; Phi2 = quantum yield efficiency for photosystem II photochemistry; PhiNPQ = quantum yields of non-photochemical exciton quenching; PhiNO = non-regulatory energy dissipation; Plt = plant height, nodes = number of nodes per plant; pods = number of pods per plant; seeds = number of seeds per plant; Hudseed = 100-seed weight; WUE = water use efficiency

Results from the PCA biplot (Figure 4.3) indicated that WUE was strongly and positively associated with grain yield; and number of seeds per plant was positively associated with biomass, which are verified by the strong and highly significant positive correlations between these traits in the correlations table (Table 4.3). Genotypes TGX1990-137F, TGX1988-70F that were mostly associated with WUE and grain yield ranked in the top five positions for WUE (Table 3.4) and in top three positions for grain yield under 70% WLS (Table 3.5), while Makwacha that was associated with 100-seed weight ranked second for this trait in the means table (Table 3.4).

Table 4.9: Principal component loadings of morphological and physiological traits under 70% water-limited stress conditions

Trait	PC1	PC2	PC3	PC4	PC5
Biomass	0.03961	0.39274	0.41725	-0.27255	-0.13368
100-seed weight	0.43633	-0.22021	0.13655	0.03836	-0.27073
Number of nodes per plant	0.13791	-0.20237	0.14158	-0.61630	0.50288
ΦII	-0.31014	0.23120	-0.36275	0.18897	0.03575
ΦNO	0.29945	0.14389	-0.19416	-0.46848	-0.46083
ΦNPQ	0.01116	-0.33814	0.47933	0.21332	0.28761
Plant height	-0.20671	-0.05203	-0.42479	-0.39228	0.28663
Number of pods per plant	-0.32186	0.35212	0.19846	0.00244	0.12764
Number of seeds per plant	-0.08242	0.48387	0.33983	-0.08903	-0.02127
SPAD	0.39941	0.11566	-0.17021	0.21369	0.06398
WUE	0.38233	0.30722	-0.10946	0.13744	0.36338
Grain yield	0.37978	0.31499	-0.10909	0.13173	0.35291
Eigenvalues	3.88	2.71	2.37	1.36	0.70
% Variation	32.30	22.62	19.72	11.37	5.85
% Cummulative	32.30	54.92	74.64	86.01	91.86

SPAD = Relative chlorophyll content; ΦII = quantum yield efficiency for photosystem II photochemistry; ΦNPQ = quantum yields of non-photochemical exciton quenching; ΦNO = non-regulatory energy dissipation; WUE = water use efficiency; PC = principal component

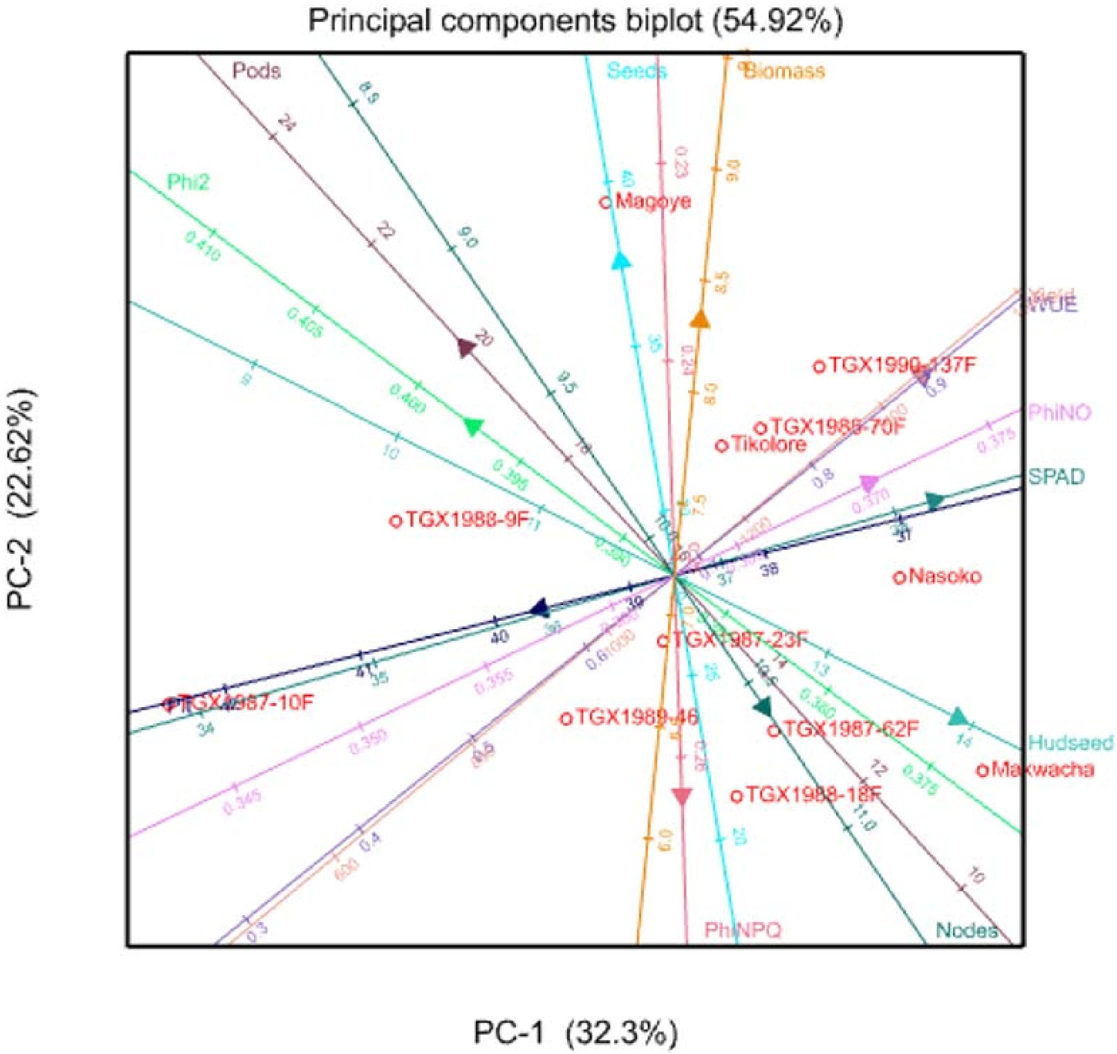

Figure 4.3: Biplot showing the first two principal components presenting the associations between grain yield, morphological and physiological traits under 70% water-limited stress conditions. SPAD = Relative chlorophyll content; Phi2 = quantum yield efficiency for photosystem II photochemistry; PhiNPQ = quantum yields of non-photochemical exciton quenching; PhiNO = non-regulatory energy dissipation; Plt = plant height, nodes = number of nodes per plant; pods = number of pods per plant; seeds = number of seeds per plant; Hudseed = 100-seed weight; WUE = water use efficiency

4.5 Discussion

Correlation, multiple regression and PCA have been previously used to determine interrelationships among traits and to identify traits that directly contribute to yield (Liu et al. 2012; Belluau and Shipley 2018; Giordani et al. 2019). Similarly, morphological traits, physiological traits and WUE were investigated using a combination of analyses to determine selection criteria and to identify traits that would contribute directly towards soybean grain yield under varied WLS regimes. Results from correlation analysis, multiple regression analysis and PCA have demonstrated relationships among traits across the different water regimes.

Plant biomass, number of pods per plant and number of seeds per plant were highly positively correlated across water regimes. SPAD and WUE were positively correlated with grain yield, while SPAD and WUE were positively correlated across water regimes. Highly negative correlations were observed between ΦNO and ΦNPQ across water regimes, and their correlation increased with an increase in the WLS level. These results were in accordance with observations made by Kobraei et al. (2011) and Giordani et al. (2019) who reported strong positive correlations between plant height and number of nodes per plant, number of seeds per plant and number of pods per plant in soybean under varied WLS regimes. In contrast Kobraei et al. (2011) observed a positive correlation between number of nodes and number of pods per plant, which was not observed in the current study.

A positive correlation between traits is useful in breeding programmes because it can provide an opportunity to implement multi-trait selection (Mwale et al. 2017). Positively correlated traits can be bred simultaneously in a genotype. For example, positive selection for increased 100-seed weight would mean an increase in SPAD and, consequently, an increase in grain yield of soybean considering that both traits are directly correlated to grain yield under WLS. Similarly under 70% WLS, SPAD, ΦNPQ and WUE showed strong positive correlations with grain yield, which indicated that a positive selection for any of these traits will consequently improve grain yield.

Results from the correlation analysis further demonstrated that plant height was positively correlated with ΦNO and negatively correlated with ΦNPQ, which means increasing plant height would simultaneously increase ΦNO and decrease ΦNPQ. However, increasing ΦNO would mean increasing the source of photo-inhibition, which is lethal to plant life. On the other hand, ΦNPQ, which is regarded as a photo-protective power (Park et al. 2010; Kuhlgert et al. 2016; Wang et al. 2016; Tietz et al. 2017), is also reduced with an increase in plant height. Results thus demonstrated that increasing plant height may not be a suitable option for improving soybean yield under WLS, as it is correlated with some undesirable traits. Also, Keep et al. (2016) found a negative correlation between grain yield and plant height in soybean. The current results support that of Mwenye (2018) who indicated that plant height on its own may not be a good trait for selection under WLS in soybean as the trait does not have enough power to significantly separate WLS tolerant genotypes from WLS sensitive genotypes. The results are in accordance with Giordani et al. (2019) who did not find a significant correlation between plant height and grain yield in soybean across WLS regimes.

With the use of multiple regression and path analysis, the current study identified traits that can be selected to improve grain yield under different water regimes. Results have demonstrated that grain yield is a function of the integrated effects of various morphological, physiological and WUE traits. Results showed that WUE, SPAD, 100-seed weight, biomass and number of pods per plant directly explained the variation in grain yield under different WLS regimes. Kobraei et al. (2011) similarly reported a highly positive correlation between number of pods per plant and grain yield both under non-WL and WLS conditions. Under non-WL, 100-seed weight, SPAD and WUE could be potential selection traits for soybean grain yield, while number of pods per plant, SPAD and WUE could be used for selection under moderate WLS (50%) and SPAD and WUE for selection under severe WLS (70%). Similar results were reported by Ghanbari et al. (2018) who found a direct contribution of seed weight to total variation in grain yield in soybean.

Results from the direct path coefficient analysis indicated that WUE explained the highest percentage of the variation (99%) across WLS regimes. Thus, WUE is regarded an important trait that needs to be considered when selecting for high yielding performing genotypes under WLS conditions. The results are in accordance with observation made by Fried et al. (2019) who reported a positive relationship between soybean grain yield and WUE under WLS conditions and concluded that genotypes with high WUE are useful genetic materials for improving yield under drought conditions. Manavalan et al. (2009) identified WUE as one of the important traits associated with drought tolerance in soybean, considering that water consumption and its efficient use by crops are related to yield. Nadeem et al. (2019) suggested that drought tolerant genotypes with improved WUE should be developed with the aim to enhance crop productivity under WLS conditions.

Except for SPAD, the physiological traits did not contribute directly to grain yield under both non-WL and WLS conditions. Although SPAD did not show a marked reduction with WLS, it showed marked differences among genotypes. Genotypes with minimum grain yield loss under WLS were associated SPAD which could have attributed to strong correlation between SPAD and grain yield. Results were in accordance with Liu et al. (2012) who found a positive correlation between SPAD and grain yield. Keep et al. (2016) further recommended the selection for chlorophyll content in plant breeding programmes as a possible means to improve genetic gain in soybean. Giordani et al. (2019) cited SPAD as one of the most important components that need to be considered when selecting soybean genotypes under WLS as it reflects photo-oxidation of pigments and chlorophyll degradation. Similar positive correlations between SPAD and yield were observed in bread wheat (Kamrani 2015).

Trait combinations for selection under WLS conditions were investigated using PCA. Under both non-WL and WLS conditions, strong positive associations were observed among traits such as biomass, respectively with number of pods per plant and number of seeds per plant; and SPAD with 100-seed weight. Grain yield, WUE, 100-seed weight and SPAD positively contributed most to the total variation in the data set for PC1 and PC2 when soybean was exposed to WLS conditions. Similarly, in the PCA biplot, grain yield, WUE and SPAD were identified as the most important traits for both 50% WLS and 70% WLS, which could indicate that these traits could be the most important for selection of high yielding genotypes in WLS environments.

The lower cumulative variation explained by PC1 and PC2 under 70% WLS (54.92%) and 50% WLS (61.46%) compared to non-WL (71.04%) could indicate the complexity of the interrelationships among traits under WLS conditions. In addition results revealed that under non-WL conditions, selection of genotypes should focus on biomass plant, number of seeds per plant and number of pods per plant, while under moderate WLS conditions, selection of genotypes should focus on grain yield, WUE, number of nodes per plant, ΦII and SPAD. However, under severe WLS, selection of soybean genotypes should focus on grain yield, WUE, SPAD, ΦNO and 100-seed weight. The results are in accordance with Kamrani (2015) who reported differences in selection of traits under varied WLS environments in bread wheat. These authors attributed the differences to differential response of genotypes under different WLS environments.

Further, PCA demonstrated strong negative associations among physiological traits and these results were confirmed by results obtained in correlation analysis. Strong negative associations were observed among ΦII, ΦNO and ΦNPQ and this could be attributed to competition for absorbed energy between ΦII, ΦNO and ΦNPQ. Baker (2008) and Tietz et al. (2017) reported competition of these three traits for the captured light in the photosystem II. The total summation value of ΦII, ΦNO and ΦNPQ is 1.0 (ΦII + ΦNPQ + ΦNO = 1) with a normal range for each parameter between 0.00 and 0.84 (Kramer et al. 2004). This means an increase in one of these photosynthetic parameters, for example ΦII, will result in a decrease in ΦNO and ΦNPQ and *visa versa*.

In addition, the magnitude of negative correlations among physiological traits increased with an increase in WLS levels, with 70% WLS giving the strongest negative correlations among physiological traits. This was indicative of the effect of WLS on photosynthesis processes. The results are in accordance with Mwale et al. (2017) who observed a negative correlation between ΦNPQ and ΦII under WLS conditions. In addition, Park et al. (2010) reported an increase in ΦNPQ with a decrease in ΦII under drought stress conditions. This could consequently have result in the negative associations between ΦNPQ, respectively with ΦNO and ΦII in the current study. This is because WLS induces stomatal closure (Hossaina et al. 2014) and photo-inhibition (Souza et al. 2013) and which ultimately increases chloroplast envelope permeability, alters chloroplast ion concentration as well as inhibits CO_2 assimilation (Souza et al. 2009). In addition, a decrease in the excitation energy trapping efficiency of PSII reaction centres (due to water deficit) results in a decrease in ΦII with an inverse increase in ΦNPQ (Zhang et al. 2016) and ΦNO, reflecting the photo-protective non-photochemical quenching and basal dissipation of light energy for other unregulated processes (Kramer et al. 2004; Baker 2008).

In attempt to identify genotypes that can be used in breeding for high yield performance under WLS conditions, the PCA biplot was used to organise genotypes based on their performance under different WLS regimes. Genotype Magoye, which was strongly associated with biomass, number of pods per plant and grain yield under non-WL, did not exhibit a strong association with grain yield under 70% WLS. Thus, Magoye can be identified as a high performing genotype under non-WL. Genotype TGX1987-62F was associated with high yielding performance under moderate stress (50% WLS), while TGX1988-18F, Makwacha, TGX1988-70F and TGX1990-137F showed average associations with grain yield performance under moderate stress. Genotypes TGX1990-137F and TGX1988-70F showed strong associations with grain yield under severe stress (70% WLS).

Considering that traits WUE and SPAD were ranked best in contributing directly to grain yield under WLS, genotypes TGX1988-70F and TGX1990-137F (that showed average associations with yield performance under 50% WLS and strong associations with yield under 70% WLS) with their strong associations with WUE and SPAD can be considered for selection and be incorporated in breeding for WLS tolerance. Kamrani (2015) and Giordani et al. (2019) similarly selected bread wheat and soybean genotypes, respectively for drought tolerance improvement based on their association with yield in a PCA biplot.

4.6 Conclusions and recommendations

Selection criteria for differed across the different water regimes. Positive associations among traits create opportunities for simultaneous improvement of these traits under WLS. Some morphological and physiological traits contributed directly to soybean grain yield improvement under WLS. WUE was the most direct contributor to yield, followed by SPAD and number of pods per plant, and therefore, these traits should be considered important in selection under

WLS conditions in soybean improvement. Genotypes TGX1988-70F and TGX1990-137F that were associated with WUE and grain yield under WLS can be recommended for incorporation in breeding programmes for high yield performance under drought stress conditions.

4.7 References

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CHAPTER 5

Assessing drought tolerance indices in soybean subjected to optimum and water-limited stress conditions

5.1 Abstract

Soybean is regarded as a short season crop, which flowers over a wide window of time and thus can tolerate both terminal and intermittent drought. However, not all soybean genotypes are early-maturing and have the ability to escape drought effects. Several drought tolerance indices have been used in soybean and other crops to select genotypes for cultivation under both non-water limited stress (non-WL) and water-limited stress (WLS) conditions. The aims of this study were to identify drought tolerance indices that are effective in selection for tolerant genotypes and to discriminate between drought tolerant and sensitive soybean genotypes used in this study. Twelve soybean genotypes were evaluated over two seasons, 2016 and 2017, using two water regimes in the field laid out in a factorial design with three replications. Grain yield under non-WL (Yp) and WLS (Ys) conditions were determined and used to calculate drought tolerance indices. Geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HM), stress tolerance index (STI) and yield index (YI) were positively and significantly ($p<0.001$) associated with both Yp and Ys ($r = 1.00$ to 0.70). GMP, MP, HM, STI and YI positively contributed most to the total variation in the first two principal components (PCs). The indices that were associated with both Yp and Ys and contributed most to the PCs could be suitable indices for discriminating drought tolerant genotypes from sensitive genotypes. Genotype TGX1990-137F was strongly associated with YI, gave the highest drought resistance index (DI) and exhibited the highest Ys. Both principal component analysis and cluster analysis grouped genotype TGX1990-137F as highly drought tolerant and TGX1987-10F as highly sensitive genotypes. Genotypes that were regarded drought tolerant are TGX1988-9F, TGX1987-23F and Magoye, while Tikolore, Nasoko, Makwacha TGX1988-18F, TGX1988-70F, TGX1987-62F were regarded as moderately drought tolerant. Statistical analytical tools efficiently complemented each other in separating drought tolerant genotypes from sensitive genotypes as well as to identify interrelationships among the tolerance indices.

Keywords: Drought tolerance indices, soybean, yield

5.2 Introduction

Soybean is one of the most important crops in the world as it provides a source of protein for both humans and animals (Ristova et al. 2010). However, soybean has been drastically affected by drought (Kananji et al. 2013) by limiting plant growth (Hossaina et al. 2014; Moloi et al. 2016), development (Mwenye 2018), performance and consequently productivity (Mabulwana 2013). Soybean production in Malawi has also been negatively affected by drought stress because the country experiences both terminal and intermittent drought stress (Kananji et al. 2013).

The conventional method to select for drought tolerance has been to compare the yield of a given genotype under non-stress conditions with the yield of the same genotype under drought stress conditions. However, such selection criteria have rendered genotypes susceptible to drought stress over time and across environments. Research has shown that selection for high yield potential under optimal conditions does not necessarily result in high yield under WLS conditions (Talebi et al. 2013). In order to combat the problems with drought that varies across environments and seasons, and which are escalated by global warming (Xu et al. 2010), effective selection criteria are needed to identify drought tolerant genotypes (Anwar et al. 2011; Agili et al. 2012). Selection indices have proven to be the most useful tools for determining high yielding and potentially stress tolerant genotypes (Papathanasiou et al. 2015). Abd El-Mohsen et al. (2015) suggested the use of a stress susceptibility index (SSI) for measurement of yield stability that captured the change in both potential and actual yields in variable environments. Fard and Sedaghat (2013) used the stress tolerance index (STI), geometric mean productivity (GMP) and mean productivity (MP) indices to select bread wheat genotypes that are tolerant to WLS conditions and the stress susceptibility index (SSI) to estimate the rate of change in yield for each genotype between WLS and non-WL conditions.

More recent indices such as the drought resistance index (DI), yield stability index (YSI) and harmonic mean (HM) have been accepted for identification of genotypes producing high yield under both WLS and non-WL conditions (Agili et al. 2012; El-Rawy and Hassan 2014; Abraha et al. 2017). High values of yield index (YI), YSI, DI and HM have been reported to characterise tolerant genotypes in wheat (Ali and El-Sadek 2016). However, identification of drought tolerant genotypes based on a single index tends to be difficult (Naghavi et al. 2013), considering that individual indices identify and rank genotypes differently for drought tolerance (Abd El-Mohsen et al. 2015). Therefore, the use of several drought tolerance indices in combination with principal component analysis (PCA), correlations and genotype clustering, would be ideal in efficient selection of drought tolerant genotypes. The aim of this study was to evaluate the ability of several drought tolerance indices to identify drought tolerant genotypes under three different water regimes. The specific objectives were to (1) identify the most suitable indices for drought tolerance of soybean genotypes under two different water regimes and (2) discriminate between drought tolerant and sensitive genotypes based on the drought tolerance indices, correlations, PCA and clustering methods.

5.3 Materials and methods

5.3.1 Plant material

The 12 soybean genotypes used in this study were described in Chapter 3, section 3.3.1 and Table 3.1.

5.3.2 Experimental location, design and water regime

The experiment was conducted at the experimental site of the DARS, specifically the Kasinthula Research Station during the 2016 and 2017 dry periods from July to October as described in Chapter 3, section 3.3.2. The same planting and trial management practices as described in Chapter 3, section 3.3.2, were followed. The experiment was laid out in a factorial design with three replications. Factor A included three water regimes, while factor B included 12 soybean genotypes. The water regimes were applied as described in section 3.2.2 to obtain data on non-WL, 50% WLS and 70% WLS levels.

5.3.3 Data collection

Mean grain yield, obtained from Chapter 3, section 3.4.2 was used to calculate the drought tolerance indices. Ten drought tolerance indices were calculated using the equations in Table 5.1. The grain yield means (kg ha^{-1}) used for the calculations included mean yield values of each genotype under non-WL (Yp) and 70% WLS (Ys) conditions, the mean yield of all genotypes under 70% WLS ($\bar{Y}s$) and the mean yield of all genotypes under non-WL ($\bar{Y}p$) conditions.

5.3.4 Data analysis

Simple correlation coefficients between Yp, Ys and all drought tolerance indices were determined to establish relationships among the indices and grain yield under both non-WL and WLS conditions. Principal component analysis was performed using the correlation matrix in order to identify patterns and highlight similarities and differences existing among grain yield and drought tolerance indices. The PCA biplot was constructed in order to increase the interpretation of the data by identifying both correlated and uncorrelated variables (Jolliffe and Cadima 2016). Hierarchical cluster analysis, using nearest neighbour by Euclidean test, was performed to classify the genotypes on the basis of drought tolerance (Naghavi et al. 2013). The correlations, PCA and clustering analysis were all performed using Genstat 19th edition statistical package (VSN International 2018).

Table 5.1: Drought tolerance indices and their equations

Index name	Outcome	Equations	Reference
Mean productivity (MP)	Genotypes with high values for this index will be more desirable	$\mathrm{MP} = \frac{(\mathrm{Ys} + \mathrm{Yp})}{2}$	Naghavi et al. (2013)
Tolerance index (TOL)	Genotypes with low values for this index are more stable over WLS and non-WL conditions	$\mathrm{TOL} = (\mathrm{Yp} - \mathrm{Ys})$	Gholinezhad et al. (2014)
Yield stability index (YSI)	Genotypes with high YSI values can be regarded as stable genotypes under WLS and non-WL conditions	$\mathrm{YSI} = \frac{\mathrm{Ys}}{\mathrm{Yp}}$	Naghavi et al. (2013)
Yield index (YI)	Genotypes with high values for this index will be suitable for WLS conditions	$\mathrm{YI} = \frac{\mathrm{Ys}}{\bar{\mathrm{Y}}\mathrm{s}}$	Naghavi et al. (2013)
Drought resistance index (DI)	Genotypes with high values for this index will be suitable for WLS conditions	$\mathrm{DI} = \frac{\mathrm{Ys}(\mathrm{Ys}/\mathrm{Yp})}{\bar{\mathrm{Y}}\mathrm{p}}$	Abd El-Mohsen et al. (2015)
Yield reduction ratio (YR)	Genotypes with low values for this index will be suitable for WLS conditions	$\mathrm{YR} = 1 - (\frac{\mathrm{Ys}}{\mathrm{Yp}})$	Gholinezhad et al. (2014)
Stress susceptibility index (SSI)	Genotypes with SSI<1 are more resistant to drought stress conditions	$\mathrm{SSI} = \frac{1 - (\mathrm{Ys}/\mathrm{Yp})}{1 - (\bar{\mathrm{Y}}\mathrm{s}/\bar{\mathrm{Y}}\mathrm{p})}$	Kargar et al. (2014)
Harmonic mean (HM)	Genotypes with high values for this index will be more desirable	$\mathrm{HM} = \frac{2(\mathrm{Yp} * \mathrm{Ys})}{(\mathrm{Yp} + \mathrm{Ys})}$	El-Rawy and Hassan (2014)
Stress tolerance index (STI)	Genotypes with high STI values will be tolerant to drought stress	$\mathrm{STI} = \frac{(\mathrm{Yp} * \mathrm{Ys})}{(\bar{\mathrm{Y}}\mathrm{p})\,2}$	Fard and Sedaghat (2013)
Geometric mean productivity (GMP)	Genotypes with high values for this index will be more desirable	$\mathrm{GMP} = \sqrt{}\ (\mathrm{Yp})(\mathrm{Ys})$	Fard and Sedaghat (2013)

WLS = water-limited stress; Ys = yield of a given genotype under WLS conditions; Yp = yield of a genotype under non-WL conditions; Ȳs = mean yield of all genotypes under WLS conditions and Ȳp = mean yield of all genotypes under non-WL conditions

5.4 Results

5.4.1 Use of drought tolerance indices and genotype rankings in grouping tolerant and sensitive soybean genotypes

Mean values for grain yield under non-WL and WLS conditions (Yp and Ys) as well as the tolerance indices for each genotype are presented in Table 5.2. In addition, genotypes were ranked for each value in descending order from number 1 as the top performing genotype to number 12 for the lowest performing genotype (Table 5.3). Genotypes that generally had high values and ranked in the top five positions for Yp, Ys, MP, YI, HM and GMP were Magoye, Nasoko, TXG1987-62F and TGX1990-137F (Tables 5.2 and 5.3). These genotypes have shown above average grain yields under non-WL and WLS conditions and showed high productivity in terms of the respective tolerance indices. However, from these genotypes, only TGX1990-137F ranked high (in the top five positions) for the rest of tolerance indices including TOL (1146), YSI (0.56), DI (0.37), YR (0.44), SSI (0.84) and STI (0.74).

Although genotype TGX1988-70F ranked in the seventh position for both Yp (2446 kg ha^{-1}) and TOL (1191.00), this genotype ranked third for Ys (1255 kg ha^{-1}) and it ranked in the top five positions for MP, YSI, YI, DI, YR, SSI, HM, STI and GMP. Thus, this genotype showed good productivity, especially under WLS conditions and ranked high for most tolerance indices. On the other hand, genotype TGX1989-46 that ranked second for Yp (2951 kg ha^{-1}) and showed high grain yield under non-WL conditions, showed low values for the tolerance indices and a poor yield under WLS conditions (883 kg ha^{-1}). Genotypes TGX1987-23F, TGX1988-9F and Tikolore showed poor yield performance with Yp and Ys values below average and low values for MP, HM and GMP; however, these genotypes ranked in the top five positions for the tolerance indices TOL, YR, SSI and DI (excluding Tikolore).

Results showed that the use of drought tolerance indices can assist in separating WLS tolerant genotypes from sensitive genotypes. Ranking of genotypes, based on their mean values, can help to efficiently differentiate WLS tolerant genotypes from sensitive genotypes with those ranking best as the most tolerant of all genotypes.

5.4.2 Use of simple correlation analysis to determine relationships among the drought tolerance indices

Significant ($P<0.05$) to highly significant ($P<0.001$) correlations (Table 5.4) were observed among drought tolerance indices. Most correlations were highly significant and positive with correlation coefficients ranging between $r = 0.66$ to $r = 1.00$. Some significant negative correlations were observed between YSI with SSI, TOL, Yp and YSI respectively, and between DI with YR. Generally, YSI was negatively correlated to all indices except for DI ($r = 0.68$). Highly significant and negative correlations were observed among drought tolerance indices SSI and YSI; and TOL and YSI. Yield under non-WL (Yp) was positively correlated respectively with GMP, HM, MP SSI, STI, TOL and YI with r-values ranging from 0.69 to 0.94. Yield under WLS (Ys) was significant and positively correlated respectively with DI, GMP, HM, MP, STI, YI and Yp, with correlation coefficients ranging from $r = 0.70$ to 1.00.

Table 5.2: Mean values for grain yield (Yp and Ys) and drought tolerance indices obtained for 12 soybean genotypes subjected to non-water-limited stress and water-limited stress conditions

Genotypes	Yp (kg ha^{-1})	Ys (kg ha^{-1})	MP	TOL	YSI	YI	DI	YR	SSI	HM	STI	GMP
Magoye	3300	1387	3037.00	1913.00	0.42	1.26	0.26	0.58	1.12	1953.10	0.88	2139.42
Makwacha	2517	1177	2435.50	1340.00	0.47	1.07	0.24	0.53	1.02	1603.96	0.57	1721.19
Nasoko	2755	1182	2559.50	1573.00	0.43	1.08	0.22	0.57	1.10	1654.26	0.62	1804.55
TGX1987-10F	1363	549	1230.50	814.00	0.40	0.50	0.10	0.60	1.15	782.73	0.14	865.04
TGX1987-23F	1272	875	1511.00	397.00	0.69	0.80	0.26	0.31	0.60	1036.80	0.21	1054.99
TGX1987-62F	2634	1250	2567.00	1384.00	0.47	1.14	0.26	0.53	1.01	1695.42	0.63	1814.52
TGX1988-18F	2281	1127	2267.50	1154.00	0.49	1.03	0.24	0.51	0.97	1508.62	0.49	1603.34
TGX1988-70F	2446	1255	2478.00	1191.00	0.51	1.14	0.28	0.49	0.94	1658.87	0.59	1752.06
TGX1988-9F	1135	897	1464.50	238.00	0.79	0.82	0.31	0.21	0.40	1002.06	0.19	1009.01
TGX1989-46	2951	883	2358.50	2068.00	0.30	0.80	0.12	0.70	1.35	1359.28	0.50	1614.23
TGX1990-137F	2626	1480	2793.00	1146.00	0.56	1.35	0.37	0.44	0.84	1893.07	0.74	1971.42
Tikolore	2137	1110	2178.50	1027.00	0.52	1.01	0.25	0.48	0.92	1461.08	0.45	1540.15

SSI = Stress susceptibility index; STI = stress tolerance index; GMP = geometric mean productivity; TOL = tolerance level; MP = mean productivity; YI = yield index; DI = drought resistance index; YSI = yield stability index; YR = yield reduction ratio; HM = harmonic mean

Table 5.3: Ranking of 12 soybean genotypes based on drought tolerance indices subjected to non-water limited stress and water-limited stress conditions

Genotypes	Yp	Ys	MP	TOL	YSI	YI	DI	YR	SSI	HM	STI	GMP	$\bar{R}$
Magoye	1	2	1	11	10	2	6	10	10	1	1	1	5
Makwacha	6	6	6	8	8	6	9	8	8	6	6	6	7
Nasoko	3	5	4	10	9	5	10	9	9	5	4	4	6
TGX1987-10F	10	12	12	3	11	12	12	11	11	12	12	12	11
TGX1987-23F	11	11	10	2	2	11	4	2	2	10	10	10	7
TGX1987-62F	4	4	3	9	7	4	5	7	7	3	3	3	5
TGX1988-18F	8	7	8	6	6	7	8	6	6	7	8	8	7
TGX1988-70F	7	3	5	7	5	3	3	5	5	4	5	5	5
TGX1988-9F	12	9	11	1	1	9	2	1	1	11	11	11	7
TGX1989-46	2	10	7	12	12	10	11	12	12	9	7	7	9
TGX1990-137F	5	1	2	5	3	1	1	3	3	2	2	2	3
Tikolore	9	8	9	4	4	8	7	4	4	8	9	9	7

Yp = Mean yield of each genotype under non-WL; Ys = mean yield of each genotype under WLS; SSI = stress susceptibility index; STI = stress tolerance index; GMP = geometric mean productivity; TOL = tolerance level; MP = mean productivity; YI = yield index; DI = drought resistance index; YSI = yield stability index; YR = yield reduction ratio; HM = harmonic mean; $\bar{R}$ = mean rank

Table 5.4 Correlation coefficients for grain yield under non-water-limited stress and water-limited stress, and the drought tolerance indices obtained for 12 soybean genotypes

	DI	GMP	HM	MP	SSI	STI	TOL	YI	Yp	YR	Ys	YSI
DI	-											
GMP	0.34	-										
HM	0.47	0.99***	-									
MP	0.33	1.00***	0.98***	-								
SSI	-0.68**	0.43	0.31	0.44	-							
STI	0.35	0.99***	0.98***	0.99***	0.43	-						
TOL	-0.32	0.76***	0.66*	0.78**	0.86***	0.76**	-					
YI	0.70**	0.91***	0.96***	0.90***	0.03	0.90***	0.42	-				
Yp	0.01	0.94***	0.87***	0.94***	0.69**	0.93***	0.94***	0.71***	-			
YR	-0.68**	0.43	0.31	0.44	1.00***	0.42	0.86***	0.03	0.69**	-		
Ys	0.70**	0.91***	0.96***	0.90***	0.03	0.90***	0.42	1.00***	0.71**	0.03	-	
YSI	0.68*	-0.43	-0.31	-0.44	-1.00***	-0.42	-0.86***	-0.03	-0.69*	-1.00***	-0.03	-

*, **, *** Significant at $P< 0.05$, $P<0.01$, $P<0.001$, respectively; WLS = water-limited stress; Yp = mean yield of each genotype under non-WL; Ys = mean yield of each genotype under WLS; SSI = stress susceptibility index; STI = stress tolerance index; GMP = geometric mean productivity; TOL = tolerance level; MP = mean productivity; YI = yield index; DI = drought resistance index; YSI = yield stability index; YR = yield reduction ratio; HM = harmonic mean

5.4.3 Grouping of genotypes based on principal component analysis and cluster analysis

The PCA grouped the drought tolerance indices into 12 components, which accounted for 100% of the variability among the genotypes (Table 5.5). The first five principal components (PC) are presented showing their contribution values, eigenvalues, % variation contributed by each PC and the cumulative %. The first two PCs had eigenvalues of more than 1.0 with the first PC having the highest eigenvalue of 7.90. The first two PCs explained 98.91% of the total variation. The first PC, which explained 65.82% of the total variation among the genotypes was positively attributed to variation in GMP, HM, MP, STI and Yp. The second PC contributed 33.09% to the total variation among the genotypes due to positive loading of DI, YI, YSI and Ys.

Genotype grouping was presented in both the PCA biplot (Figures 5.1) and the dendrogram (Figure 5.2). In the PCA biplot, tolerance indices that were associated with high mean productivity and those that contributed most to the total variation in PC1 were clustered together in quadrant Z of the PCA biplot (Figure 5.1). Strong positive associations were observed among MP, GMP, HM, STI and Yp, as well as between SSI and YR in the PCA biplot and these were verified by the strong and highly significant positive correlations between these indices in the correlations table (Table 5.4). The strong and significantly negative association between YSI and SSI was confirmed by results from the correlations table.

Genotype TGX1987-10F that formed its own cluster, Cluster 1 in the PCA biplot (Figure 5.1) as well as Cluster I in the dendrogram (Figure 5.2), was characterised by low Yp and Ys values and ranked in the bottom two positions for most drought tolerance indices. This genotype is, therefore, regarded as poor yielding under both non-WL and WLS conditions and is sensitive to WLS conditions. Genotype TGX1990-137F that clustered separately in Cluster 2 in the PCA biplot and Sub-cluster I in the dendrogram, was positively associated with Ys and YI followed by GMP, HM, MP and STI in the PCA biplot. Genotype TGX1989-46 that formed its own cluster, Cluster 3 in the PCA biplot and Sub-sub group A in the dendrogram, was positively associated with Yp but ranked in the bottom positions for Ys and the tolerance indices. Thus, this genotype is regarded as sensitive and high yielding under non-WL conditions only. Genotypes TGX1988-9F and TGX1987-23F that formed Cluster 4 in the PCA biplot and were grouped among other genotypes in Group I in the dendrogram, ranked in the bottom positions for Yp and Yp but ranked highly for the tolerance indices TOL, YSI, DI, YR and SSI. Thus, these genotypes were poor yielding under both WLS and non-WL conditions but showed more tolerance to WLS conditions.

Table 5.5 Loadings of grain yield under non-water limited stress and water-limited stress, and the drought tolerance indices on the first five principal components analysed based on correlation matrix

Tolerance indices	Principal components (PC)				
	PC1	**PC 2**	**PC 3**	**PC 4**	**PC 5**
DI	0.05659	0.49338	0.15985	0.30443	0.75321
GMP	0.34908	0.09644	-0.03916	0.06811	-0.21843
HM	0.33568	0.1638	0.14637	0.00752	-0.39415
MP	0.34963	0.09004	-0.13419	0.14847	-0.02886
SSI	0.21289	-0.39851	0.3197	0.06623	0.14504
STI	0.34674	0.09863	-0.03673	-0.88806	0.28225
TOL	0.30822	-0.22923	-0.60595	0.1736	0.16966
YI	0.28899	0.29014	0.22995	0.09285	-0.16023
YR	0.21274	-0.39864	0.32100	0.07442	0.14597
YSI	-0.21274	0.39864	-0.32100	-0.07442	-0.14597
Yp	0.34915	-0.07114	-0.38849	0.17056	0.07299
Ys	0.28899	0.29014	0.22995	0.09285	-0.16023
Eigenvalues	7.90	3.97	0.11	0.01	0.01
% Variation	65.82	33.09	0.92	0.12	0.05
% Cumulative	65.82	98.91	99.83	99.95	100.00

WLS = Water-limited stress; Yp = mean yield of each genotype under non-WL; Ys = mean yield of each genotype under WLS; SSI = stress susceptibility index; STI = stress tolerance index; GMP = geometric mean productivity; TOL = tolerance level; MP = mean productivity; YI = yield index; DI = drought resistance index; YSI = yield stability index; YR = yield reduction ratio; HM = harmonic mean

Genotype Magoye that formed Cluster 5 in the PCA biplot and Group II in the dendrogram was highly and positively associated with Yp, MP, HM, GMP and STI, followed by YI. Thus, this genotype showed high yield performance under both non-WL and WLS conditions and ranked in the top two positions for grain yield (Yp and Ys) performance. However, this genotype ranked in the lower positions for TOL, YSI, YR, SSI and DI and was, therefore, regarded as sensitive. Genotypes that formed Cluster 6 in the PCA biplot and some of which were grouped in Group 1 of the dendrogram were good performing in terms of Yp and Ys under non-WL and WLS conditions, MP, HM, STI and GMP (except for Makwacha and Tikolore). However, they showed ranking changes in terms of the other tolerance indices.

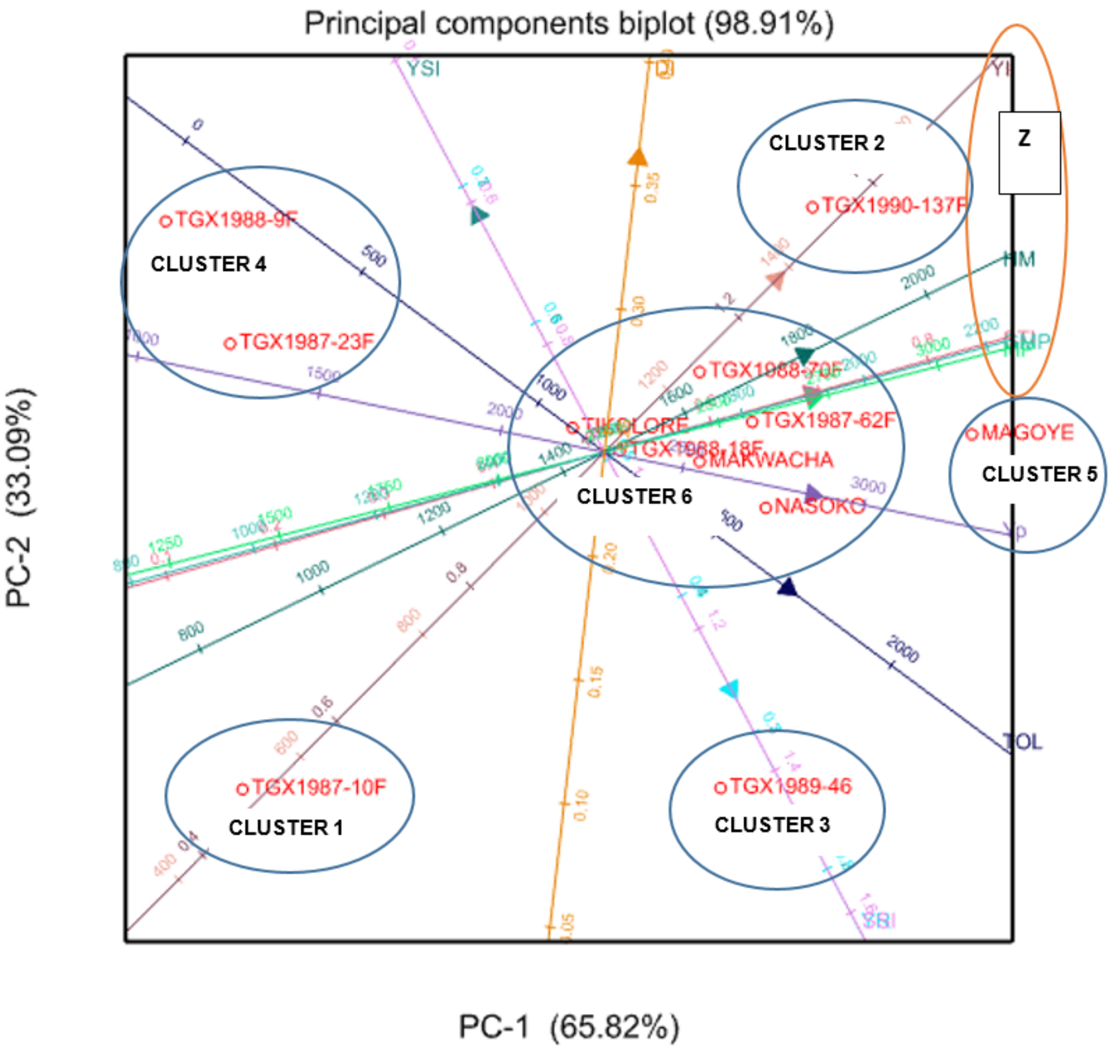

Figure 5.1: Biplot showing the first two principal components to present the associations between grain yield and drought tolerance indices for 12 soybean genotypes subjected to water-limited stress. Yp = Mean yield of each genotype under non-WL; Ys = mean yield of each genotype under WLS; SSI = stress susceptibility index; STI = stress tolerance index; GMP = geometric mean productivity; TOL = tolerance level; MP = mean productivity; YI = yield index; DI = drought resistance index; YSI = yield stability index; YR = yield reduction ratio; HM = harmonic mean

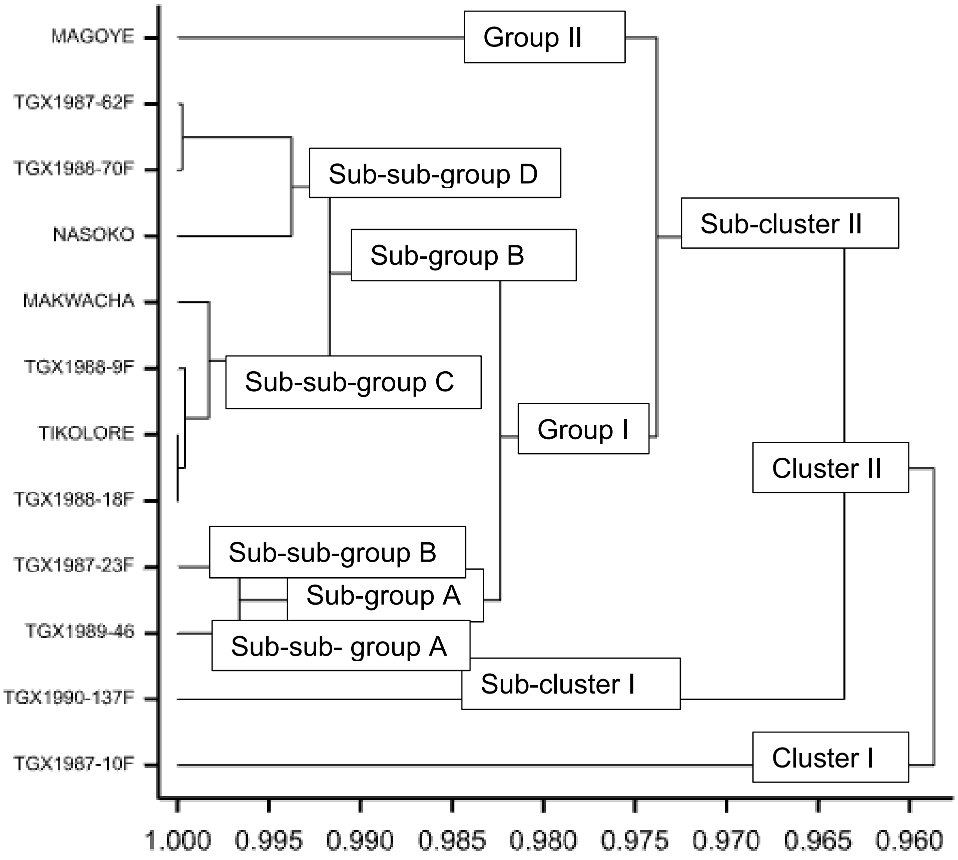

Figure 5.2: Dendrogram obtained from hierarchical cluster analysis using drought tolerance indices showing classification of 12 soybean genotypes based on similarity coefficients among genotypes subjected to water-limited stress

The results obtained from both the PCA biplot and dendrogram showed that soybean genotypes can be clustered and separated based on their level of tolerance or sensitivity to WLS by using the drought tolerance indices. A pictorial view of the PCA biplot showed the interrelationships among the drought tolerance indices as well as the association between genotypes and the indices.

5.5 Discussion

The correlation coefficients, PCA and clustering analysis of soybean grain yield and the drought tolerance indices under non-WL and WLS conditions were determined in order to determine relationships among tolerance indices and to separate drought tolerant genotypes from sensitive genotypes. These analytical tools were used as a way of identifying the most desirable and efficient drought stress selection criteria. Genotypes with high GMP are desirable since this indicates their relative performance over seasons and environments considering that drought stress tends to vary in severity across environments and over years (Fard and Sedaghat 2013). El-Rawy and Hassan (2014) and Gholinezhad et al. (2014) indicated that a desirable drought tolerant genotype, selected based on drought indices, should be one with high values of MP, GMP, YI, YSI, DI, STI, low values of TOL, YR and a SSI value of <1. Abd El-Mohsen et al. (2015) and Yahoueian et al. (2017) described genotypes with high YSI and low TOL as more stable both under well-watered and WLS environments. In addition, Naghavi et al. (2013) described genotypes with SSI values of <1 as more tolerant to drought conditions. Such traits were exhibited in genotype TGX1990-137F, which had higher mean grain yields coupled with high values of MP, HM, GMP, STI, YSI, YI and DI and low values of TOL, YR and SSI value of 0.84. This genotype may, therefore, be more desirable for drought stress conditions than other tested genotypes.

Genotypes TGX1989-46 and TGX1987-10F that ranked in the bottom five positions for Ys, and all the tolerance indices showed that they have low productivity under WLS conditions and were drought sensitive. Although, these two genotypes were bred for drought tolerance, they still showed sensitivity under WLS which could be attributed to the intensity and period of the stresses that the genotypes were exposed to (70% WLS from 4 weeks after planting up to harvesting). Similar observations were made on soybean (Kargar et al. 2014; Van der Merwe et al. 2018) and other crops such as wheat (Anwar et al. 2011; Farshadfar et al. 2012) and maize (Menezes et al. 2014) where genotypes with low values of GMP, Ys, YSI, MP and STI were identified as drought sensitive. On the other hand, genotypes TGX1987-23F and TGX1988-9F showed the poorest productivity under both non-WL and WLS conditions but these showed some tolerance since they ranked in the top one to four positions for TOL, YSI, DI, YR and SSI, respectively.

Results from the current study have shown that mean rank, based on indices, can effectively be used to discriminate between drought tolerant and sensitive genotypes. The results are in accordance with Naghavi et al. (2013) and Abd El-Mohsen et al. (2015) who efficiently used mean rank in separating drought tolerant from sensitive genotypes of maize and bread wheat, respectively. Mean rank has also been used in soybean (Yahoueian et al. 2017) in separating drought tolerant from sensitive genotypes. Genotype TGX1990-137F that ranked in the top five positions across all indices, was identified as the most tolerant and best performing genotype. Genotypes TGX1987-10F and TGX1989-46 were the poorest performing under

WLS and were sensitive to WLS as they ranked in the bottom positions for all tolerance indices. The use of ranking to identify drought tolerant genotypes has been used in bread wheat (Farshadfar et al. 2012) and confectionery sunflower (Gholinezhad et al. 2014).

The tolerance indices GMP, HM, MP, STI and YI were highly correlated with each other. The significant positive correlations between these indices were confirmed by the high and positive r-values ranging from 1.00 to 0.66. These indices were positively correlated with yield under both non-WL (Yp) and WLS (Ys) with r-values ranging from 1.00 to 0.71. The results are in agreement with findings of Mwenye (2018), Yahoueian et al. 2017 and Van der Merwe et al. (2018) in soybean and these indices have the ability to identify genotypes producing high yields under both WLIS and non-WLIS conditions. TOL that showed significant positive correlations with MP, GMP and HM and yield under non-WL (Yp) did not show any significant correlation with yield under WLS (Ys). TOL was more associated with yield under non-WL environments compared to WLS environments and, therefore, can be used as a selection criterion under non-WL environments. Similar observations were reported by Menezes et al. (2014) in grain sorghum who suggested that TOL cannot be used to identify genotypes with good yield in both environments.

Principal component analysis has been previously used to indicate associations between tolerance indices and genotypes in order to separate drought tolerant genotypes from sensitive genotypes (Mwenye 2018). The cosine of the angle between the vectors of two indices in a biplot approximates the correlation coefficient between them (Menezes et al. 2014). Even though the cosine of the angles does not precisely translate into correlation coefficients considering that biplots do not explain all of the variation in a dataset, the angles are informative enough to allow a whole picture about the interrelationships among the indices (Abd El-Mohsen et al. 2015). SSI with an angle of >90° to YSI are strongly negatively correlated. Strong positive associations were observed between GMP, HM, MP, STI and YI with angles of <90°. Results from the current study are in accordance with Yahoueian et al. (2017) who determined associations among GMP, HM, MP and STI based on angles between them and their alignment in relation to Yp and Ys in soybean. Similar observations were reported for sorghum (Menezes et al. 2014) where positive associations between Yp and Ys with MP, GMP, HM and STI were also identified by acute angles.

PCA results supported of correlation results. All strong associations observed in the PCA biplot were significant in the correlation results. For example, indices GMP, HM, MP, YI that were significantly and positively correlated with grain yield both under non-WL (Yp) and WLS (Ys) conditions in the correlation table showed strong associations with Yp and Ys in the PCA biplot. In addition, these indices that showed highly significant correlations and strong positive associations among each other in correlation analysis and PCA biplot contributed the most to the variation in the first two PCs. Indices that made strong positive contributions to the variations in PC1 were associated with increased mean productivity, while indices that made strong positve contributions to the variation in PC2 were associated with increased yield stability index. As such, indices that contributed most to both PC1 and PC2 were more desirable and may have the best ability to differentiate between high yielding, drought tolerant and sensitive genotypes. Therefore, all indices that were in quadrant Z (YI, HM, STI, and GMP MP) can be considered as suitable drought selection indices for soybean crop improvement.

Besides soybean (Naghavi et al. 2013; Mwenye 2018; Yahoueian et al. 2017; Van der Merwe et al. 2018), indices including MP, GMP, YI and HM have been reported to be associated with selection under both non-WL and WLS conditions in other crops. Such crops include orange-fleshed sweet potato (Agili et al. 2012), bread wheat (Fard and Sedaghat 2013), confectionery sunflower (Gholinezhad et al. 2014), grain sorghum (Menezes et al. 2014) and maize (Papathanasiou et al. 2015).

Mwenye (2018) and Van der Merwe et al. (2018) considered PC1 and PC2 in a PCA biplot as indicators of yield potential and yield stress torelant demessions respectively under both non-WL and WLS conditions. This is confirmed in the current study where genotype TGX1990-137F in Cluster 2, which was positively associated with Ys, YI, GMP, HM, MP and STI had both high contributions in PC1 and PC2, was situated in the direction of high yield potential. Similarly, genotypes TGX1988-9F and TGX1987-23F in Cluster 4 in the biplot, with low contributions to PC1 but high contributions to PC2, had low yield but they exhibited higher tolerance to WLS.

Genotypes TGX1988-9F and TGX1987-23F were the top two genotypes for TOL, YSI, YR and SSI. This indicated that although these were low yielding genotypes, they they exhibited higher drought tolerance than other genotypes under WLS conditions. Such genotypes need to be considered when selecting genotypes for soybean improvement programmes for drought tolerance by hybridising with high yielding genotypes. Results from the current study was supported by previous reports that indicated the usefulness of PCA as a tool in determining both real genotype performances and drought tolerance levels (Golabadi et al. 2015; Ganança et al. 2018).

Cluster analysis further classified genotypes based on drought tolerance indices. The nature of clusters obtained in the PCA biplot were generally similar to clusters obtained in the dendrogram. Naghavi et al. (2013) used cluster analysis to classify maize inbred lines and grouped those with the highest MP, GMP, YI, YSI, DI and STI in the tolerant group, those with average indicator values as semi-tolerant and those with high SSI values as sensitive to drought. A similar trend was observed in the results of the current study. Genotypes that were classified as having below average grain yield under WLS and non-WL conditions, were found in Cluster 1 (TGX1987-10F) and Cluster 4 (TGX1988-9F and TGX1987-23F) in the PCA biplot and Cluster I and Group I (Sub-group and Sub-sub group C, respectively) in the dendrogram. These genotypes were highly dissimilar from genotypes that had high and above average grain yields in Cluster 2 (TGX1990-137F) and Cluster 5 (Magoye) in the PCA biplot and Sub-sub cluster I and Group II in the dendrogram, respectively. Genotype TGX1990-137F with an acute angle of <90° was positively associated with YI, YSI and DI, while Magoye was positively associated with GMP, MP, HM and STI.

Genotype TGX1989-46 was not positively associated with any of the tolerance indices although it had a good yield under non-WL conditions. This genotype formed its own group, Cluster 3 in the PCA biplot and clustered in Sub-sub-group A in the dendorgram. Genotypes, TGX1987-62F, TGX1988-70F, Nasoko and Makwacha TGX1988-18F, Tikolore and TGX1988-18F grouped in Cluster 6 of the PCA biplot and Group I in the dendrogram. These were good to average performing in terms of Yp and Ys under non-WL and WLIS conditions, MP, HM, STI and GMP. However, they showed ranking changes in terms of the other tolerance indices.

When cluster analysis is performed on drought tolerance indices, two main clusters with either three or four sub-groups are formed (Gholinezhad et al. 2014; Abd El-Mohsen et al. 2015; Van der Merwe et al. 2018). Genotypes were clustered as drought tolerant, semi-tolerant, sensitive and/or highly sensitive. Similarly, two main clusters were observed in the dendrogram, which clustered genotypes in Cluster I as sensitive and Cluster II as semi-tolerant to tolerant genotypes. Genotypes in Cluster II were further divided into groups and sub-groups as tolerant and moderately tolerant genotypes. Results have demonstrated that both clustering and PCA biplot analysis have the capacity to separate drought tolerant genotypes from sensitive genotypes in soybean.

5.6 Conclusions and recommendations

The drought tolerance indices MP, GMP, HM, STI and YI were superior indices for identifying high yielding and drought tolerant genotypes to be grown under WLS conditions because they were able to effectively characterise genotype performance both under non-WL and WLS conditions. Based on PCA and clustering analysis, genotypes were separated into two main groups. Group A consisted of the most drought tolerant, highest yielding genotype under WLS conditions (TGX1990-137F) and average yields as well as low yield but moderate drought tolerant (Magoye, Tikolore, Nasoko, Makwacha TGX1988-18F, TGX1987-62F, TGX1988-70F TGX1988-9F and TGX1987-23F). Group B consisted of genotypes that are high yielding under non-WL but highly sensitive to drought and genotypes that are low yielding and highly drought sensitive under WLS conditions (TGX1989-46 and TGX1987-10F). Further trials are necessary in order to determine genotype performance and stability across environments and seasons when WLS occurs at specific growth stages.

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CHAPTER 6

Genotype by environment interaction of soybean under optimum and water-limited stress conditions

6.1 Abstract

Soybean is cultivated in wide range of environments in Malawi. Selection of genotypes that show high and predictable yields with wide adaptability is the main objective of breeding programmes for Malawi. The aims of the study were to identify high yielding and stable genotypes for each of three water-limited stress (WLS) treatments (a non-WL, WLS at flowering and WLS at pod-filling) and to identify the ideal environment (location) for WLS testing. Grain yield stability of six soybean genotypes under three WLS treatments were evaluated over the two seasons of the 2016 and 2017 and across three different environments (Bvumbwe, Masenjere and Kasinthula). The experiment was laid out in a split plot designl with four replications. Highly significant ($P<0.001$) differences were observed between genotypes for grain yield across WLS treatments, environments and seasons. Grain yield was largely influenced by environment and environment by season interaction, which indicated the adaptability of specific genotypes to specific environments. Nasoko, TGX1988-70F and TGX1987-62F showed high yield potential and were ranked as the highest yielding genotypes under non-WL, WLS at flowering stage and WLS at pod-filling, respectively. Both additive main effects and multiplicative interaction analysis (AMMI) and genotype main effects and genotype by environment interaction analysis (GGE) effectively identified stable genotypes. Genotypes TGX1988-9F, TGX1989-46 and TGX1987-62F were stable under non-WL, WLS at flowering and WLS at pod-filling, respectively. Masenjere was a more discriminating and representative environment than the other environments and, therefore, the best site for soybean grain yield evaluation under WLS at pod-filling.

Keywords: AMMI, GGE, grain yield, stability, water-limited stress

6.2 Introduction

Drought stress is one of numerous factors, which limit crop growth and productivity and results in significant yield losses worldwide (Tuberosa 2012). In soybean, drought has a negative impact on grain yield and stability (Mwenye 2018). Kobraei et al. (2011) reported that the critical growth stages for drought stress in soybean are initial flowering (R1 growth stage) and initial seed-fill (R5 growth stage) and, therefore, water shortage during these growth stages will result in a large reduction of grain yield (Ku et al. 2013; Moloi et al. 2016).

The development of high yielding and stable soybean genotypes that are adapted to drought conditions has been a challenge (Cucolotto et al. 2007; Mwenye 2018). This is because drought tolerance is a complex trait that is controlled by numerous genes with small effects (Anithakumari et al. 2012; Mir et al. 2012). Breeding for drought tolerant genotypes is further hampered by the complex interaction that exists between the genotype and its surrounding environment. It has been reported that genotype by environment interaction (GEI) affects genetic gain, selection and recommendation of genotypes with wide adaptability (Namorato et al. 2009; Kivuva 2013). In addition, the occurrence of drought in nature is unpredictable, since it can be permanent, periodic or random and can be either in the early, middle or late season (Pathan et al. 2010; Shashidhar et al. 2013; Rukundo et al. 2014). This has made genotype selection and recommendation even more difficult. Malawi experiences both intermittent and terminal drought stress (Kananji et al. 2013). As such, it would be important to identify adapted genotypes that can give optimum yield when WLS occurs at specific growth stages and at specific locations.

Selection of high yielding and stable genotypes has been possible despite the presence of GEI. This has been achieved by partitioning GEI into adaptability and stability (Cucolotto et al. 2007; Dlamini 2016) through statistical methods such as regression analysis, AMMI and GGE biplot analysis (Yan et al. 2007; Zhe et al. 2010; De Leon et al. 2016). The use of AMMI and GGE biplot analysis are effective for gaining accuracy and informative with regard to environments and genotype performance in GE studies (Cucolotto et al. 2007; Frutos et al. 2014). The AMMI has dual function as it consists of additive and multiplicative components that explain GEI more effectively (Cucolotto et al. 2007; Dlamini 2016). The AMMI has principal component analysis (IPCA 1) that provides interpretation on the residual matrix (Kivuva 2013). On the other hand, the GGE has the inner product property of the biplot and the discriminating power versus representativeness view (Namorato et al. 2009). GGE biplot analysis is effective in evaluating test environments (Namorato et al. 2009; Frutos et al. 2014) and is considered as a powerful tool for visualisation and understanding of complex GEI (Zhe et al. 2010). Therefore, such methods need to be deployed when selecting for genotype stability. The use of these statistical methods, to partition GEI, will help to efficiently characterise the studied genotypes and environments. This will help to effectively select high yielding and stable genotypes that can perform well in both non-WL and WLS environments.

Soybean is widely cultivated across all agro-ecological zones in Malawi (Monyo 2013; Nzima and Dzanja 2015). However, yield per hectare has remained stagnant over years, mainly because soybean in Malawi is constrained by biotic and abiotic factors of which drought is one of the major abiotic stress factors (Kananji et al. 2013). In addition, genotype sensitivity to GEI has not been investigated and documented for the genotypes that are currently being used by Malawian farmers. As such, understanding the nature of GEI and quantifying their magnitude is paramount for breeding, genotypes release and identifying the most discriminating and representative test environments in Malawi. Therefore, the aims of the study were to evaluate the presence of GEI and to determine grain yield stability of six soybean genotypes subjected to WLS induced at two growth stages across three environments and two seasons. The specific objectives were to (1) identify high yielding and stable genotypes for each WLS level, (2) identify the most ideal genotypes and environments under optimum and WLS conditions.

6.3 Materials and methods

6.3.1 Plant material

The plant material used in this study consisted of six soybean genotypes of which two were released genotypes (Monyo 2013) and four were promising lines from IITA germplasm (Table 6.1).

Table 6.1: Characteristics of soybean genotypes used as experimental material

Name of genotype	Description of genotype	Source
Makwacha	It was recommended for production in Malawi in 2003. Medium to late maturity (120 to 140 days), exhibits indeterminate growth and yields up to 3000 kg ha^{-1}.	Zimbabwe
Nasoko	It was recommended for production in Malawi in 2002. Medium to late maturity (120 to 140 days), exhibit indeterminate growth and yields up to 3000 kg ha^{-1}.	Zimbabwe
TGX1988-70F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance.	IITA
TGX1988-9F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance.	IITA
TGX1989-46	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance	IITA
TGX1987-62F	Bred by IITA for drought tolerance. Advanced promising lines for drought tolerance.	IITA

6.3.2 Experiment location, design and water regime

The experiment was conducted at three research stations, Bvumbwe, Masenjere and Kasinthula of the Department of Agricultural Research Services (DARS) in Malawi. The experiment was carried out from July to October during dry period with no rainfall both in 2016 and 2017 to ensure easy management of WLS treatments. The geographical locations and climate characteristics of each research station are described in Table 6.2.

The experiment was laid out in split plot design with four replications. Factor A which was a main plot included three WLS treatments; a non-WL (control), WLS at flowering and WLS at pod-filling, while factor B which was a sub-plot included six soybean genotypes. Seed were sown by hand and spaced 5 cm within rows and 50 cm between rows. Each entry in a replication in the field was represented by four rows, containing 100 plants per row of 5 m. The two outer rows were guard rows and data were collected from two inner middle rows (net plot) with 200 plants per treatment. Fertilizer (NPK 8:18:15) was incorporated in the soil at the rate of 18 g fertiliser per meter length of the ridge by making a groove in the middle of the ridge (Kananji et al. 2013). Soybean was not inoculated with rhizobium in order to mimic farmers' field conditions because most farmers in Malawi do not use inoculants.

Table 6.2: Climate characteristics of experimental locations

Research station	Altitude	Latitude	Longitude	Mean annual rainfall	Mean temperature during experimental period
Bvumbwe	1146 m	15^0 55' S	35^0 04' E	1219 mm	29.45°C
Masenjere	72 m	16^0 20' 0' S	35^0 6' 0' E	850 mm	37.80°C
Kasinthula	60 m	16^0 00' S	34^0 05' E	700 mm	39.21°C

To determine the timing and rate of irrigation, soil moisture storage was estimated from the available water holding capacity as described in Chapter 3, section 3.3.2. In order to apply WLS treatments, the plants were subjected to three water regimes; a non-WL treatment as a control, WLS imposed at the flowering growth stage (WLSF) and WLS imposed at the pod-filling growth stage (WLSP). For the non-WL treatment, plants were well-watered at field capacity soil moisture level throughout the growth cycle until maturity. For the WLSF treatment, plants were well-watered at field capacity soil moisture level up to flowering stage and the plants did not receive water thereafter until maturity. In the WLSP treatment, plants were well-watered at field capacity soil moisture level up to the pod-filling stage and the plants did not receive water thereafter until maturity.

6.3.3 Data collection

At maturity, plants from the middle two rows per plot were harvested. Seeds were manually threshed and weighed in order to calculate grain yield in kg ha^{-1}.

6.3.4. Data analysis

The data collected was subjected to ANOVA. The mean values of genotypes were used to analyse yields under non-WL and WLS treatments. Least significant difference (LSD) test was used to separate the means at 5% level of significance. To determine genotype main effect (G), GEI as well as stability, meta-analysis was performed using AMMI and GGE analysis based on statistical models as described by Kivuva (2013) and Dlamini (2016). Both ANOVA and meta-analysis were performed using GenStat 19th Edition (VSN International 2018).

6.4 Results

6.4.1 Genotype response to water-limited stress across environments and seasons

The combined ANOVA (Table 6.3) performed on the soybean grain yield in response to three WLS treatments across three environments and two seasons showed highly significant ($P<0.001$) effects for genotypes (G), WLS treatments, environments (L) and all interactions. Season (Y) effect was not significant. The largest variation in grain yield was contributed by the WLS treatment effects (23.53%), followed by environment (23.01%) and GxWLSxYxL interaction effects (18.19%). The genotypic main effect contributed minimally to the total variation (2.61%) compared to other main effects, while season (0.28%) contributed least of all the main effects.

Due to the significant interactions of genotypes with stress treatments, ANOVA was done for the separate stress treatments. The mean square values of yield for six genotypes, subjected to three separate WLS treatments across three environments and two seasons are presented in Table 6.4. Under non-WL, highly significant effects were observed for genotype, environment and all interactions, while the season effect was non-significant. The largest contribution to the variation in grain yield was due to interaction between environment and season (LxY) (44.89%), followed by the environment main effect (29.41%).

Results for WLS imposed at flowering (WLSF), showed highly significant effects for genotype ($P<0.01$), environment ($P<0.001$), as well as the interactions GxL ($P<0.001$), GxY ($P<0.01$) and GxLxY ($P<0.001$) (Table 6.4). However, the season effect was non-significant. The environment (68.06%) contributed the largest percentage to the total variation followed by the GxL interaction (10.87%). ANOVA for WLS imposed at the pod-filling (WLSP), showed highly significant ($P<0.001$) effects for genotype, environment, season and all their interactions. The environment (46.54%), followed by the LxY interaction (31.92%) contributed the largest percentages to the total variation. Results from both the combined ANOVA and separate ANOVA have identified the importance of considering the environment main effect when selecting soybean genotypes to be grown under different WLS conditions.

Table 6.3: Combined analysis of variance showing the mean square values of grain yield for six soybean genotypes subjected to water-limited stress across three environments and two seasons

Source of variation	Degrees of freedom	Yield (kg ha^{-1})	% Contribution
Replication	3	59470	0.08
Genotype (G)	5	1938532***	2.61
WLS	2	17499343***	23.53
Season (Y)	1	209134	0.28
Environment (L)	2	17118969***	23.01
GxWLS	10	1459140***	1.96
GxY	5	557450***	0.75
WLSxY	2	1080563**	1.45
GxL	10	416685***	0.56
WLSxL	4	1936196***	2.60
YxL	2	12130828***	16.31
GxWLSxY	10	542743***	0.73
GxWLSxL	20	892622***	1.20
GxYxL	10	760361***	1.02
WLSxYxL	4	4158055***	5.59
GxWLSxYxL	20	13530472***	18.19
Residual	281	92731	0.12
CV (%)		28.8	

***, **, * Significant at P<0.001, P<0.01 and P<0.05, respectively; WLS = water-limited stress

Table 6.4: Analysis of variance showing grain yield mean square values of six soybean genotypes subjected to water-limited stress at two growth stages across three environments and two seasons

Source of variation	Degrees of freedom for Non-WL	Non-WL	% Contribution	Degrees of freedom for WLSF	WLSF	% Contribution	WLSP	Degrees of freedom for WLSFP	% Contribution
Replication	3	198529	0.59	3	116575	2.33	18197	3	0.11
Genotype (G)	5	4238906***	12.60	5	168092**	3.36	263454***	5	1.58
Environment (L)	2	9891946***	29.41	2	3401520***	68.06	7780377***	2	46.54
Season (Y)	1	137701	0.41	1	151445	3.03	2101807***	1	12.57
GxL	10	1286301***	3.82	10	543468***	10.87	439699***	10	2.63
GxY	5	1087218***	3.23	5	170897**	3.42	412854***	5	2.47
LxY	2	15096914***	44.89	2	29772	0.60	5336473***	2	31.92
GxLxY	10	1536119***	4.57	10	485877***	9.72	329684***	10	1.97
Residual	91	160016	0.48	86	46398	0.93	33313	98	0.20
CV (%)		27.40			26.40		20.70		

***, **, * Significant at P<0.001, P<0.01 and P<0.05, respectively; WLS = water-limited stress; WLSF = WLS at flowering; WLSP = WLS at pod-filling

The mean grain yield for each genotype at each WLS treatment, environment and season is presented in Table 6.5. Across environments and seasons, the genotype mean yield ranged from 1034.23 kg ha^{-1} (Makwacha) to 2118.74 kg ha^{-1} (Nasoko) under non-WL, from 719.19 kg ha^{-1} (TGX1989-46) to 942.81 kg ha^{-1} (TGX1988-70F) under WLSF and from 697.05 kg ha^{-1} (TGX1989-46) to 986.10 kg ha^{-1} (TGX1987-62F) under WLSP. Compared to non-WL, WLS both at flowering and pod-filling resulted in a reduced grain yield across all tested genotypes.

Although genotype rankings changed across the three WLS treatments, TGX1987-62F was the only genotype that ranked in the top three positions for mean grain yield across all three WLS treatments. In addition, TGX1988-70F ranked second for non-WL and first for WLSF, respectively.

Across genotypes and seasons, the environment mean yield ranged from 1102.84 kg ha^{-1} (Kasinthula) to 1970.23 kg ha^{-1} (Masenjere) under non-WL, from 576.50 1970.23 kg ha^{-1} (Bvumbwe) to 1102.98 kg ha^{-1} (Masenjere) under WLSF and from 472.69 kg ha^{-1} (Kasinthula) to 1277.65 kg ha^{-1} (Masenjere) under WLSP. Although environment rankings changed across the three WLS treatments, Masenjere ranked in the top position for mean grain yield for all three WLS treatments.

At Bvumbwe the WLS treatment mean for the 2017 season was significantly higher than the 2016 season under both non-WL and WLSP, while under WLSF the means for the 2016 and 2017 seasons were not largely different (Table 6.5). At Kasinthula, a similar trend was observed where the means for the 2017 season was significantly higher than the 2016 season for both non-WL and WLSP, while under WLSF the means for 2016 and 2017 were not largely different. At Masenjere, the WSL treatment means for 2016 were significantly higher than for 2017 across all three WLS treatments.

Table 6.5: Mean grain yield (kg ha^{-1}) of six soybean genotypes subjected to water-limited stress at two growth stages across three environments and two seasons

Genotype	Bvumbwe		Kasinthula		Masenjere		Mean
	2016	**2017**	**2016**	**2017**	**2016**	**2017**	
Non-water-limited stress							
Makwacha	907.40	1312.68	691.55	733.41	1748.75	811.60	1034.23(6)
Nasoko	961.16	2122.40	3350.18	1686.60	3203.29	1388.82	2118.74(1)
TGX1988-70F	1163.65	2801.20	414.04	1595.09	3103.63	1409.41	1747.84(2)
TGX1988-9F	1020.24	1028.38	253.48	1181.79	2428.72	1505.34	1236.32(4)
TGX1989-46	879.88	952.14	280.61	563.24	2401.27	1507.75	1097.48(5)
TGX1987-62F	667.75	1833.47	379.04	2105.03	2965.55	1168.56	1519.90(3)
Treatment mean	**933.35**	**1675.05**	**894.82**	**1310.86**	**2641.87**	**1298.58**	**1459.08**
Environment mean	**1304.20**		**1102.84**		**1970.23**		
LSD$_{0.05}$ (G)							229.38
LSD$_{0.05}$ (L)							162.20
LSD$_{0.05}$ (Y)							132.43
Water-limited stress at flowering							
Makwacha	511.53	265.44	839.63	784.08	1097.42	1245.51	790.60(3)
Nasoko	1178.79	824.40	619.11	305.40	642.72	1032.69	767.18(5)
TGX1988-70F	722.89	779.77	320.45	910.12	1755.90	1167.75	942.81(1)
TGX1988-9F	306.22	464.52	1524.49	364.59	1007.58	1066.82	789.04(4)
TGX1989-46	456.92	340.93	415.20	997.07	997.02	1107.98	719.19(6)
TGX1987-62F	578.78	487.73	1172.13	1000.63	1140.18	974.06	892.25(2)
Treatment mean	**625.86**	**527.13**	**815.17**	**726.98**	**1106.80**	**1099.14**	**816.85**
Environment mean	**576.50**		**771.08**		**1102.98**		
% yield reduction							60.38
LSD$_{0.05}$ (G)							123.70
LSD$_{0.05}$ (L)							87.47
LSD$_{0.05}$ (Y)							71.42
Water-limited stress at pod-filling							
Makwacha	742.44	682.84	227.85	1160.51	1907.97	908.55	938.36(3)
Nasoko	434.54	1181.52	216.42	795.79	1745.32	720.78	849.06(5)
TGX1988-70F	578.80	783.36	213.81	859.55	1795.92	937.87	861.55(4)
TGX1988-9F	683.31	1517.16	112.69	368.90	1515.52	1525.82	953.90(2)
TGX1989-46	441.75	1630.95	72.42	605.82	654.93	776.43	697.05(6)
TGX1987-62F	534.81	1500.60	174.57	863.95	1630.34	1212.30	986.10(1)
Treatment mean	**569.28**	**1216.07**	**169.63**	**775.75**	**1541.67**	**1013.63**	**881.00**
Environment mean	**892.68**		**472.69**		**1277.65**		
% yield reduction							55.98
LSD$_{0.05}$ (G)							104.56
LSD$_{0.05}$ (L)							73.94
LSD$_{0.05}$ (Y)							60.37

LSD = Least significant difference; G = genotype; L = environment; Y = season; number in brackets are ranking number running downwards from 1 as the best

6.4.2 Genotype by environment interaction and stability analysis for grain yield using AMMI analysis

The effects of genotypes, environments and their interactions on soybean grain yield under non-WL, WLS at flowering (WLSF) and WLS at pod-filling (WLSP) are presented in Table 6.6. In this analysis, seasons were considered as blocks and showed non-significant effects for non-WL, WLSF and WLSP, indicating that genotype performance was similar across seasons. Both genotypes and environment effects were highly significant ($p<0.001$) for grain yield under non-WL, WLSF and WLSP, but genotype effect was not significant under WLSP. The first interaction principal components axes (IPCA1) was highly significant for grain yield under WLSF, and significant ($p<0.05$) under both non-WL and WLSP. The second interaction principal components axes (IPCA2) was only significant ($p<0.01$) for grain yield under WLSF. Environments were the largest source of variation, accounting for 41.83%, 53.47% and 67.28% of the treatments sums of squares (SS) for grain yield under non-WL, WLSF and WLSP, respectively. GEI effects were larger than the genotype effects under WLSF and WLSP but not under non-WL. The first IPCA the captured highest portion of the GEI, explaining 86.69%, 76.22% and 62.35% of the total GEI for grain yield under non-WL, WLSF and WLSP, respectively. Results obtained were similar to separate ANOVA (Table 6.4).

The AMMI biplots were created by plotting IPCA1 scores against IPCA2 scores for both genotypes and environments. The AMMI biplot explained 100% of the total GEI (Figure 6.1) across all three WLS treatments. Under non-WL, genotype G6 (TGX1987-62F) was closer to the point of origin, indicating that it did not interact with the environment. Genotypes G4 (TGX1988-9F) and G5 (TGX1989-46) were positively associated with Masenjere (MAS), G3 was positively associated with Bvumbwe (BARS) and G2 (Nasoko) was associated with Kasinthula (KAS). Under WLSF, genotype G5 (TGX1989-46) was closer to the point of origin, indicating that it was more stable across environments compared to other genotypes. Genotypes G6 (TGX1987-62F) and G4 (TGX1988-9F) were positively associated with Kasinthula (KAS), G2 (Nasoko) was positively associated with Bvumbwe (BARS) and G3 (TGX1988-70F), G5 (TGX1989-46) and G1 (Makwacha) were all three associated with Masenjere (MAS). Under WLSP, genotype G6 (TGX1987-62F) was closer to the point of origin. Genotype G4 (TGX1988-9F) was positively associated with Masenjere (MAS) and Bvumbwe (BARS) while G2 (Nasoko), G1 (Makwacha) and G3 (TGX1988-70F) were all three associated with Kasinthula (KAS).

Table 6.6: AMMI analysis of variance for soybean grain yield of six genotypes evaluated over three environments and two seasons with water-limited stress induced at two growth stages

Source of variation	**Non-water-limited**			**Water-limited stress at flowering**			**Water-limited stress at pod-filling**		
	SS	**%SS**	**% of interaction**	**SS**	**%SS**	**% of interaction**	**SS**	**%SS**	**% of interaction**
Total	115092663			21540758			44601684		
Treatments	54888995***			13286708***			18866800***		
Genotypes	21754987***	39.63		1393593***	10.49		1860838	9.86	
Environments	22958585***	41.83		7104707***	53.47		12694349***	67.28	
Block	2703625			633250			616949		
Interactions	10175424	18.54		4788408***	36.04		4311613	22.85	
IPCA1	8820902*	16.07	86.69	3649936***	27.47	76.22	2688275*	14.25	62.35
IPCA2	1354522	2.47	13.31	1138472**	8.57	23.78	1623338	8.60	37.65
Error	57500043			7620799			25117935		

SS = sums of squares

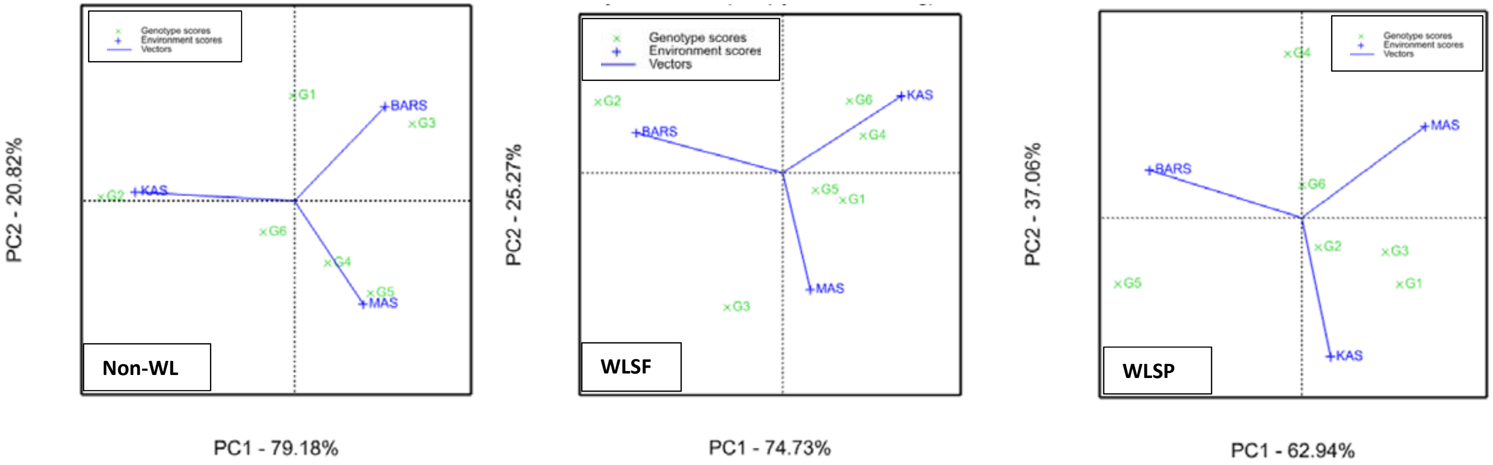

Figure 6.1: AMMI 2 biplot showing IPCA1 against IPCA2 scores for each of the three water-limited stress treatments. G1 = Makwacha; G2 = Nasoko; G3 = TGX1988-70F; G4 = TGX1988-9F; G5 = TGX1989-46; G6 = TGX1987-62F; BARS = Bvumbwe; KAS = Kasinthula; MAS = Masenjere; Non-WL = plants were subjected to non-water-limited condition; WLSF = plants were subjected to water-limited stress at flowering stage; WLSP = plants were subjected to water-limited stress at pod-filling

6.4.3 Genotype by environment interaction and stability analysis using GGE biplot analysis

The GGE biplot analysis was performed to understand the relation among test environments, to identify mega-environments and discriminating/representative environments as well as to identify winning genotypes for each mega-environment. The scatter plot (Figure 6.2) explained 91.86%, 87.09% and 96.20% of the total GEI for grain yield under non-WL, WLSF and WLSP, respectively. These biplots captured less GEI compared to those explained by the AMMI analysis of variance (Table 6.6 and Figure 6.1).

The vector views of the GGE biplot (Figure 6.2) were used to identify the interrelationships between test environments in relation to the grain yield by determining the vector angles between tested environments. Under non-WL, environments Bvumbwe (BARS) and Masenjere (MAS) had an angle of <90° between them, indicating similarities of these environments while Kasinthula (KAS) was the outlier environment. Under WLSF, environments Masenjere (MAS) and Kasinthula (KAS) had an angle of <90°between them, indicating similarities of these environments while Bvumbwe (BARS) had right angle (±90°) with these environments. Under WLSP, environments Bvumbwe (BARS) and Masenjere (MAS) had a more or less right angle between them, as well as Masenjere (MAS) and Kasinthula (KAS). However, Bvumbwe (BARS) and Kasinthula (KAS) had a straight angle between them, indicating dissimilar response of genotypes at these locations.

Although some similarities and dissimilarities were identified for some environments across the different WLS treatments, the associations between environments changed across the different WLS levels as well as genotype responses to these environment. Thus, there is a need to identify specific adaptation of genotypes to environments for each WLS treatment.

The polygon view of the GGE biplot (Figure 6.3) showed both mega-environments as well as the wining genotype (at the vertex) for each mega-environment. Under non-WL, environments Bvumbwe (BARS) and Masenjere (MAS) were located in one mega-environment with TGX1988-70F (G3) as the dominant genotype. Under WLSF, Masenjere (MAS) and Kasinthula (KAS) formed one mega-environment with TGX1988-70F (G3) as the dominant genotype, while Bvumbwe (BARS) formed another mega-environment with Nasoko (G2) being the dominant genotype. Under WLSP, environments Bvumbwe (BARS) and Masenjere (MAS) were located in one mega-environment with TGX1988-9F (G4) as the dominant genotype while Kasinthula (KAS) formed one mega-environment with Makwacha (G1) as the dominant genotype.

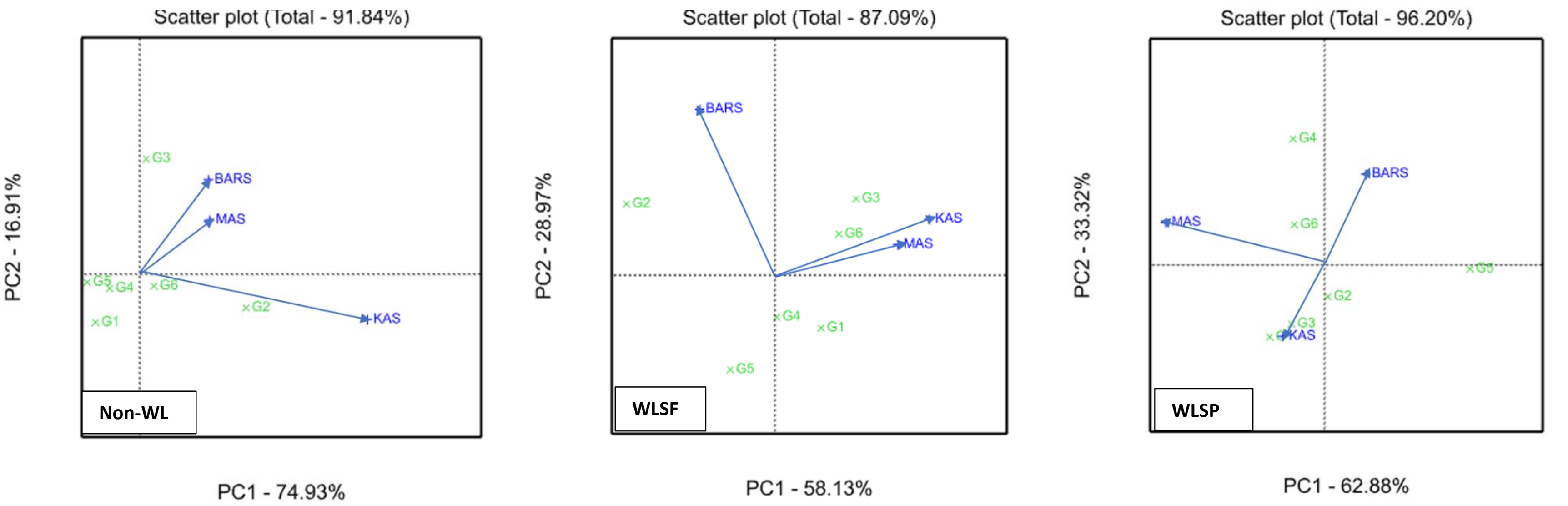

Figure 6.2: GGE biplot showing associations amoung environments for each of the three water-limited stress treatments. G1 = Makwacha; G2 = Nasoko; G3 = TGX1988-70F; G4 = TGX1988-9F; G5 = TGX1989-46; G6 = TGX1987-62F; BARS = Bvumbwe; KAS = Kasinthula; MAS = Masenjere; Non-WL = plants were subjected to non-water-limited condition; WLSF = plants were subjected to water-limited stress at flowering stage; WLSP = plants were subjected to water-limited stress at pod-filling

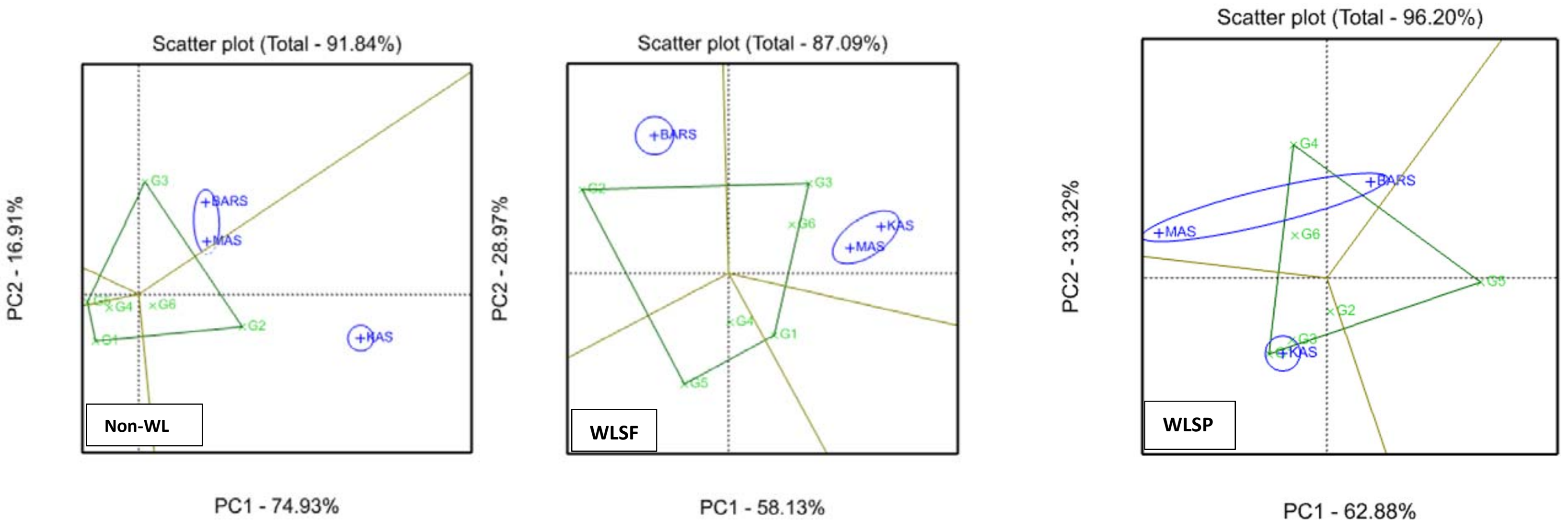

Figure 6.3: Polygon view of the GGE biplot showing mega-environments and which soybean genotype wins where. G1 = Makwacha; G2 = Nasoko; G3 = TGX1988-70F; G4 = TGX1988-9F; G5 = TGX1989-46; G6 = TGX1987-62F; BARS2016 = Bvumbwe 2016; BARS2017 = Bvumbwe 2017; KAS2016 = Kasinthula 2016; KAS2017 = Kasinthula 2017; MAS2016 = Masenjere 2016; MAS2017 = Masenjere 2017; Non-WL = plants were subjected to non-water-limited condition; WLSF = plants were subjected to water-limited stress at flowering stage; WLSP = plants were subjected to water-limited stress at pod-filling

The comparison view of the GGE biplot (Figure 6.4) indicates the discriminating ability and representativeness of test environments and assist in identifying the ideal environment. Under non-WL, test environment Kasinthula (KAS) had the longest vector compared to the other test environments and was regarded the most discriminating environment of the three. In addition, it is closer to the centre of the concentric circles and were thus regarded more ideal than the other environments. However, the cosine of the angle between the environment vector and abscissa of average environment axis (AEC) was smaller for environments Masenjere (MAS) and Bvumbwe (BARS), indicating that these environments were most representative of the mega-environment.

Under WLSP, Bvumbwe (BARS) had the longest vector; however, the cosine of the angle between this environment vector and abscissa of the AEC axis was large. Kasinthula (KAS) had the second longest vector, with a smaller cosine of the angle between the vector and the AEC axis. Of the three environments, Masenjere (MAS) was the most representative. Of the three test environments Kasinthula (KAS) was regarded more ideal as it was closer to the centre of the concentric circles. Under WLSF, Masenjere (MAS) was the most ideal environment as it was closest to the centre of the concentric circles, it was the most representative of the mega-environments and had the strongest discriminating power.

Genotype yield performance mean versus stability was evaluated from the AEC and is shown in Figure 6.5. Under non-WL based on yield performance genotypes were ranked in the order G2>G3>G6>G4>G5>G1. However, genotypes G2 (Nasoko) and G3 (TGX1988-70F) had the largest genotype projections from the AEC ordinate axis of all genotypes even though they had above average grain yield. These genotypes were thus regarded as high yielding but unstable. Genotype G1 (Makwacha), G4 (TGX1988-9F) and G6 (TGX1987-62F) had the smallest projections from the AEC coordinate axis and were, therefore, regarded as the most stable genotypes. However, these genotypes were below average performing in terms of grain yield except G6 (TGX1987-62F).

Under WLSF (Figure 6.5), yield performance of genotypes was ranked in the order G3>G6>G4>G1> G5>G2. Genotype G3 was the highest yielding with a relative good stability. However, genotype G5 (TGX1989-46) was the most stable genotype but was below average performing in terms of grain yield. Under WLSP, yield performance of genotypes was ranked in the order G6>G4> G3>G1>G2>G5. Genotype G4 (TGX1988-9F) was most unstable of all genotypes even though it was the second highest yielding genotype. Genotype G6 (TGX1987-62F) was the most ideal genotype with the highest yield and the smallest projection from the AEC coordinate axis.

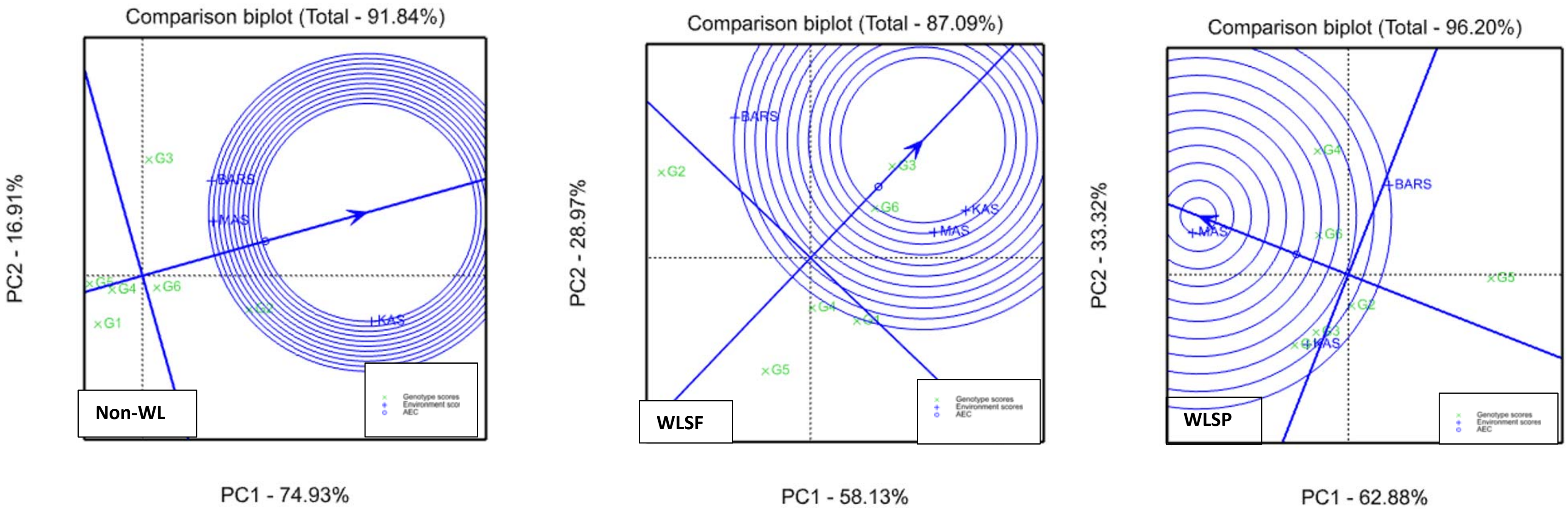

Figure 6.4: GGE biplot showing the discriminating versus representative environment for each water-limited stress treatment. BARS = Bvumbwe; KAS = Kasinthula; MAS = Masenjere; Non-WL = plants were subjected to non-water-limited condition; WLSF = plants were subjected to water-limited stress at flowering stage; WLSP = plants were subjected to water-limited stress at pod-filling

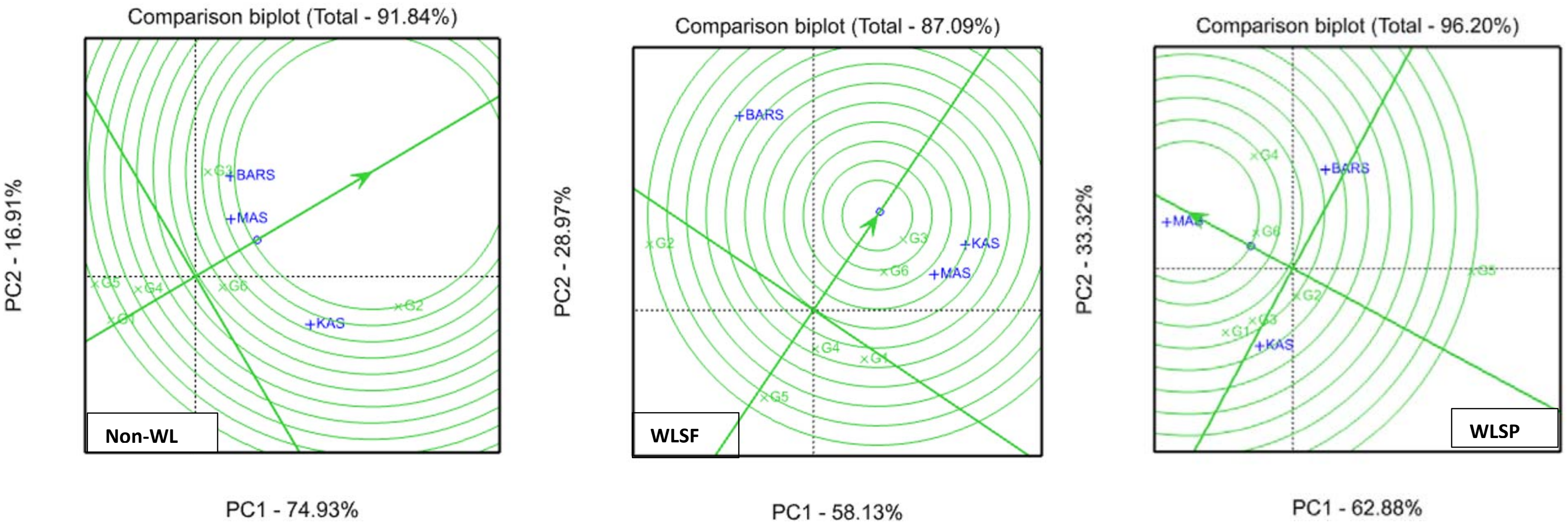

Figure 6.5: GGE biplot showing ranking of genotypes for both mean yield and stability performance. G1 = Makwacha, G2 = Nasoko, G3 = TGX1988-70F, G4 = TGX1988-9F, G5 = TGX1989-46, G6 = TGX1987-62F, BARS = Bvumbwe, KAS = Kasinthula, MAS = Masenjere, Non-WL = plants were subjected to non-water-limited condition; WLSF = plants were subjected to water-limited stress at flowering stage; WLSP = plants were subjected to water-limited stress at pod-filling

6.5 Discussion

One of the problems associated with GEI is that when GEI is significant, genotypes may perform differently across environments and this makes the selection of top performing genotypes difficult (Edugbo et al. 2015; Ramos Junior et al. 2017). In an ideal situation, the top performing genotype should achieve the yield across many environments (Ngalamu et al. 2013; Edugbo et al. 2015). However, yield as the single most important measure of plant productivity, is obscured by a mixture of all environmental conditions over the season, which determine how the plant achieves its end of the season state (De Leon et al. 2016).

Results of the combined ANOVA indicated that WLS treatment, genotype, environment and their interaction effects were highly significant, indicating the importance of determining environmental factors where a genotype grows in order to obtain optimum soybean grain yield. The results are in accordance with De Leon et al. (2016) and Neto et al. (2018) who indicated that phenotypic expression is a function of the genotype, the environment and GEI. Water availability is one of the most important environmental factors that has a large impact on soybean yield (Ngalamu et al. 2013). Drought stress has been reported as the most complex and devastating environmental factor to crop yield (Anithakumari et al. 2012).

Similarly, results from the separate ANOVA indicated grain yield variation under non-WL as a result of interaction between season and environment, followed by environment and genotypes. Environment effects were highly significant and contributed most to the total phenotypic (grain yield) variation among genotypes both at flowering and pod-filling stages. Reduction in grain yield was observed among all tested genotypes when plants were exposed to WLS both at flowering and pod-filling stages with mean yield reduction of 60.38% and 55.98%, respectively. The highest yield reduction was recorded in genotype TGX1989-46 across WLS treatments. A grain yield reduction of up to 88% in soybean at pod development has been reported by Ku et al. (2013). The results are in accordance with Keep et al. (2016), Ramos Junior et al. (2017) and Neto et al. (2018) who reported that soybean grain yield differs across environments and among genotypes. This resulted in differences in genotype ranking across environments in the current study. For example, under non-WL, Nasoko was the top yielding genotype but it ranked second last under both WLSF and WLSP. This genotype was followed by TGX1988-70F for yield performance under non-WL. Also, under WLSF TGX1988-70F, followed by TGX1987-62F, were the top performing genotypes while under WLSP TGX1987-62F, followed by TGX1988-9F were the top performing genotypes.

Even within WLS treatments the genotype rankings changed from one environment and season to the other, indicating high diversity in environmental conditions. Such significant interactions as well as differences between environments and genotypes were also reported by Karasu et al. (2009) and Tefera (2018) when studying soybean genotype stability. Atnaf et al. (2013), Edugbo et al. (2015) and Ramos Junior et al. (2017) attributed differences in genotype ranking across sites and seasons to cross-over GEI effects. Significant GY and GL interactions reflects changes in the ranking of the genotypes for grain yield (Karasu et al. 2009). As such demonstration of significant superiority of any genotype tends to be difficult since their relative rankings usually do not remain the same when genotypes are grown at several locations to test their performance (Namorato et al. 2009; Neto et al. 2018).

Highly significant interactions observed between genotypes, WLS treatments, environments and seasons, therefore, necessitated further characterisation of genotypes and their test environments in order to determine adaptability and stability of the tested genotypes and to identify mega-environments. As such meta-analysis was performed using AMMI and GGE biplots. These statistical models have been consistently reported as useful in identifying high yielding and stable genotypes, for wide adaptation as well as mega-environments (Cucolotto et al. 2007; Zhe et al. 2010; Frutos et al. 2014).

AMMI has been used as a multi-dimensional environmental characterisation tool (De Leon et al. 2016) to analyse GEI and to determine genotype stability in crops such as soybean (Cucolotto et al. 2007), maize (Namorato et al 2009) and sweetpotato (Kivuva 2013; Andrade et al. 2016). ANOVA results obtained from the AMMI were in accordance with results obtained in both the combined and separate ANOVAs where environments, followed by interaction effects, had the largest effects on grain yield. The large variability obtained between test environments resulted in ranking changes of genotypes across WLS treatments. Genotype TGX1989-46 was most stable under WLSF, while TGX1987-62F was as the most stable under non-WL and WLSP, with AMMI scores closer to zero and they were situated closer to the point of origin in AMMI2 biplots. Andrade et al. (2016) also reported changes in rankings among genotypes when investigating genotype stability of sweetpotato subjected to non-WL and WLS. The current results are in agreement with previous findings where higher environmental effect than GEI was reported in sweetpotato (Kivuva 2013) and sugarcane Dlamini (2016). In contrast Atnaf et al. (2013) and Edugbo et al. (2015) noted higher magnitudes of GEI than environmental and genotypic effects in soybean, citing the possibility of obtaining mega-environments.

Mega-environments are segments in a polygon containing two or more similar environments/group of environments (Ramos Junior et al. 2017). Identification of mega-environments helps in extrapolation of genotype performances within mega-environments, which ultimately reduces the number of test environments by removing non-discriminating testing environments within mega-environments (Dlamini 2016). The GGE biplots has consistently been used in identifying mega-environments (Edugbo et al. 2015; Ramos Junior et al. 2017). Angles and distances between environments in a GGE biplot have been used to identify similar environments (mega-environments) (Kivuva 2013; Tefera 2018). Environments with acute angles between them were identified as similar and those with obtuse to straight angles were said to be negatively associated and thus dissimilar (Dlamini 2016). Such differences in angles and distances between environments were also observed in the current study.

Based on the polygon view of the GGE biplot, two mega-environments were consistently identified for each of the three WLS treatments. Under both non-WL and WLSP, Bvumbwe and Masnjere formed one mega-environment and Kasinthula forming another, while under WLSF Kasinthula and Masenjere formed one mega-environment and Bvumbwe forming another. The presence of more than one location in a mega-environment suggests the possibility of removing other sites, which may not be more discriminating for grain yield. However, there was no association between Bvumbwe and Masenjere under WLSP, suggesting that genotype data obtained from Masenjere may not accurately predict genotype performance in Bvumbwe. Thus, there is a need to test these mega-environments for their representativeness.

Winning genotypes are superior genotypes with the highest yield in specific mega-environments and are the vertex genotypes of the polygon (Zhe et al. 2010). These reveal either the best or poorest genotypes in one or the other mega-environment (Frutos et al. 2014). Results of the polygon view of the GGE biplots generally identified genotypes TGX1988-70F, TGX1988-9F, Makwacha and Nasoko as high yielding for specific mega-environments. Genotype TGX1988-70F was the winning genotype under non-WL for the Bvumbwe-Masenjere mega-environment as well as under WLSF for the Masenjere-Kasinthula mega-environment. TGX1988-70F was also recorded as the highest and second highest yielding genotype under WLSF and non-WLF treatments. As such genotype TGX1988-70F can be considered as a promising high yielding genotype specifically for three environments under both non-WL and WLSF conditions. Results obtained from the GGE biplot are in agreement with results from the AMMI that showed similar associations between environments and genotypes. The results are in accordance with findings reported by Kivuva (2013) and Dlamini (2016), who identified high yielding genotypes for mega-environments in sweetpotato and sugarcane, respectively using AMMI and GGE biplot analyses by correlating environments and genotypes as well as by identifying vertex genotypes.

In the comparison view of the GGE biplot, discriminating and representative environments are identified by the sizes of their vectors as well as the angle between the environment vector and abscissa of the average environment axis (Atnaf et al. 2013). The vector length is a measure of the discriminating ability and the ability increases with an increase in vector length (Frutos et al. 2014; Dlamini 2016). The representativeness is measure by the cosine of the angle between the vector and the abscissa of the average environment axis. As such, environments with the most representativeness of the mega-environment, coupled with the greatest discriminating power, tend to have the longest vectors and smallest angle between the environment vector and AEC abscissa point (Atnaf et al. 2013; Edugbo et al. 2015).

In the current study, Masenjere was identified as the most representative environment for grain yield evaluation under non-WL in the Masenjere-Bvumbwe mega-environment, while Kasinthula was the most ideal environment with the most discriminating power. Under WLSF, Kasinthula was the most ideal environment and the most representative of the Kasinthula-Masjenjere mega-environment, while Bvumbwe was the most discriminating environment. Under WLSP, Masjenjere was the most ideal environment with the most discriminating power and it was the most representative of all environments. Thus, the selection of an environment is important in giving more information about genotype differences and these biplots can, therefore, help in to select superior genotypes (Ramos Junior et al. 2017; Tefera 2018).

High yielding and stable genotypes are located on or close to the AEC ordinate axis, which reflects points in the direction of higher mean performance of the genotypes and represents main effects of the genotypes (Yan et al. 2007). An ideal genotype is supposed to be found where the arrow (centre of the concentric circles) is and its projection on the AEC is zero (Frutos et al. 2014). The smaller the genotype projection from the AEC ordinate axis that nears zero, the less interaction with the environments and the more stable the genotype is (Kivuva 2013; Dlamimi 2016; Ramos Junior et al. 2017). Under non-WL, genotypes TGX1988-9F and Makwacha were the most stable genotypes, however, they had mean grain yields below the grand mean. On the other hand, the top two performing genotypes, Nasoko and TGX1988-70F were highly unstable, while the third ranking genotype TGX1987-62F was relatively stable. TGX1987-62F was also identified as stable under non-WL in the AMMI2 biplot.

Under WLSF, TGX1988-70F and TGX1987-62F were the top two performing genotypes and they were relatively stable for grain yield. However, genotype TGX1989-46 was the most stable but with a grain yield below the grand mean. Under WLSP, TGX1987-62F and Nasoko were the most stable genotypes, however, of these two only TGX1987-62F was high yielding and ranked in the top position for grain yield across environments and seasons. These findings are in line with previous studies (Karasu et al. 2009; Namorato et al. 2009; Ngalamu et al. 2013; Neto et al. 2018) where yield performance ranking of genotypes and stability for grain yield changed from one WLS treatment to the other.

6.6 Conclusions and recommendations

Results have demonstrated significant variation in genotypes' response to environment and WLS treatments. Yield reduction was observed due to WLS effects and was more pronounced when WLS was applied at flowering stage. Grain yield was significantly influenced by environmental effects and environment by season interaction effects. Genotype TGX1987-62F was ranked as the most stable under non-WL and WLS at pod-filling, while TGX1989-46 was the most stable under WLS at flowering. However, across WLS treatments, TGX1987-62F was high yielding with general adaptability. The most ideal environments were Kasinthula under non-WL and WLS at flowering, while Masenjere was the ideal and the most discriminating and representative amongst tested environments for grain yield under WLS as pod-filling. These results have indicated that grain yield is very sensitive to GEI effects, and breeding for yield stability is very important.

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CHAPTER 7

Estimation of combining ability and mode of gene action in soybean under optimum and water-limited stress conditions

7.1 Abstract

Development of improved genotypes requires acquiring adequate information on genetic background and nature of gene action for planning appropriate breeding and selection strategies. The aims of this study were to determine the gene action of various soybean traits, to obtain estimates of general and specific combining ability as well as to identify the best combiners to be used as donor parents in developing drought tolerant soybean genotypes. Six genotypes, selected for their high grain yield potential and variability under two different water-limited stress (WLS) treatments, were crossed using the North Carolina II mating design. The F_2 population, together with their parents, were evaluated under non-WL and WLS conditions imposed at flowering in a RCBD using a factorial layout with three replications. Significant (P<0.05) to highly significant (P<0.001) differences were observed among genotypes for all traits both under non-WL and WLS. Families TGX1990-137F x Nasoko performed the best for number of pods per plant, TGX1988-70F x Makwacha gave the highest 100-seed weight and TGX1990-137F x Makwacha was best performing for grain yield under non-WL and WLS. TGX1990-137F x Nasoko and TGX1990-137F x Makwacha gave the highest number of seeds per plant under non-WL and WLS, respectively. TGX1990-137F showed positive and high general combining ability (GCA) estimates for all traits, except 100-seed weight, while TGX1988-70F showed positive and high GCA estimates for 100-seed weight both under non-WL and WLS. TGX1988-70F x Makwacha and TGX1988-70F x Nasoko showed the highest specific combining ability (SCA) estimates for grain yield under non-WL and WLS, respectively. Both additive and dominance gene effects were important in soybean WLS improvement with the predominance of non-additive genetic effects for all four traits tested. Broad-sense heritability estimates were higher than narrow-sense heritability for all traits under non-WL and WLS, indicating a low transfer of genes from parents to offspring.

Keywords: Combining ability, drought tolerance, gene effects, heritability, water-limited stress

7.2 Introduction

Drought stress is one of the most serous economical constraints (Hirut et al. 2017) to crop production in the world. It is even more critical in Sub-Saharan Africa where crop farming is predominantly rain fed (Mwije et al. 2014). Malawi is highly prone to drought stress (Kananji et al. 2013). The rainfall pattern fluctuates from year to year due to the *El Niño* Southern

Oscillation (ENSO) phenomenon (Vincent et al. 2014; PDNA 2017). This ENSO has an influence on the climate of Malawi such that the country often receives erratic and below average rainfall, coupled with prolonged dry spells. This has resulted in severe crop failure of which soybean is inclusive (Chabvunguma et al. 2015). The impact, frequency and spread of drought stress have increased in Malawi in the past 10 years, which resulted in 1% loss of gross domestic product annually, with 60% of the loss being due to crop failure (PDNA 2017). Thus, there is an urgent need to address the drought stress problem in Malawi through crop improvement programmes. Development of drought tolerant genotypes would be one of the appropriate strategies.

Development of improved genotypes requires an understanding of the genetic background and nature of gene action governing the stress factor in the germplasm that is used for breeding. This information is necessary for planning appropriate breeding and selection strategies (Zhang et al. 2005). Soybean genetic effects are reported to be controlled by embryonic, cytoplasmic and maternal effects (Chiari et al 2006; Wen-Xia et al. 2006). The narrow genetic base of soybean genotypes (Bi et al. 2015), coupled with low heritability of grain yield (which is controlled by polygenes) under WLS conditions (Akaogu et al. 2017), make improvement of soybean under drought stress difficult. Other strategies of indirect selection using secondary traits have been employed and was effective (Manavalan et al. 2009). As such, a combination of grain yield and yield components such as number of pods, number of seed and 100-seed weight in soybean for selection of drought tolerance sources could be employed in soybean. These agronomic traits have high heritability (Gavioli et al. 2008; Zeinnab and Helal 2014) and are highly correlated with grain yield (Kobraei et al. 2011; Ghanbari et al. 2018), which makes it suitable for selection under WLS.

The North Carolina mating design has extensively been used to obtain information regarding genetic background and nature of gene action (Nyadanu et.al. 2012; Golabadi et al. 2015; Hirut et al. 2017; Mwale et al. 2017b). Analysis of this design partitions the total variation of population data into general combining ability (GCA) and specific combining ability (SCA) of the crosses (Nduwumuremyi et al. 2013; Salami and Agbowuro 2016). Thus, GCA provides estimates of additive gene effects while SCA estimates non-additive gene action (Machikowa et al. 2011). The North Carolina II mating design further provides information for two independent GCAs (male and female) variances (Fasahat et al. 2016), which gives a better estimation of additive genetic effects. Since it is a bi-parental design, it creates heterozygosity as well as providing information on additive genetic variance and dominance genetic variance (Rodrıguez et al. 2018). The North Carolina II mating design is the most efficient breeding procedure that enables selection of superior genotypes in self-pollinating crops like soybean

(Kiryowa et al. 2008; Fasahat et al. 2016). In soybean, this mating design has been applied to estimate the magnitude of genetic parameters controlling soybean rust resistance and to estimate narrow-sense heritability of the resistance (Kiryowa et. al. 2008). Although studies to understand the mode of gene action and inheritance of important traits in soybean have been conducted under normal production conditions, little has been reported on WLS conditions. The aims of the study were to determine the inheritance and gene action of various traits controlling the expression of soybean grain yield under water-limited stress (WLS) conditions and to identify the best combiners to be used as donor parents in developing drought tolerant soybean genotypes. The specific objectives were to (1) estimate the general and specific combining ability effects of parents and crosses on number of pods, number of seed, 100-seed weight and grain yield and (2) determine the gene action of grain yield components in soybean.

7.3 Materials and methods

7.3.1 Parental material and crosses

The plant material used in this study consisted of six soybean genotypes of which two were released genotypes (Monyo 2013) and four were promising lines from IITA germplasm (Table 7.1). The genotypes used were selected based on their good attributes as discussed in the Chapter 3 and their drought tolerance levels determined and discussed in the Chapter 5.

The parents and progeny were planted both in the field and glasshouse at Bvumbwe Research Station (described in Chapter 6, section 6.3.2) during October 2016 to March 2017. At flowering, crosses were made using the North Carolina II mating design (Kiryowa et al. 2008; Rodrıguez et al. 2018). F_1 plants were grown and left to self-pollinate in the glasshouse during the months of October 2017 to January 2018 to obtain the F_2 population in order to generate enough seed for testing in field condition.

7.3.2 Experiment location, design and water regime

The F_2 population together with their parents were planted at Masenjere and Kasinthula Research Stations (described in Chapter 6, section 6.3.2) from June to October 2018. The experiment was laid out in RCBD using a factorial arrangement with three replications. Seeds were planted by hand and spaced 5 cm within rows and 50 cm between the rows. Each entry in a replication in the field was represented by two rows, containing 20 plants per row. The WLS treatments included a control (non-WL) and WLS treatment. For the non-WL treatment, plants were well-watered at field capacity soil moisture level throughout the growth cycle until maturity. Normal irrigation was supplied, which was kept at field capacity soil moisture based on crop water use computed from climatic data using soil water balance schedule as described in Chapter 3, section 3.3.2 (Fandika et al. 2011). For, the WLS treatment, plants were well-watered at field capacity soil moisture level up to flowering stage and the plants did not receive water thereafter until maturity.

Table 7.1: Male and female parental soybean genotypes with their associated attributes

Parents	Source	Tolerance to drought	Attributes preferred by farmers
		Males	
Makwacha	Zimbambwe - Recommended for production in Malawi in 2003.	Moderate	High 100-seed weight
Nasoko	Zimbambwe - Recommended for production in Malawi in 2002.	Moderate	High 100-seed weight and high grain yield
		Females	
TGX1990-137F	IITA - Advanced promising lines	Highly tolerant	High number of pods and high grain yield
TGX1987-23F	IITA - Advanced promising lines	Moderately tolerant	High number of pods
TGX1988-70F	IITA - Advanced promising lines	Moderately tolerant	High number of pods and high grain yield
Tikolore	IITA - Recommended for production in Malawi in 2011	Moderately tolerant	High number of pods

7.3.3 Data collection

Data were collected from the two rows planted with 40 plants per treatment. At maturity, plants were harvested to determine number of pods per plant, number of seeds per plant, 100-seed weight and soybean grain yield. Plants from the two rows per plot were harvested. Five plants were randomly picked from which number of pods per plant and number of seeds per plant were counted. The weight of 100 seeds was determined by weighing 100 seeds in a beam balance weighing scale in grams. Seeds from plants of the two harvested rows were manually threshed to calculate grain yield (kg ha^{-1}).

7.3.4 Data analysis

Data analyses included combining ability, estimates of genetic parameters and heritability (Table 7.2). ANOVA was done using Analysis of Genetic Designs with R, version 5.0. Genetic estimations were computed using the Henderson's balanced method, which eliminates biases on the estimation of variance components and displays more variance components than restricted maximum likelihood (REML) (Doğan and Kiliç 2014; Rodrıguez et al. 2018). Combing ability ratio was calculated using Baker's ratio as described by Mwije et al. (2014).

Table 7.2: Variance components computed using Henderson's balanced method

Variance components	Genetic interpretations
$\sigma^2 g$	$\frac{1}{r}(MS_g - MS_e)$
$\sigma^2 m$	$\frac{1}{rm}(MS_m - MS_{mf})$
$\sigma^2 f$	$\frac{1}{rf}(MS_f - MS_{mf})$
$\sigma^2 sca$	$\frac{1}{rm}(MS_{mf} - MS_e)$
$\sigma^2 gca$	$(m-1)MS_m+(f-1)MS_f-(m+f-2)MS_{mf})/r(2mf-m-f)$
$\sigma^2 A$	$4\sigma^2 gca$
$\sigma^2 D$	$4\sigma^2 sca$
$\sigma^2 E$	$\frac{\boldsymbol{\sigma}^2 e}{r}$
$h^2 b$	$\frac{\boldsymbol{\sigma}^2 A + \boldsymbol{\sigma}^2 D}{\boldsymbol{\sigma}^2 A + \boldsymbol{\sigma}^2 D + \boldsymbol{\sigma}^2 E}$
$h^2 n$	$\frac{\boldsymbol{\sigma}^2 A}{\boldsymbol{\sigma}^2 A + \boldsymbol{\sigma}^2 D + \boldsymbol{\sigma}^2 E}$

$\sigma^2 g$ = Genotype variance; $\sigma^2 m$ = male variance; $\sigma^2 f$ = female variance; $\sigma^2 sca = \sigma^2_{mf}$ = male x female variance (SCA); $\sigma^2 gca$ = GCA variance; $\sigma^2 A$ = additive genetic variance; $\sigma^2 D$ = dominance genetic variance; $\sigma^2 E$ = environmental variance; $h^2 b$ = broad-sense heritability; $h^2 n$ = narrow-sense heritability (Rodrıguez et al. 2018)

7.4 Results

7.4.1 Analysis of variance for agronomic traits determined under optimum and water-limited stress conditions

The analysis of variance for number of pods per plant, number of seeds per plant, 100-seed weight and grain yield for parents and F2 population was performed using Henderson's method to investigate the response of different genotypes to WLS treatment effects. The WLS levels effects was significantly (P<0.001) the highest contributor of the variation that was observed across all traits which was explained by the high mean squares. The interaction effects for WLS x G was only significant ($P<0.01$) for number of pods and seed per plant while WLS levels effects x SCA effect was significantly ($P<0.01$; $P<0.05$) present for all traits except for 100-seed weight (Table 7.3). GCA (Female) effect contributed most to the total variation under both non-WL and WLS. The GCA/SCA ratio was general high with non-WL recording higher ratios than WLS. However, all GCA/SCA ratios for the traits were ≥0.5 under WLS except for number of pods per plant (Table 7.4).

Table 7.3 Analysis of variance showing the mean square values for agronomic traits of Male x Female across two water regimes

Source of variation	DF	Number of pods per plant	Number of seeds per plant	100-seed weight (g)	Grain yield (kg ha^{-1})
Water regime (WR)	1	64252.66***	316445.41***	772.24***	199800111.87***
Rep x WR	4	637.78	2613.99	6.35	1800316.19
Genotypes (G)	7	5568.90***	18731.34***	130.12***	6186914.45***
GCA $_{\text{Male}}$	1	556.22	10173.93	221.65***	27448.14
GCA $_{\text{Female}}$	3	9367.19***	35265.14***	181.93***	14200950.47***
SCA $_{\text{(male x female)}}$	3	3399.23***	5169.93	51.48*	245580.03
WR x G	7	1649.82**	8195.67**	17.03	1784322.40
WR x GCA $_{\text{Male}}$	1	2400.19	1125.14	49.84	713528.91
WR x GCA $_{\text{Female}}$	3	1213.68	7296.90*	5.70	173097.41
WR x SCA $_{\text{(male x female)}}$	3	1878.12**	11331.38**	13.74	3732932.40*
Residuals	559	649.55	2749.65	19.00	1250475.45

***, **, * Significant at $P<0.001$, $P<0.01$ and $P<0.05$; DF = Degrees of freedom, WLS = water-limited stress, WR = Water limited levels/regimes

Table 7.4: Analysis of variance showing the mean square values for agronomic traits of F_2 progeny and their parents subjected to non-water-limited and water-limited stress conditions

Source of variation	Number of pods per plant	Number of seeds per plant	100-seed weight (g)	Grain yield (kg ha^{-1})
		Non-WL		
Replication	889.85	4094.19	1.49	3116942.16
Genotype	4137.31***	16572.26***	104.87***	3655983.23*
GCA_{Male}	557.03	6712.21	211.57**	609292.50
GCA_{Female}	6852.63***	26789.75***	128.43**	7213090.82**
$SCA_{(male\ x\ female)}$	2615.42*	9641.45	45.74	1114439.22
Residual	896.80	3985.60	20.55	1792404.77
GCA/SCA Ratio		0.55	0.63	0.77
		WLS		
Replication	385.72	1133.78	11.21	483690.22
Genotype	3081.41***	10354.76***	42.28*	4315253.62***
GCA_{Male}	3586.04**	3605.03	38.84	1166031.92
GCA_{Female}	3332.68***	16099.57***	66.24**	6816174.60***
$SCA_{(male\ x\ female)}$	2661.93***	6859.85**	19.48	2864073.21**
Residual	365.22	1328.31	17.23	627256.74
GCA/SCA Ratio		0.22	0.50	0.63

***, **, * Significant at P<0.001, P<0.01 and P<0.05; WLS = water-limited stress

7.4.2 Performance of F_2 progeny and their parents under non-water-limited and water-limited stress conditions

Under non-WL (Table 7.5), among the F_2 progeny, TGX1990-137F x Nasoko performed the best for both number of pods per plant (75.15) and number of seeds per plant (147.43) but second best for grain yield (2895.34 kg ha^{-1}). In addition, this progeny performed better than the parents for these traits under WLS. TGX1990-137F x Makwacha was best performing for grain yield (2896.25 kg ha^{-1}), while the performance of TGX1990-137F x Nasoko, in terms of 100-seed weight, was lower than other progeny. TGX1987-23F x Makwacha gave the highest 100-seed weight (19.25 g), followed by TGX1988-70F x Makwacha (18.65 g) and both of these progeny were better than their parents used in the cross.

Table 7.5: Mean values for agronomic traits of F_2 progeny and their parents under non-water-limited stress conditions

F_2 Progeny / parents	Number of pods per plant	Number of seeds per plant	100-seed weight (g)	Grain yield (kg ha^{-1})
		F_2 progeny		
TGX1990-137F x Makwacha	61.76	104.84	15.93	2896.25
TGX1987-23F x Makwacha	52.39	93.44	19.25	2304.06
TGX1988-70F x Makwacha	53.45	92.87	18.65	2474.60
Tikolore x Makwacha	72.66	126.77	17.27	2171.59
TGX1990-137F x Nasoko	75.15	147.43	14.05	2895.34
TGX1987-23F x Nasoko	54.10	107.25	15.70	2070.68
TGX1988-70F x Nasoko	43.96	80.19	18.25	2072.17
Tikolore x Nasoko	58.43	123.80	17.06	2313.03
		Parents		
Makwacha	60.06	104.48	17.77	2461.62
Nasoko	57.91	114.67	16.27	2337.80
TGX1990-137F	68.45	126.13	14.99	2895.80
TGX1987-23F	53.25	100.34	17.47	2187.37
TGX1988-70F	48.71	86.53	18.45	2273.38
Tikolore	65.54	125.29	17.17	2242.31
Mean	59.57	108.66	17.18	2377.22
LSD $_{0.05}$	13.86	28.71	1.16	411.93

LSD = Least significant difference

Among the parents, TGX1990-137F was the best for number of pods per plant (68.45), number of seeds per plant (126.13) and grain yield (2895.80 kg ha^{-1}), while TGX1988-70F and Makwacha were the top performing parents in terms of 100-seed weight (18.45 g) and (17.77 g), respectively.

Similarly, under WLS (Table 7.6), among the F_2 progeny, TGX1990-137F x Nasoko performed the best for number of pods per plant (52.40). TGX1988-70F x Makwacha gave highest 100-seed weight (16.33 g), while TGX1990-137F x Makwacha gave the highest values for number of seeds per plant (96.52) and grain yield (1974.83 kg ha^{-1}), and these progeny were better than their parents used in the crosses. Among the parents, TGX1990-137F was the best for number of pods per plant (50.20), number of seeds per plant (90.63) and grain yield (1769.43 kg ha^{-1}), while TGX1988-70F was the top performing parent in terms of 100-seed weight (15.91 g). However, all agronomic traits were reduced under WLS for both progeny and parents.

7.4.3 Combining ability

Both positive and negative GCA effects were observed across the two WLS treatments (Table 7.7). When comparing non-WL and WLS, the direction of the GCA estimates has changed from positive to negative for some of the traits. Under both non-WL and WLS conditions, female parent TGX1990-137F showed positive and high GCA estimates for all traits, except 100-seed weight, while TGX1988-70F showed positive and high GCA estimates for 100-seed weight. Male parent Nasoko was a good general combiner under WLS with positive and high GCA estimates for all traits, except 100-seed weight. On the other hand, Makwacha showed a positive GCA effect for 100-seed weight. Under non-WL, Nasoko had negative GCA estimates for number of pods per plant, 100-seed weight and grain yield, while Makwacha had positive GCA estimates for these three respective traits.

Both positive and negative SCA effects were observed across the two WLS treatments (Table 7.8). When comparing non-WL and WLS, the direction of the SCA estimates has changed from positive to negative for some of the traits. Under both non-WL, TGX1990-137F x Nasoko showed positive and high SCA estimates for number of pods per plant and number of seeds per plant. TGX1988-70F x Nasoko was the best parent combination for 100-seed weight and showed high and positive SCA estimates for grain yield. TGX1988-70F x Makwacha showed positive SCA estimates for grain yield and ranked as highest for grain yield.

Under WLS, the best combinations for number of pods per plant were Tikolore x Makwacha, for number of seeds per plant it was TGX1988-70F x Nasoko, followed by Tikolore x Makwacha and TGX1990-137F x Makwacha, while the best parent combination specifically for 100-seed weight was Tikolore x Nasoko. TGX1988-70F x Nasoko was also the best parent combination for grain yield followed by TGX1990-137F x Makwacha.

Table 7.6: Mean values for agronomic traits of F_2 progeny and their parents under water-limited stress at flowering

F_2 Progeny / parents	Number of pods per plant	Number of seeds per plant	100-seed weight (g)	Grain yield (kg ha^{-1})
F_2 progeny				
TGX1990-137F x Makwacha	48.00	96.52	14.11	1974.83
TGX1987-23F x Makwacha	28.32	50.31	15.44	946.97
TGX1988-70F x Makwacha	29.05	45.27	16.33	1055.13
Tikolore x Makwacha	42.41	66.69	14.21	1037.58
TGX1990-137F x Nasoko	52.40	84.73	13.03	1564.03
TGX1987-23F x Nasoko	38.74	60.55	14.53	1183.67
TGX1988-70F x Nasoko	48.12	72.62	15.49	1599.96
Tikolore x Nasoko	30.06	45.84	15.50	738.72
Parents				
Makwacha	36.95	64.70	15.02	1253.63
Nasoko	42.33	65.93	14.64	1271.60
TGX1990-137F	50.20	90.63	13.57	1769.43
TGX1987-23F	33.53	55.43	14.98	1065.32
TGX1988-70F	38.59	58.95	15.91	1327.54
Tikolore	36.23	56.26	14.85	888.15
Mean	38.45	62.79	14.86	1769.43
LSD $_{0.05}$	12.59	21.92	1.47	431.83

LSD = Least significant difference

7.4.4 Variance components and heritability

The genetic components of the traits analysed are shown in Table 7.9. The genotype variance (σ^2g) was higher in non-WL than WLS for all traits, except for 100-seed weight and grain yield. Under both non-WL and WLS, the variance estimates of females (σ^2f) were greater than that of the males (σ^2m) across all traits.

Table 7.7: General combining ability estimates for agronomic traits under non-water-limited and water-limited stress conditions

Parents	Number of pods per plant	Number of seeds per plant	100-seed weight (g)	Grain yield (kg ha^{-1})
		Non-WL		
Makwacha	0.50	-5.18	0.60	84.40
Nasoko	-1.66	5.01	-0.91	-39.42
TGX1990-137F	8.89	16.47	-2.19	518.57
TGX1987-23F	-6.32	-9.32	0.30	-189.85
TGX1988-70F	-10.86	-23.13	1.27	-103.84
Tikolore	5.97	15.63	-0.01	-134.91
		WLS		
Makwacha	-1.51	1.90	0.16	53.95
Nasoko	3.88	3.14	-0.23	71.92
TGX1990-137F	11.75	27.83	-1.29	569.75
TGX1987-23F	-4.93	-7.36	0.12	-134.36
TGX1988-70F	0.13	-3.85	1.05	127.86
Tikolore	-2.22	-6.53	-0.01	-311.53

WLS = water-limited stress

The SCA variance (σ^2sca) was higher in WLS than non-WL for all traits (Table 7.9). The GCA variance (σ^2gca) was generally higher in non-WL than WLS for all traits, except for 100-seed weight. Under WLS, the SCA variance was greater than GCA variance for number of pods per plant (63.47; 9.09) and number of seeds per plant (152.86; 75.77), respectively. On the other hand, under non-WL, the GCA variance was higher than SCA variance for 100-seed weight (1.07; 0.65) and grain yield (46060.34; 0.00), respectively.

Under non-WL, the additive genetic variance (σ^2A) was higher than dominance genetic variance (σ^2D) for 100-seed weight (4.29; 2.61) and grain yield (184241.38; 0.00), respectively (Table 7.9). Under WLS, dominance genetic variance was higher than the additive genetic variance for all traits. The environmental variance (σ^2e) was generally larger than the additive and dominance genetic variance under both non-WL and WLS for all traits, except for number of pods per plant, number of seeds per plant and grain yield under WLS (where the dominance variance was the largest for all three traits).

Broad-sense heritability (h^2_b) was generally higher than narrow-sense heritability (H^2_n) for all traits across the two WLS treatments (Table 7.9). Broad-sense heritability varied between 0.70 (number of pods per plant under WLS) and 0.24 (grain yield under non-WL). In general, the broad-sense heritability estimates under WLS was larger than under non-WL, except for 100-seed weight. Narrow-sense heritability varied between 0.22 (number of seeds per plant under WLS) and 0.09 (number of pods per plant under WLS). Narrow-sense heritability was larger under non-WL than WLS for all traits, except for number of seeds per plant.

Table 7.8: Specific combining ability estimates for agronomic traits under non-water-limited and water-limited stress conditions

F_2 Progeny	Number of pods per plant	Number of seeds per plant	100-seed weight (g)	Grain yield (kg ha^{-1})
		Non-WL		
TGX1990-137F x Makwacha	-7.19	-16.12	0.34	-83.95
TGX1987-23F x Makwacha	-1.35	-1.73	1.18	32.29
TGX1988-70F x Makwacha	4.25	11.52	-0.40	116.81
Tikolore x Makwacha	6.62	6.66	-0.49	-155.12
TGX1990-137F x Nasoko	8.35	16.29	-0.03	38.97
TGX1987-23F x Nasoko	2.51	1.90	-0.86	-77.27
TGX1988-70F x Nasoko	-3.09	-11.35	0.71	-161.80
Tikolore x Nasoko	-5.46	-6.49	0.80	110.14
		WLS		
TGX1990-137F x Makwacha	-0.69	4.00	0.38	151.45
TGX1987-23F x Makwacha	-3.70	-7.03	0.30	-172.30
TGX1988-70F x Makwacha	-8.03	-15.58	0.26	-326.36
Tikolore x Makwacha	7.69	8.52	-0.80	95.48
TGX1990-137F x Nasoko	-1.68	-9.04	-0.31	-277.32
TGX1987-23F x Nasoko	1.33	1.98	-0.23	46.44
TGX1988-70F x Nasoko	5.66	10.54	-0.19	200.50
Tikolore x Nasoko	-10.06	-13.57	0.87	-221.35

WLS = water-limited stress

Table 7.9: Estimates of variance component and heritability under non-water-limited and water-limited stress conditions

Variance component	Non-WL	WLS	Non-WL	WLS	Non-WL	WLS	Non-WL	WLS
	Number of pods per plant		**Number of seeds per plant**		**100-seed weight (g)**		**Grain yield (kg ha^{-1})**	
σ^2g	83.90	75.06	325.87	249.44	2.18	325.87	48247.99	101913.56
σ^2m	0.00	6.38	0.00	0.00	1.07	0.00	0.00	0.00
σ^2f	54.85	9.27	221.98	127.66	1.07	221.98	78946.95	54605.89
σ^2sca = σ^2_{mf}	44.50	63.47	146.43	152.86	0.65	146.43	0.00	61811.85
σ^2gca	27.58	9.09	125.61	75.77	1.07	125.61	46060.34	31461.91
σ^2A	110.32	36.38	502.43	303.08	4.29	502.43	184241.38	125847.62
σ^2D	177.98	253.87	585.72	611.43	2.61	585.72	0.00	247247.42
σ^2E	298.93	121.74	1328.53	442.77	6.85	1328.53	597468.26	209085.58
h^2_b	0.49	0.70	0.45	0.67	0.50	0.45	0.24	0.64
h^2_n	0.19	0.09	0.21	0.22	0.31	0.21	0.24	0.22

WLS = water-limited stress

7.5 Discussion

The creation of genetic variability is one of the most important approaches to improve crop productivity as it helps to achieve long-term selection gain (Hyten et al. 2006; Mwale et al. 2017b). As such identifying genetic variability in the already existing germplasm of a particular crop is the first step in crop improvement. This is usually achieved through the use of different breeding methods such as mutation, hybridisation and polyploidy (Wanderi 2012; Kole et al. 2015). Yield and yield components have successfully been used to determine genetic variability in crop germplasm (Mwale et al. 2017a). Six genotypes were used to determine genetic variability, mode of gene action and heritability of agronomic traits in soybean genotypes found in Malawi. Hybridisation was used to determine combining ability of genotypes for numbers of pods and seeds per plant, 100-seed weight and grain yield in response to two WLS treatments.

The significant differences between genotypes in terms of grain yield and yield components observed under non-WL and WLS indicated genetic variation among the parents and their F2 progenies. This significant variation showed the possibility of identifying drought tolerant genotypes with desired grain yield and yield components. Such variability among parents and F2 progenies has previously been reported in soybean (Gavioli et al. 2008; Kiryowa et al. 2008; Ojo and Ayuba, 2013). In addition, the significant interaction effect between genotypes, GCA (females) and SCA (male x female) with water treatment effect signifies the need to determine the role of environment (drought stress) in crop improvement. Mohammed (2009) and Makanda et al. (2010) attributed the interaction effect observed to the significance of environment (drought) stress in altering both the additive and non-additive gene effects. This implies that progenies need to undergo series of selection across varied WLS levels for GCA and SCA in order to identify the best parents and potential F2 progenies before recommendation for drought tolerance. Akaogu et al. (2017), who observed significant interaction between both GCA and SCA effects with the environment under both rainfall and drought conditions, suggested testing for different environments to identify F2 progenies with consistent performance across contrasting environments. Furthermore, SCA (male x female) effect that were not significant under non-WL became highly significant under WLS. This suggests that breeding and selection for drought tolerance should be done under WLS conditions as under non-WL the progenies significant differences may not be detected. The higher GCA/SCA ratio in non-WL treatment than WLS indicates higher additive genetic control in expression of the traits under non-WL treatment than WLS. However, the presence of ≥0.5 GCA/SCA ratios in all traits except for number of pods per plant under WLS indicates a degree of additive gene action present in the expression of the traits.

Mwije et al. (2014), Mwale et al. (2017b) reported that GCA/SCA ratios of >0.5 signifies the predominance of additive genetic control on the trait which may translate into higher heritability of a trait.

Large variability was observed between F2 progenies in terms of yield components and grain yield under both non-WL and WLS conditions. This large variability has also been observed between the parents in terms of yield components and grain yield under both non-WL and WLS conditions. Such variability in grain yield performance among parents and crosses have also been reported in crops such as maize (Mohammed, 2009), faba bean (Zeinab and Helal, 2014), sunflower (Golabadi et al. 2015) and sweetpotato (Rukundo et al. 2017). Although some similarities in terms of ranking performances across the two WLS treatments were observed, the best performing progenies under non-WL are not necessarily the best under WLS signifying the role of drought stress effect on different genotypes and genetic variability in response to drought stress.

Although, the rankings of most parents changed across the water treatments, parents TGX1990-137F and Makwacha, generally showed stable responses across the two water treatments and respectively performed in the top one, and top two to four positions for NPP, NSP and grain yield. Considering that parent TGX1990-137F produced the highest NPP, NSP and grain yield under WLS and its progeny were among the top performing F2 progenies for these traits, this would imply that it might have traits and thus genes involved in drought tolerance, which are passed on to the progeny. Thus, TGX1990-137F can be promoted for use in areas with WLS and be incorporated in a breeding program to improve drought tolerance.

The GCA is the average performance of a genotype in a hybrid combination (Salami and Agbowuro, 2016). The general combining ability of a plant measures its breeding value (Rukundo et al. 2017) which is a function of the additive gene action (Zeinab and Helal 2014). The additive genes are directly transported from parents to the offspring (Fasahat et al. 2016) which implies that the higher the positive GCA effect the higher will be the possibility of desired genes being transferred from parents to offspring. Results have shown variability in GCA effects across genotypes when exposed to different WLS treatments. Most genotypes that had higher GCA effect under non-WL showed reduced GCA under WLS except genotype TGX1990-137F which showed increased GCA when exposed to WLS than under non-WL. In addition, the GCA effects changed from positive to negative for most parents across the traits (except for 100-seed weight).

Thus implies that parents that have good general combining ability under non-WL might be poor general combiners under WLS conditions. The implication thereof is that if you want to select parents that are good combiners in drought tolerance breeding programs, these parents need to be identified under WLS conditions. The only parent that did not show this trend was TGX1990-137F, which had the highest positive GCA effects under both non-WL and WLS.

The GCA effects for all yield components, except 100-seed weight for parent TGX1990-137F under WLS, were positive and significant. This is an indication that TGX1990-137F can contribute towards expression of number of pods per plant, number of seeds per plant and grain yield under WLS. Parent TGX1988-70F showed positive and high GCA estimates for 100-seed weight under WLS, indicating that it can be used as a donor parent for 100-seed weight under WLS. TGX1988-70F also showed positive GCA for number of pods per plant under WLS. Parent Makwacha had significant positive effects for 100-seed weight and grain yield under WLS. Therefore, besides parent TGX1990-137F, Makwacha that had high and positive GCA effects for grain yield under WLS can be also used as a parent in crosses to improve soybean grain yield in a wide range of WLS environments. Parents that might be generally good combiners under both non-WL and WLS conditions for grain yield and its components include, TGX1990-137F and Nasoko both for NPP, NSP and grain yield, Makwacha for grain yield only and TGX1988-70F for 100-seed weight and grain yield.

The SCA of a cross gives an indication of the proportion of loci that shows dominance and interaction (Zeinab and Helal, 2014). The SCA effects indicates how much cross performance deviates from the performance predicted from the parents' GCA effects (Su et al. 2017). Specific Combining Ability effects (SCA) information is very essential in breeding because it can help to identify combinations that perform relatively better than expected on the average. Results revealed both positive and negative SCA effects under WLS which gave the possibility of identifying better combination performers. Similar SCA effects have been reported in soybean which have been used to select best combiners for number of seed per plant, 100-seed weight and grain yield (Cho and Scott 2000; Karyawati et al. 2015) and other crops such sweetpotato (Kivuva 2013), maize (Akaogu et al. 2017), cowpea (Mwale et al. 2017b) for drought tolerance and chrysanthemum (Su et al. 2017) for waterlogging tolerance. However, the results from this study indicated that not all parents that had positive and high GCA effects managed to produce progeny with high and positive SCA effects under WLS environment. Similar observations were made by Mwije et al. (2014) who suggested the possibility of the presence of modifier genes that are involved in the expression of traits and affirming the role of additive gene action.

The results have further indicated that the direction of the SCA estimates can change from positive to negative (and from negative to positive) for some traits in the F2 progenies under the different WLS treatments indicating the importance of selecting progenies under similar conditions to which it will be subjected after recommendation for cultivation. In addition, some genotypes with positive GCA effects under WLS that were used in a cross with another parent with negative GCA effects under WLS produced F2 progeny with positive SCA effects for some traits under WLS conditions. These results are in accordance with Kivuva (2013) and Mwije et al. (2014) in sweetpotato who suggested that parents should not be disqualified only based on negative GCA effects. They indicated that the positive contribution of non-additive gene action to the expression of a trait in the progeny may not depend on the parental GCA effects.

The higher contribution of SCA variance to the total variance, compared to GCA variance, under WLS supports results obtained on additive variance compared to dominance variance. When comparing additive variance to dominance variance between the non-WL and WLS treatments, under non-WL, the additive genetic variance was larger than dominance genetic variance for 100-seed weight and grain yield under non-WL. This indicated the importance of additive genetic variance over dominance genetic variance in expression of these traits under non-WL. However, under WLS, the additive genetic variance was larger than dominance genetic variance for only 100-seed weight. This indicated the importance of dominance genetic variance over additive genetic variance in expression of the rest of the traits under WLS. The results are in accordance with those of Ghiday and Tizzazu (2017) who reported higher non-additive effects than additive effects in soybean for number of pods per plant and grain yield. Wanderi (2012) and Karyawati et al. (2015) reported higher additive genetic than non-additive variance for number of pods per plant, number of seed per plant, 100-seed weight and grain yield while Mebrahtu and Devine (2009) reported higher additive genetic effects for number of pods per plant and Ghiday and Tizzazu (2017) reported higher additive effects on 100-seed weight in soybean. Besides soybean, higher additive genetic variance has been reported in 100-seed weight and grain yield of sunflower (Machikowa et al. 2011) and grain yield of maize (Akaogu et al. 2017; Fasahat et al. 2016) under drought stress conditions.

The presence of both additive and dominance genetic variance for all studied traits under WLS signifies the importance of these gene actions in the genetic control on the measured traits under WLS. Apart from yield and it's components, the presence of both additive and dominance genetic effects have been reported in soybean for other traits such as total isoflavone (Bi et al. 2015), resistance to rust disease (Kiryowa et al. 2008; Wanderi, 2012) as well as sugar composition (Mebrahtu and Devine, 2009), which indicate the important role of both these gene actions in soybean traits. However, under WLS dominance genetic effects played a larger role in number of pods per plant, number of seeds per plant and grain yield than additive genetic effects. For both 100-seed weight and grain yield, additive genetic effects played a larger role than dominance genetic effects under non-WL, while the opposite was true for under WLS.

Although the environmental effects for sites was not accounted (since the data was performed as combined analysis), the water regimes effect that represented environmental effect was accounted which could have contributed to the higher environmental variance in the present study. As such, this change in gene action between additive and dominance when genotypes are exposed to different WLS treatments could be attributed to environmental variance. In addition, drought stress is associated with other environmental factors such as temperature, salinity, relative humidity as well as vapour pressure (Makanda et al. 2010; Lweendo et al. 2017) that could also have played a bigger role in gene action in the present study. Le et al. (2012) stated that, within species, numerous genes may be responsible for regulation of drought responses in a specific manner, based on both developmental stages and the stress effect.

Considering that the material used in the present study were the F2 population, this high environmental variance could also be attributed to high segregation in the material. A similar high magnitude of environmental variance was reported in soybean by Kiryowa et al. (2008) who attributed it to use of a highly segregating F2 population. As a result, Mebrahtu and Devine (2008) who observed a similar high environmental variance in soybean when using F2 population, suggested selection of superior lines in F5 or F6 generations, citing the importance of repeatable and GEI unbiasedness in subsequent generations. In addition, the use of the single seed descent technique to minimize environmental variance was proposed by Mohammed (2009) as this technique helps to rapidly advance the population to homozygosity (Mebrahtu and Devine, 2009). Higher broad-sense than narrow-sense heritability in this study may be an indication of a higher contribution of dominance genetic variance, coupled with environmental variance, to the total genotypic variance (Mwale et al. 2017b). This indicated that a high percentage of genes involved were not directly transferred to the offspring. The relatively high broad-sense heritability estimates observed for number of pods per plant, number of seeds per plant and grain yield would help in the selection of appropriate parents for soybean improvement under WLS. Similar findings were reported by Zeinab and Helal (2014) in faba bean genotypes for number of pods per plant, suggesting that such traits with high heritability would respond to selection. Low values of narrow-sense heritability indicate a low contribution of additive genetic variance to total genotypic variance (Salami and Agbowuro, 2016).

7.6 Conclusions and recommendations

There is potential for breeding progenies with high grain yield and yield-related traits that are superior to those that are currently available in Malawi which can improve soybean productivity under both non-WL and WLS. Based on the mean values from F_2 families, the top three families with high values for most agronomic traits were TGX1990-137F x Nasoko, TGX1990-137F x Makwacha and TGX1988-70F x Nasoko under WLS. Parents TGX1990-137F and Nasoko were identified as good combiners that would produce, after crossing, offspring that would perform well in terms number of pods plant, number of seeds per plant and grain yield under WLS. TGX1988-70F was identified as the best combiner for 100-seed weight under both non-WL and WLS. Based on SCA, combinations TGX1988-70F x Makwacha and TGX1990-137F x Nasoko under non-WL, and parent combinations TGX1990-137F x Makwacha, TGX1987-23F x Nasoko and TGX1988-70F x Nasoko under WLS were identified as the best combinations for most agronomic traits. These can, therefore, be recommended as promising cross combinations that are suitable for soybean breeding programmes in WLS environments. Both additive and dominance genes were important for expression of grain yield and yield related traits in soybean under WLS. However, dominance genetic effects played a major role in expression of traits, indicating that most of the genes involved were not transferred from parents to the offspring. As such employment of specific crossing combinations would be necessary.

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CHAPTER 8

General conclusions and recommendations

Soybean is one of the most important leguminous crops in the world as it provides a source of vegetable oil, protein, feed for animals as well as soil fertility. The soybean industry in Malawi has an enormous opportunity to expand since there is a high demand for soybean feed due to expansion of both the poultry and fish industries. The private sector has shown interest, especially processors, to support and enhance soybean production to meet local demand. There is also a political will and government has put initiatives in place to enhance soybean production. Despite the growing demand, the yields realised by farmers are four times lower than the potential yield. Soybean production is faced with numerous challenges among which drought is one of the most important. Drought is one of the most devastating environmental factors reducing crop productivity by limiting plant growth, development and crop yield.

Identification and development of drought tolerant genotypes is amongst the best possible strategies of dealing with drought effects. One of the challenges in selecting genotypes is the identification of traits contributing to improved stress tolerance. Yield per se has been used with a number of limitations rooting from both environmental and genetic factors. Therefore, this study was implemented to investigate the possibility of using multiple soybean traits (grain yield, morphological, physiological) and multidimensional statistical tools in identifying and developing WLS tolerant genotypes.

Soybean genotypes, of which four (Makwacha, Nasoko, Magoye and Tikolore) were released genotypes and eight (TGX1987-10F, TGX1987-23F, TGX1987-62F, TGX1988-18F, TGX1988-70F, TGX1988-9F, TGX1989-46 and TGX1990-137F) were advanced promising lines for drought tolerance from IITA germplasm, were evaluated for drought tolerance using morphological and physiological traits. The morphological traits included plant height, number of nodes per plant, number of pods per plant, biomass, number of seeds per plant, 100-seed weight, soybean grain yield and WUE. Physiological traits were SPAD, ΦII, ΦNPQ and ΦNO. Multiple statistical methods were used to determine tolerance in the genotypes ranging from drought tolerance indices, correlation analysis, regression analysis, PCA, AMMI as well as GGE.

All traits were significantly associated with WLS, except for number of nodes per plant and SPAD. Genotype effects were highly significant for all traits, except for ΦII. Different WLS levels negatively affected all soybean genotypes. Plant height, number of nodes per plant, 100-seed weight, WUE and SPAD exhibited less percentages of reduction under WLS and were considered as less sensitive traits. Determining the response of plants to WLS, using both morphological and physiological traits, proved helpful in identifying good performing genotypes for cultivation under WLS as genotypes TGX1990-137F and TGX1988-70F were identified as better performing under WLS conditions. However, interaction observed between WLS levels and genotypes called for additional studies to better characterise how these genotypes perform under WLS conditions.

The 12 genotypes were further evaluated to determine breeding selection criteria and identify traits that can be used to improve yield. Genotypes that can be incorporated in a breeding program for good yield performance under WLS have been identified through the use of correlation analysis, regression analysis and PCA. Selection criteria differed from one WLS level to the other, both for traits and genotypes. Traits WUE, SPAD, number of pods per plant and 100-seed weight highly correlated with grain yield. The results were in line with literature that links WUE to grain yield in soybean with an explanation of 99% variation in grain yield across WLS levels. Therefore, these traits can be used in soybean breeding programmes for WLS genotype improvement. Genotypes TGX1988-70F and TGX1990-137F were strongly and positively associated with grain yield and WUE under 70% WLS. TGX1988-70F and TGX1990-137F can, therefore, be used in breeding for high yield performance under drought stress conditions to obtain higher genetic gains.

The conventional method of selecting drought tolerant genotypes has been to compare the yield under non-WL with the yield under WLS in a specific environment. However, this method has proved futile since the occurrence and intensity of drought stress varies across environments, seasons and is worsened by global climate change. As such, drought tolerance indices that have been previously reported be effective for selection of drought tolerant genotypes to be cultivated both under non-WL and WLS conditions, were used to further dissect drought tolerance. Twelve genotypes and a total of 10 drought tolerance indices were used.

Geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HM), stress tolerance index (STI) and yield index (YI) were positively and significantly associated with both non-WL and WLS. These indices positively contributed most to the total variation in the principal components and can, therefore, be used to discriminate drought tolerant soybean genotypes from sensitive genotypes. Genotype TGX1990-137F which was strongly associated with YI, gave the highest drought resistance index (DI) and exhibited the highest yield under WLS. Both PCA and cluster analysis grouped TGX1990-137F as highly tolerant and TGX1987-10F as highly sensitive genotypes. Genotypes TGX1988-9F, TGX1987-23F and Magoye were ranked as tolerant, and Tikolore, Nasoko, Makwacha TGX1988-18F, TGX1988-70F, TGX1987-62F as moderately tolerant. The results have showed that using a combination of several analytical tools, where multiple variables are involved, would be important for efficient interpretation of results and select suitable indices and genotypes.

Soybean is grown widely in Malawi and, therefore, determination of yield stability is required before the release of a genotype for cultivation. Grain yield stability of six soybean genotypes were evaluated using AMMI and GGE with WLS induced at two growth stages for two seasons across three different environments to identify stable genotypes. Genotypes performed significantly different regarding grain yield across WLS treatments, environments and seasons. Grain yield was mainly influenced by environment and environment by season interaction. Nasoko, TGX1988-70F and TGX1987-62F showed high yield potentials and were ranked as the top yield performing genotypes under non-WL, WLS at flowering and WLS at pod-filling, respectively. Genotypes TGX1988-9F, TGX1989-46 and TGX1987-62F were identified as stable genotypes under non-WL, WLS at flowering and WLS at pod-filling, respectively. Masenjere was a more discriminating and representative environment and, therefore, better site for soybean grain yield evaluation under WLS at pod-filling.

Six genotypes, selected for their high grain yield potential and variability under WLS conditions, were further hybridised in order to determine the gene action of drought tolerance in soybean and to identify the best combiners to be used as donor parents in developing drought tolerant soybean genotypes. Crosses TGX1990-137F x Nasoko, TGX1988-70F x Makwacha TGX1990-137F x Makwacha were ranked as the best combinations for number of pods per plant, 100-seed weight and grain yield, respectively under non-WL and WLS conditions. TGX1990-137F showed positive and high GCA estimates for all traits, except for 100-seed weight, while TGX1988-70F showed positive and high GCA estimates for 100-seed weight, both under non-WL and WLS conditions, which indicated that these genotypes can be used as donor parents for the respective traits.

TGX1988-70F x Makwacha and TGX1988-70F x Nasoko showed the highest SCA estimates for grain yield under non-WL and WLS, respectively. Both additive and dominance gene effects were important in improvement of soybean under WLS. However, current findings point at non-additive gene action as predominant for expression of the number of pods per plant, number of seeds per plant, 100-seed weight and grain yield. However, results need to be further validated under multiple environments at an advanced stage of selection. Broad-sense heritability estimates were higher than narrow-sense heritability for all traits under non-WL and WLS, indicating a low transfer of genes from parents to offspring. Superior families that were identified in terms of grain yield, yield components, and their high and positive GCA and SCA effects have the potential to be used in breeding to develop drought tolerant genotypes. In addition, further studies are needed to investigate the mechanisms these genotypes use to cope with drought tolerance in order to better understand the drought tolerance response of soybean genotypes in Malawi.