Evaluation of soybean (*Glycine Max* L. Merr.) lines for grain yield and drought-tolerance

By

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General Abstract

Soybean (*Glycine max* L. Merr.) is ranked as the fourth-highest commercial agronomic seed crop in South Africa. An increase in animal feed demand has mainly driven the significant growth in demand for soybean oilcake and oil. This demand is also contributed by increasing demand for protein-rich foods, especially among the middle class. However, soybean production has always been variable in different seasons in South Africa mainly due to the occurrence of droughts in some provinces causing the yields to decline. Therefore, enhancing grain yield and drought tolerance would preserve farmers' profits at large and small-scale farms. The present study was undertaken during 2019 and 2020 summer growing seasons in the field and greenhouse trials to: i) screen 36 soybean genotypes for drought-tolerance using morphological and physiological traits, ii) assess drought-tolerance in soybean genotypes using drought-tolerance indices and iii) estimate the variance components and heritability of yield and yield components of soybean under well-watered and water-stressed. Thirty-six soybean lines obtained from the International Institute of Tropical Agriculture (IITA) were screened for drought-tolerance in the field and greenhouse under water-stressed and well-watered regimes using morphological, physiological traits and yield-based selection indices. The targeted traits were; plant height (PH), stem diameter (STD), leaf width (LW), leaf length (LL), seed moisture content (SMOI), stomatal conductance (STC), chlorophyll content (CC), 100 seed weight (SW) and biomass Yield (BMS). High genetic variation was observed in grain yield and morpho-physiological traits under both well-watered and water-stressed regimes. Genotype effect was significant for PH, LL, LW, STD, BM, SW and GY. The water regime indicated a significant effect for PH, LL, LW, STD, SMOI and GY. The environment effect was significant for all morphological traits PH, LL, LW, STD, FLW, SMOI, SW and GY. The environment by water regime interaction showed a significant effect for PH, FLW, SMOI, SW.

 A significant reduction in agronomic traits was observed for G10, G12, G22 and G29, which were the best potential genotypes for improving drought-tolerance. The PH, LL, LW, STD; GY, SMOI, BM and SW could effectively be used for selection in the yield improvement of soybeans under water stress conditions, since they were positively correlated with GY. The Principal components analysis (PCA) and cluster plot analysis approach was very helpful in identifying high-yielding, drought-tolerant genotypes, discriminating and grouping genotypes based on their responses to water stress. The principal components indicated that first dimension (Dim1) was consistently correlated with PH, LL, LW and STD. The SW, CC, FLW, STC, SMOI, BM and GY were either associated with second dimension (Dim2) or third dimension (Dim3). The cluster plot showed that G1, G10, G12, G20, G22, G25 and G29 under WS in the field experiment had high means values and were consistency associated with STD, LL, STC, FLW, SMOI, BM, SW and GY based

on principal components and cluster plot, represented as cluster II. Whistle, G5, G7, G10, G12, G13, G14, G17, G21, G22, G23, G27, G29 and G31, showed significantly high mean values and association with PH, STD, LW, LL, STC, CC, BM, SW and GY in the greenhouse environment.

The selection for drought-tolerance among 36 soybean lines under well-watered and water-stressed regime was performed using yield based selection indices, including Drought intensity index (DII), Stress susceptibility index (SSI), yield index (YI), Stress tolerance index (STI), Mean relative performance (MRP), GMP-Geometric mean of productivity (GMP), Yield stability index (YSI), Mean productivity(MP), TOL-Stress tolerance (TOL), Harmonic mean (HM) and Relative efficiency index (REI). The ANOVA indicated that the main effects due to the environment, genotype and water regime were significant for GY at the level of significance of ($P \leq 0.05$), ($P \leq 0.001$) and ($P \leq 0.001$).

The drought-tolerant indices with significantly positive correlation with the grain yield under well-watered and water-stressed regimes were MRP, GMP, MP, MRP, HM and REI ($P < 0.001$-$P < 0.05$). These indices were comparably effective than SSI, YI, STI, YSI and TOL in selecting and predicting better grain-yielding soybean genotypes under a well-watered and water-stressed regime. Most of the soybean genotypes studied resembled water stress tolerance, including G22, G4, G8, G1, G23, G5, G20, G24, G27, G25, G16, G14, G7, G2, G28, G11, G6, G34, G10, G30, G3, G15, G19, G36, G17, G21, G31, G18, G33, G35, G13, based yield reduction rankings. Among these genotypes, G1, G19, G13, G33, G31 showed high mean performance, tolerance and association with SSI, STI, MRP, GMP, MP, TOL, HM and REI. However, G26, G32, G9, G29, G12 were considered moderately susceptible to water stress and G7, G8, G14, G22, G34 had low mean performance values and low association with indexes.

The 36 imported lines from the International Institute of Tropical Agriculture (IITA) were assessed in the field and greenhouse environments, using a 6×6 alpha-lattice design with two replications. Water stress was applied up two weeks after 50% flowering for each genotype and a well-watered regime was used as a control treatment. The genotypes were screened using morphological and physiological traits including; PH, STD, LW, SMOI, STC, CC, SW, BM and GY for estimating variance components and broad-sense heritability.

The present study showed the existence of genetic variability among 36 soybean genotypes examined. Hence, one can examine the presence of variability in these soybean lines for crop improvement programs through indirect selection. According to the results, a higher genotypic coefficient was observed for grain yield under both water regimes, consistent with wide-ranging heritability. The PCV was higher than the GCV for all traits across environments and water regimes, thereby suggesting the significance of the environmental expression for all traits. The PCV was higher than the GCV for all traits in all environments

and water regimes, suggesting the importance of the environmental effect for all traits. The PCV values ranged from 7.03 to 92.84, while the GCV values ranged from 0.07 to 60.77.

GY and BM showed high PCV and GCV values in each environment and under the respective water regimes, which signifies a considerable genotypic variation in these traits. Additionally, the phenotypic expression of these traits would help identify genotypic potential and are efficient further to be used to improve breeding plants. Because of the reduced effects of environmental stress, there was no clear trend in the traits examined for heritability in both environments and water households. Heritability estimates under WW ranged from -0.34 to 55%, while under WS, they ranged from -0.29 to 43%. Overall, most traits had low heritability in both water regimes. Consequently, one should be careful in selecting for drought-tolerance using the traits examined.

Declaration

I, Thubelihle Lungelo Mathonsi, declare that,

1. The work presented in this dissertation, except where otherwise indicated, is my original research.
2. This dissertation has not been submitted for any degree or examination at any university.
3. This dissertation does not contain other person's data, pictures, graphs, or other information unless expressly acknowledged as being sourced from other persons.
4. This dissertation does not contain another person's writing unless expressly acknowledged as being sourced from 1other researchers. Where other written sources have been quoted, then: their specific words have been re-phrased, while the general information attributed to them has been referenced; where their exact words have been used, then their writing has been replaced in italics and inside quotation marks and referenced.
5. This dissertation does not contain text, graphs, or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the dissertation reference sections.

Signature……………██████████………………Date…………26/08/2021…

Thubelihle Lungelo Mathonsi

As the candidate's supervisors, we agree with the submission of the dissertation:

Signature……………█████████………. Date………26/08/2021………………………

Professor Julia Sibiya (Main supervisor)

Signature………………………………… Date…………………………………

Dr. Aleck Kondwakwenda (Co-supervisor)

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Dedication

This dissertation is dedicated to:

my mother:

Funaziphi, **Denty Mathonsi**

Who in times that I wanted to give up was nothing but my source of inspiration and strength through her prayers, who continuously supported me morally, spiritually and financially

My one and only brother:

Dumisani Enock Mathonsi

Who advised and supported me to get my Master's degree and encouragement to complete this degree,

my best sisters**:**

Zandile, Silindani and twins Thobeka and Xolile Mathonsi

Who supported me morally from the beginning to the end and finally

My ancestors

For guidance, strength, power of the spirit, protection and to give me good health.

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Under WS in the field environment, the genotypic correlation for STD showed a significant and positive correlation with PH (r=0.90; P≤0.01. The STD, indicated a significant and positive correlation with LW (r=0.56; P≤0.01), LL (r=0.89; P≤0.01) and GY (r=0.27; P≤0.01). The LL, showed a significant and positive correlation with PH (r=0.91; P≤0.01) and STD (r=0.36; P≤0.01). The STC, showed a negative and

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List of Acronyms and Abbreviations

ANOVA: Analysis of variance

BMS: Biomass Yield

CC: Chlorophyll content

DF: Days to flowering

DI: Drought resistance index

DII: Drought intensity index

DR: Drought resistance

FAO: Food and Agriculture Organisation

GEI: Genotype by environmnent interaction

GPP: Grain weight per plot

G_s: Stomatal coductance

GY: Grain yield

HM: Harmonic mean

HSD: Honest significant difference test

IITA: International Institute of Tropical Agriculture

LL: Leaf length

LW: Leaf width

MC: Moisture content

PC: Principal component

PCA: Principal Components Analysis

PH: Plant height

P_n: Photosynthetic rate

REI: Relative efficiency index

SD: Stem diameter

SSA: sub-Saharan Africa

SSI: Stress susceptibility index

STI: Indices stress tolerance

SW: 100 seed weight

T_r : Transiration rate

VC: Cotyledon

VE: Emergence

WS: Water-stressed

WW: Well-watered

YI: Yield index

YSI: Yield stability index

Chapter one

Introduction

1.1 BACKGROUND

Soybean (*Glycine max. L* Merri) is an economically important annual leguminous crop widely cultivated for its edible seeds, highly rich in protein, among other important nutrients (Sharma et al., 2014). Hence, in South Africa (SA), soybean is considered as one of the important food crops with great potential to eliminate protein malnutrition in underprivileged households. In the past 50 years, soybean production in SA has increased with an average of 80.6%, while yield increased with 55.4% and the area planted to soybeans has expanded rapidly by 80%. The increase in production, yield and planted area to soybean is reinforced by the agricultural environment that has encouraged the commercialization and utilization of agricultural biotechnologies. This facilitates a smooth transition for commercial farmers to be able to rotate soybean as other top important grain crops to maximize profits.

Despite the significant growth in soybean industry and its importance, soybean production still faces several biotic, abiotic and economic constraints. Climate change exacerbated chronic and episodic drought conditions are the major limiting factors in South Africa. Developing drought-tolerant cultivars can help to reduce the impact of drought on soybean production and yield. This section of the dissertation presents the importance and the utilization of soybean in South Africa, the soybean production trends in the world and in South Africa and highlight the impact of drought among other biotic, abiotic and economic production constraints in South Africa.

1.2 IMPORTANCE AND THE UTILIZATION OF SOYBEAN

Soybean is one of the most affordable, stable and prosperous sources of high-quality protein for human consumption and animal feed worldwide. It is an essential source of antioxidants and phytoproteins, which are praised for numerous health benefits (Asif & Acharya, 2013). Soybean seed comprises of 17% oil, 50% proteins and 63% of fibre (DAFF, 2010). It also contains several essential vitamins, amino acids and minerals, including vitamin K1, manganese (Mn), copper (Cu), phosphorus (P), folate and thiamine (El-Shemy, 2011; Hassan, 2013). Furthermore, consumers that are lactose intolerant, consume soymilk in place of cow milk. Soybean plays an important role in the ecosystem by improving soil fertility through nitrogen fixation (Hungria & Mendes, 2015).

Although soybean has incredible nutritional value, its utilization in South Africa is relatively low due to minimal soybean usage in the human food market (Dlamini et al., 2014b; Joubert & Jooste, 2013). According to (DAFF, 2010), the livestock sector is the largest soybean consumer with an estimate of 60% (Figure 1.1). Meanwhile, soybean consumption for oil and oilcake is estimated at 32%, protein at 25% and human consumption at 8% (Figure 1.1) (DAFF, 2017). In addition, soybean is consumed in a vast range of food products including soya sauce, soups, in a form of nutritious cereals, as yogurt and flavoured soymilk products (Dlamini et al., 2014b).
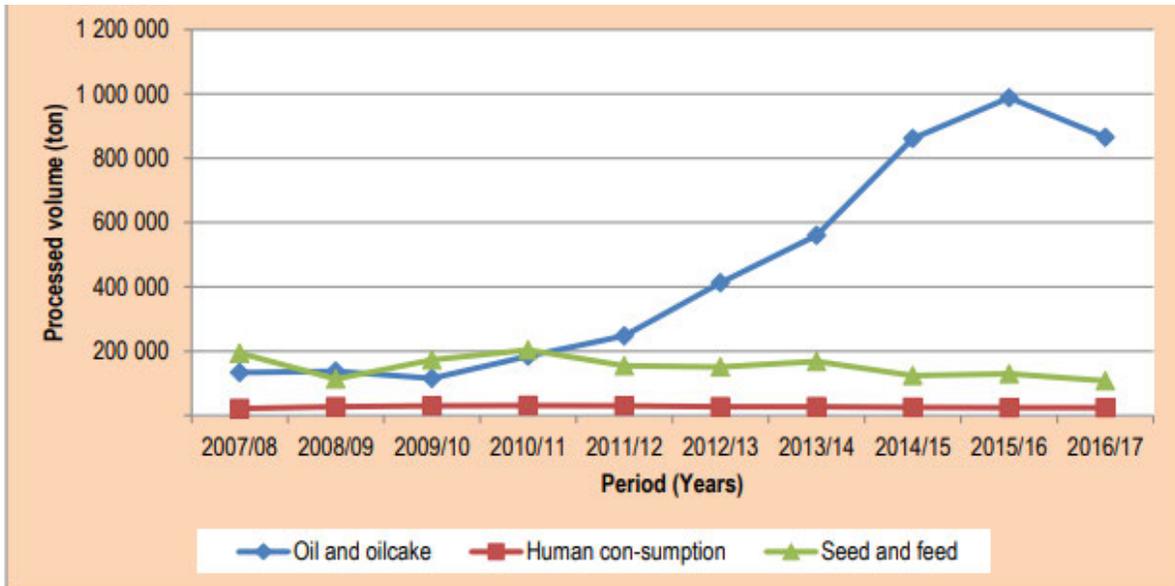
Figure 1.1: Soybean consumption and utilization in South Africa from 2007 to 2017, adapted from (DAFF, 2010)

1.3 SOYBEAN PRODUCTION IN THE WORLD AND SOUTH AFRICA

Soybean is one of the world's most essential pulse crops with an annual production of 362.52 million metric tons for the 2019/2020 season, globally (Strong, 2019). It is the leading seed oil crop worldwide, accounting for about 35 % of the total harvested area devoted to annual and perennial oil crops (Thoenes, 2004). The top three soybean producers in the world are the United States of America (U.S.A, 16.41 %), Brazil (3.97 %) and China (3.31 %). These three countries collectively harvested 81% of the world's production in 2006 (Hartman et al., 2011; Leng & Hall, 2019). Brazil alone has gained the status as the leading exporter and one of the largest soybean producers. Recently, Brazil overtook U.S.A to be the leading producer of soybean with 124 million metric tons for the 2019/2020 (Shahbandeh, 2020).

African countries are also significant producers of soybean and account for 5% of the world production (Dlamini et al., 2014a). South Africa, Nigeria, Zambia and Uganda are the leading soybean producers in sub-Saharan Africa (SSA) (Dlamini et al., 2014a). In 2009, Nigeria was the leading soybean producer in Africa, followed by South Africa, Uganda, Zimbabwe and Ethiopia (Khojely et al., 2018). However, recently according to USDA global market analysis, South Africa is the leading country for soybean production in the African continent with 1.30 million metric tons USDA (2019).

 Soybean production history in SSA

Soybean were introduced to SSA by the Chinese traders in the early 19th Century (Khojely et al., 2018). According to the Cedara Memoirs, the first soybean import to South Africa was in 1903 and cultivated as an economic crop (Shurtleff & Aoyagi, 2009). However, the early cultivars had poor germination, early plant development and early pod shattering. In the early 1950s, a research institute under the name of Geduld in the region of Potchefstroom, South Africa, developed and released new lines that were well adapted and resistant to shattering before harvesting. These new lines were sustainable and profitable until the 1980s (Khojely et al., 2018).

The South African soybean production improvement program was initiated by the Protein Research foundation in 1996 with a target of 100 000 tons of soybean before the year 2000 (Scholtemeijer, 2017). The production was successful, and exceeded the target; yielding 120 000 tons from 87 000 ha in the same year. A one-million-ton mark was reached for the first time in the 2014/2015 season, which saw 1 04 million tons from 687 300 ha (Scholtemeijer, 2017). In 2015/2016 season, domestic soybean production declined drastically by 30% from 107 000 tons to 74 550 tons, due to drought stress. Despite the decline, in the same year, South Africa was still leading in soybean production in Africa (Figure 1.2) (Cornelius & Goldsmith, 2019).

In 2016 the production increased by 66% (Manthata, 2018), while in the 2017/2018 season, the soybean commercial production and planted area figures increased by 17% (1 540 000 tons) and 37% (787 200 hectares), respectively (Human, 2017). These figures made a mark as the highest recorded in South African soybean production history. However, the average national yields in the same season decreased by 14% to 1.96 t/ha, closer to the ten-year average of 1.75 t/ha. The losses continued in the 2018/2019 season with commercial soybean crop production decreasing by 24% (1 170 345 tons) and the average yield by 18% from 1.96 t/ha to 1.60 t/ha (Nortjé, 2020; Sibulali & Morokong, 2019). Despite the losses in production and yields in that season, soybeans remained in second place in terms of total planted area and as the third-highest in production record figures, as shown in Figure 1.3 (Nortjé, 2020). The primary soybean-producing

provinces in South Africa, contributing to the total crop, are Mpumalanga and Free State in all the respective seasons mentioned above.
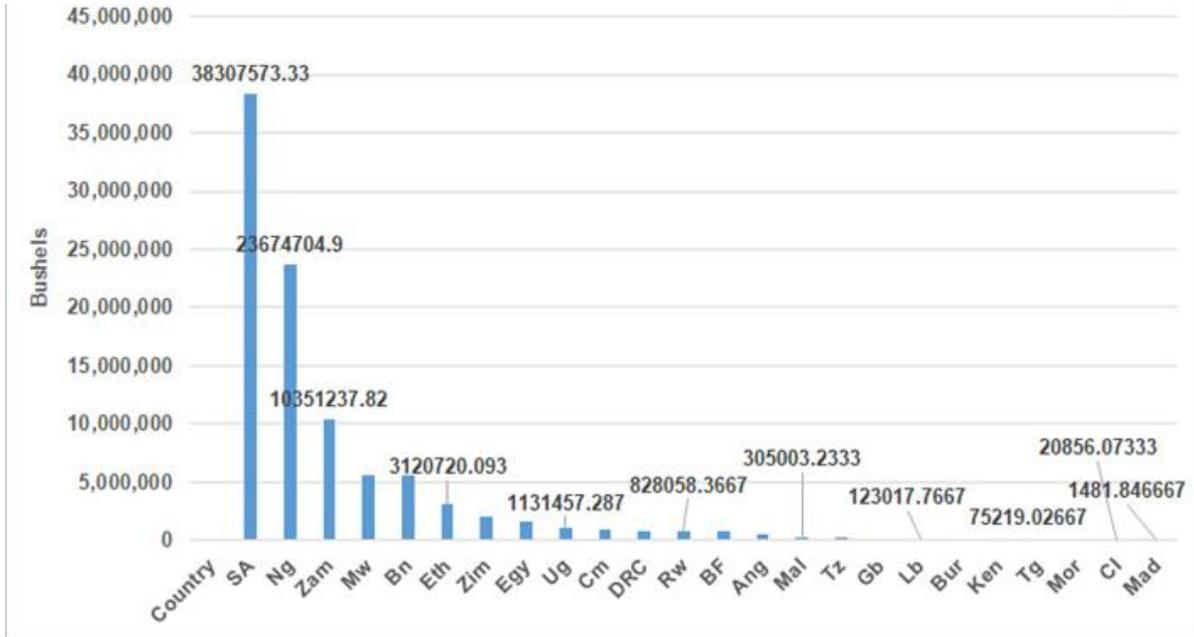
Figure 1.2: Soybean production in Africa by country averages from 2015 to 2017 season. Adapted from (Cornelius & Goldsmith, 2019).
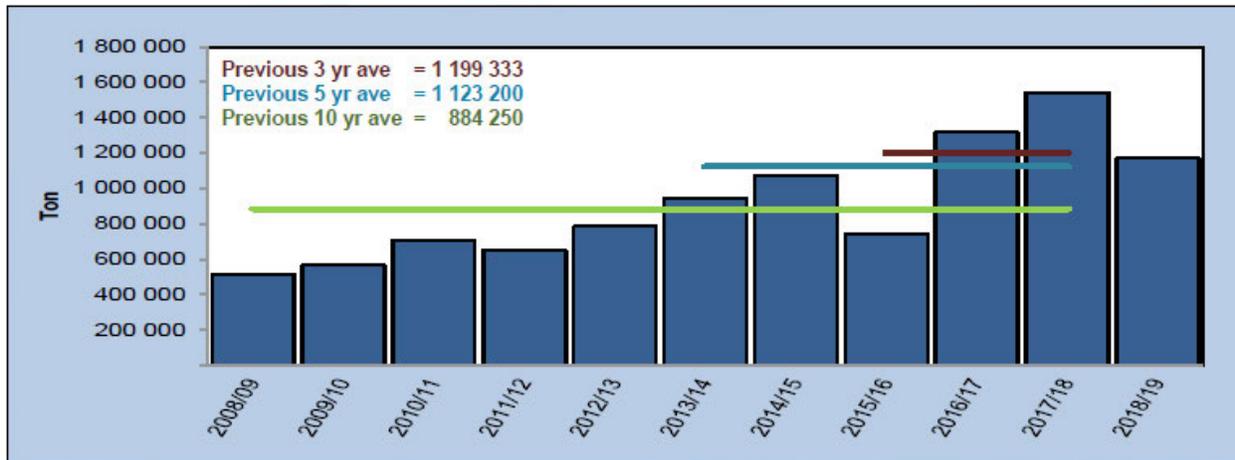
Figure 1.3: South African soybean production from 2008 to 2019 seasons. Adapted from (Nortjé, 2020)

Soybean production areas in South Africa

Soybeans in South Africa are produced at both small and large scale. The soybean production started at small scale in the late 1990s and expanded to large scale production (Dlamini et al., 2014b). The production of soybeans in large quantities is still a growing component of South Africa's agricultural economy. Although soybeans are produced in nearly all the nine provinces of South Africa, Mpumalanga is the leading province with an estimate of 44% production, followed by Free State (33%), KwaZulu-Natal (8%), Limpopo (4%), North West with (4%), Gauteng with (6%) and Western Cape (1%) (Figure 2.1) (Nortjé, 2020). However, Northern Cape, in the past 25 years, compared to other provinces, has been attaining the highest production yields of about 1.56 t/ha (DAFF, 2010; Dlamini et al., 2014a).
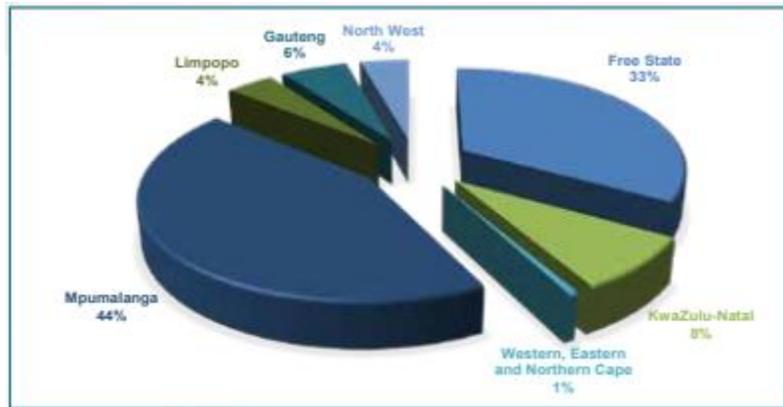
Figure 1.4: Soybean output (%) per Province in South Africa. Adapted from (Nortjé, 2020).

The average yields in South Africa range from 1.48 to 2.29 t/ha (Schulze & Maharaj, 2007). These yields are 40% lower than the average yields of the three primary producers, globally (Manthata, 2018). In 2017, soybean yield (2.29 t/ha) in South Africa increased by 55.4 %, which was the highest yield on record (Motaung, 2018). However, in 2018/2019, there was a decrease in average yield with an estimated 1.60 t/ha (Sector, 2020). The last season 2019/2020, the average yield was1.79 t/ha, indicating a slight increase, while in the 2020/2021 season, the average yields increased to 1.81 t/ha (Sector, 2020).

1.4 PRODUCTION CONSTRAINTS

Abiotic and biotic stresses are important constraints that directly and indirectly reduce the yield and quality of soybean worldwide (Hartman et al., 2011). About 70% of crop production is affected by abiotic and biotic factors (Cramer et al., 2011). Moreover, it is challenging to estimate the exact impact of these constraints on crop production (Cramer et al., 2011). The most documented abiotic crop production

constraints are drought and high salinity, toxicity as a result of overfeeding of fertilizer (More than 50% of the world's crop yields are reduced by the above abiotic factors (Anami et al., 2020).

Recent studies have reported that one major abiotic factor expected to affect soybean production is land availability (Murithi et al., 2016). As a result of soil degradation and making the soil unusable due to erosion by wind and air. Other factors contributing to land losses for agricultural activities is the conservation for urban use including highways, housing and industrialisation (Scherr & Yadav, 1996). In tropical regions, the major abiotic factors include soil fertility, poor nodulation and seed longevity (Hartman et al., 2011). Biotic factors causing severe losses and adverse effects on soybean's quality are related to geological and environmental factors, including diseases, insect pests (Heinrichs & Muniappan, 2018) and weeds (Vivian et al., 2013). Kawukiet al. (2013) indicated that in SSA regions, the major soybean diseases are soybean rust, bacterial pustule, frogeye leaf spot, red leaf blotch and bacterial blight (Kawuki et al., 2003; Murithi et al., 2016). Among them, soybean rust poses the greatest threats to soybean production globally and its outbreak in Africa has caused tons of yield losses (Murithi et al., 2016). According to the FAO, in Africa, abiotic and biotic stress cause yield reduction of <1 t/ha (FAOSTAT, 2016).

1.5 DROUGHT CHALLENGE TO SOYBEAN

Among the various abiotic stresses, drought is the most destructive both economically and agronomically. Drought is the primary cause of impaired growth, development, crop yield and production (Sadeghipour & Abbasi, 2012; Wang et al., 2020). Drought stress and high temperatures reduce national cereal production by 9 to 10% (Kim et al., 2019; Lesk et al., 2016). According to the FAO, drought caused 83% of economic losses, equivalent to US$29 billion (FAO, 2017). The production losses caused by drought are significantly related to harvested area, and effects of extreme heat to yields (Lesk et al., 2016). In the same study, the findings indicated that the recent occurrence of droughts causes more damage in developing countries than in developed counties with an estimate of 8 to 11% production losses. On the other hand, with an increasing population expected to be 10 billion by the year 2050, drought stress may be the main cause of severe food shortages (Sadeghipour & Abbasi, 2012). Furthermore, global climate change worsens this condition in many regions. Thus, there is an urgent need to develop crop cultivars that are tolerant to these conditions to improve crop yield and food security.

Drought is known as the main constraint to soybean productivity and drought stress occurring at R4 (full pod) to R7 (beginning of maturity) causes greater yield losses than in any other stage (Staton, 2012). Drought stress reduces soybean yields by 40% (Yan et al., 2020). These yield losses are associated with a reduction in the number of pods by 20% due to flower abortion, which is the major source of yield loss (Licht et al., 2013). In addition, yield losses can be correlated with reduced Nitrogen (N) fixation by nodules

(Licht et al., 2013). Drought stress inhibits the process of nitrogen fixation leading to the failure of meeting the soybean's nitrogen fixation demand for maximum growth by 40-60% (Basal & Szabo, 2020). Breeding for soybean drought-tolerance offers an alternative to reducing the impact of drought. The following sections and literature review section discuss drought-tolerance breeding in soybean.

1.6 BREEDING SOYBEANS FOR DROUGHT-TOLERANCE

The primary breeding objectives associated with soybeans include developing superior soybean cultivars, improved yields, seed quality and resistance to biotic and abiotic stresses (Chigeza et al., 2019). The development of improved soybean lines has been achieved through conventional and molecular breeding (Ahmar et al., 2020). Breeding for quantitative traits such as yield for drought-tolerance using the conventional approach is time consuming and labour intensive because yields are quantitative traits with low heritability and are highly affected by environmental variations (Manavalan et al., 2009; Rauf et al., 2016). Moreover, conventional breeding programs are time-consuming, laborious and require a large amount of land for both field and greenhouse trials. However, despite these challenges, conventional breeding for drought-tolerance is still feasible through use of secondary traits that are correlated to yield. This breeding approach can be used for the screening and selection of drought-tolerant genotypes under well-watered and water-stressed conditions (Mwadzingeni et al., 2016). Thus, comprehensive phenotyping and screening of secondary traits forms the basis of drought-tolerance breeding (Ahmar et al., 2020).

To determine genotypes that are drought-tolerant, yield selection based-indices can be used to measure the extent of tolerance in genotypes to drought stress (Sareen et al., 2014). Drought indices can be used to characterize water stress based on timing, duration and intensity. Several drought indices have been identified to quantify drought stress, including indices stress tolerance (STI) geometric mean productivity (GMP), mean productive (MP), yield index, yield stability index (YSI), drought resistance index, stress susceptibility index relative efficiency index and Harmonic mean (Sánchez-Reinoso et al., 2020). Good estimation of variance components under both droughts stressed and well-watered conditions is key for the evaluation of the genotypes and prediction of the success of the breeding program. Hence in this study, variance components were determined among soybean genotypes evaluated under water-stressed and well-watered conditions. Drought-tolerance was determined using yield-based selection indices.

1.7 PROBLEM STATEMENT

Food insecurity remains a prominent striking feature for many South African households (Clover, 2003). Soybean production in South Africa is not meeting the demand for consumption and industry, while drought stress is the major contributing factor to the low soybean productivity in the country. Few drought-resistant

cultivars have been developed so far (Mabulwana, 2013). The gap in breeding for drought-tolerant cultivars could be because plant responses to drought stress are not well understood due to the complexity of their physiology, morphology and molecular responses. Therefore, developing new progenies that perform better under water stress is important to boost soybean productivity in South Africa.

In this study, the performance of 36 soybean lines under water-stressed and well-watered conditions was evaluated to identify promising soybean lines that are drought tolerant and select the best, efficient and effective breeding strategies for drought-tolerant breeding programs.

1.8 RESEARCH RATIONALE AND MOTIVATION

As discussed in the preceding introduction, food insecurity and malnutrition are among the most significant challenges in developing countries, including South Africa. The most affected households by food insecurity are in rural areas (Machethe et al. 2004). According to Demetre et al. (2009), about 35% of the South Africa's population suffer from a lack of food security. From the above estimation, one-quarter of this population are children under five years with stunted growth due to malnutrition (Smith et al., 2000; Svedberg, 2011).

 Soybean is among the highly rated important food crops and with great potential to eliminate protein malnutrition prevailing in underprivileged regions of South Africa. It is an important food source and oil. The consumption of soybean in South Africa is estimated at 32% for oil and oilcake, 60% for animal feed (the broiler and egg industries) and 8% for direct human consumption (DAFF, 2017). The food products made from soybean have tremendous health benefits and consist of high-quality proteins at an affordable price. These benefits have made soybean to be recognized as one of the most valuable agronomic crops in the world (Khan et al., 2017; Agarwal, et al., 2013). Hence, this warrants more research to increase its yield under different conditions, including drought stress. Soybean is also recognized as a genetically diverse plant species since it can be cultivated in various agro ecological environments (Khan et al., 2017). Understanding the genetic variation among genotypes will aid in the improvement of soybean lines for desired traits (Agarwal et al., 2013).

Currently, there are fewer varieties in South Africa that are drought-tolerant; therefore, justifying the need of drought-tolerance improvement. In this study, drought-tolerant soybean genotypes were selected through the identification and understanding of soybean physio-morphological responses and mechanisms to drought stress. The effects of drought stress on reproductive stages and yield were evaluated and compared with performance under well-watered conditions.

1.9 OBJECTIVES

The overall objective of the study was to assess the performance of 36 soybean lines under well-watered and water-stressed conditions in order to identify promising lines for drought-tolerance breeding program.

1.10 SPECIFIC OBJECTIVES

Specifically, the present study aimed to:

1) Screen soybean genotypes for drought-tolerance using morphological and physiological traits.
2) Assess drought-tolerance in soybean genotypes using drought-tolerance indices.
3) Estimate the variance components and heritability of yield and yield components of soybean under well-watered and water-stressed conditions.

1.11 HYPOTHESES

The hypotheses tested were:

i) Morphological and physiological traits discriminate soybean genotypes under well-watered and water-stressed conditions.
ii) Yield-based selection indices are effective in selecting drought-tolerant soybean genotypes.
iii) Variance components and heritability of yield and yield components in soybean genotypes decrease under water-stressed conditions compared to the well-watered conditions.

1.12 DISSERTATION OUTLINE

The present dissertation comprises of the literature review and four experimental chapters, which were summarised into a discrete and interdependent papers, according to the University of KwaZulu-Natal's dissertation format. Noteworthy that there are overlaps and unavoidable repetitions of information, precisely introductory information and references in the background, literature review and chapters.

Table 1.1: Dissertation outline

Chapter	Title
1	Dissertation introduction (background)
2	A review of the literature
3	Morph-physiological responses of soybean (*Glycine Max* L. Merr.) genotypes under well-

	watered and water-stressed conditions and yield-based indices for drought-tolerance
4	Assessment of drought-tolerance in soybean (*Glycine max* L. Merr.) genotypes using drought-tolerance indices
5	Estimation of the variance components and heritability of yield and yield components in soybean (*Glycine max* L. Merr.) genotypes under well-watered and water-stressed
6	A general overview of findings and implications of the present study.

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Chapter two

Literature Review

ABSTRACT

Drought is the leading cause of impaired growth and development, and crop yield reduction. Soybeans are sensitive to drought stress during flowering and pod filling, causing reductions in secondary traits and ultimately reducing seed yield. The integration in breeding programs of genetic and phenotypic screening ensures accuracy in selecting superior genotypes with high yielding potential under water stress. Genotypes are assessed for drought-tolerance in water-stressed and under well-watered conditions for characterization purposes. The morphological and physiological traits that are highly correlated with yield in soybean include plant height (PH), canopy (CNP), chlorophyll content (CC), biomass yield (Y), 100 seed weight (SW), pods per plant (PDP) and biomass (BM). The selection for drought-tolerance under well-watered and water-stressed conditions can be more effective and successful when yield based selection indices including, Drought intensity index (DII), Stress susceptibility index (SSI), yield index (YI), Stress tolerance index (STI), Mean relative performance (MRP), GMP-Geometric mean of productivity (GMP), Yield stability index (YSI), Mean productivity (MP), TOL-Stress tolerance (TOL), Harmonic mean (HM) and Relative efficiency index (REI) are used. The genetic variability, heritability and correlation of genotypes are fundamental components in the breeder's selection programs to improve yields and related traits under drought stress. Therefore, drought-tolerance in soybean lines can be improved using the above approaches.

Keywords: soybeans, water stress, morphological traits, physiological traits, indices, broad sense heritability.

2.1 INTRODUCTION

The chapter reviews the literature on various topics in relation to the objectives of the study mentioned in the previous section. The review gives a broad view on soybean origin, history and distribution, botanical characteristics, growth stage, developmental stages, growth and water requirements. It further highlights the effects of drought stress on plants including soybean and their adaptation mechanisms to survive water stress. In addition, a detailed account of recently used plant breeding strategies to improve crop performance under water stress are explained (phenotyping, drought-tolerance yield selection based indices, estimation of variance components and broad sense heritability). The last section of the literature review highlights the analysis tools and methods used in the study. Drought stress causes severe yield reduction in soybean from flowering to the beginning of maturity resulting in changes in soybean growth, morphological features and physiological processes. However, under water stress, soybeans have developed adaptive mechanisms such as drought resistance, i.e., drought-tolerance.

2.2 THE CROP SOYBEAN

2.2.1 Crop origin, history and distribution

Soybean (*Glycine max.* L Merri) is an annual leguminous crop native to East Asia, particularly in the North and Central China. It is commonly grown for its edible bean and other various uses (Hartman et al., 2011; Oqba, 2017). The crop further spread to other developing countries, including Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Myanmar, Nepal, and North India (Dwevedi & Kayastha, 2011). In the early 17th century, soybean was introduced in Europe, reached the United States in 1765 and became popular in the mid-20th century in the South and Midwest. Brazil and Argentina are the primary producers of soybean (Hymowitz, 2004).

The soybean genus comprises of two respective subgenera, *Glycine* and *Soja* Moench F.J Herm (Ratnaparkhe et al., 2010). The subgenus *Glycine* is the wild type, which consists of 26 perennial species (Singh, 2017). The subgenus *Soja* is classified into two economically important species, the annual cultivated soybean (*Glycine max* L. Merri) and the wild annual *G. soja* Sieb and Zucc. *Glycine soja* Sieb is dominant in China, Japan, Korea and Russia and *Glycine max L.* Merri is dominant in the Northern Central of China and East Asia (Hymowitz, 2004).

2.2.2   Botanical characteristics of soybean

Soybeans have an erect, bushy, annual growth habit with woody stems and can potentially grow up from 0.2 to 1.5 m tall depending on the variety (Singh, 2017). The soybeans consist of leaves possessing four

different leaf structures, including seed leaves (cotyledon), followed by primary leaves called unifoliate (Figure 1.6), trifoliate and small pairs of leaves prophylls (Liu, 2004). Soybean leaflets are oval or lance-like in shape with entire margins, compound 3 leaflets that are alternately arranged and usually are 3-15 cm long and 2-7 cm wide (Liu, 2004). The erect stems are covered with thick brown hair; the soybean plant produces small purple and white flowers that are inconspicuous, stalk-less, and embedded singly or in clusters located in the plant axils (Ratnaparkhe, 2011). The fruits are called pods, curved seed pods, which are 3-15 cm long that usually turn into yellow to brown when fully matured and bearing 3 to 5 seeds per pods (Figure 1.2). The soybean seeds vary in colour: yellow, green, black, or mottled combinations (Shurtleff & Aoyagi, 2013). All soybeans cultivars have the above botanical characteristics, however, they differ in

growth habits.

Figure 2.1 : a) and b) Soybean clustered white and purple flowers on plant axils were captured at R1 to R2 stage during early summer in 2019 at the faculty of Agriculture, University of Kwazulu-Natal Natal (29°37'32.9"S 30°24'18.8"E), Pietermaritzburg campus, greenhouse and c) image of soybean pods at R6 growth stage.

There are three types of growth habits among soybean cultivars: determinate, semi-determinate and indeterminate (Liu, 2004). Determinate growth habit is associated with growth that proceeds only during the vegetative season and then ceases at the flowering stage. For indeterminate cultivars, their vegetative organs continue to grow even after the flowering stage, while semi-determinates have indeterminate stems that terminate vegetative growth after the flowering stage.

2.2.3 Growth and developmental stages

The soybean's growth stages are divided into vegetative (V) and reproductive growth stages (G). The vegetative stage in soybean begins from the emergence of the young seedling and ends at the start of flowering and these stages are classified by the number of nodes on the main stem (Liu, 2004). There V stages are subdivided into three designated stages V1, V2 and V3. However, there are two crucial stages before these stages, called the emergence (VE) and cotyledon (VC), as shown in table 1.1.

Table 2.1: Subdivisions of soybean plant vegetative and reproductive stages. Adapted from (Wright & Lenssen, 2013)

Vegetative Stages	Reproductive Stages
VE = emergence	R1 = beginning bloom
VC = cotyledon	R2 = full bloom
V1 = first node	R3 = beginning of pod
V2 = second node	R4 = full pod
V3 = third node	R5 = beginning seed
V4 = fourth node	R6 = full seed
V5 = fifth node	R7 = beginning of maturity
V(n) = nth node	R8 = maturity

The VE is when the cotyledon has pulled out of the soil and in soybean, emergence occurs from 4 to 5 days after sowing depending on the genotype (Purcell et al., 2014). The cotyledon stage refers to the unfolding of unifoliate leaflets opposite the stem. V1 is the set of the unfolded trifoliate that appears (Pedersen et al., 2004), followed by the V2 stage, which is the two sets of the unfolded trifoliate at the node above unifoliate. Also, at this stage, the nitrogen fixation in the root is activated. The last stage of vegetative growth in soybean is the "nth" trifoliate V(n), which involves the unfolding of trifoliate. This stage depends on the soybean variety and environmental conditions (Purcell et al., 2014).

The subdivisions of the soybean plant's reproductive stage (R) are subdivided as R1 to R8, as described in Table 1.2. Flowering (R1) begins on the 3rd to the 6th node of the main stem and occurs approximately 6 to 8 weeks after emergence, depending on the environment and genotype (Casteel, 2010). During flowering, nitrogen fixation increases gradually with the nodulation (Pedersen et al., 2004). As stresses occur over the following two weeks, many flowers will abort instead of developing into pods. After 15 to 20 days, the pods develop slowly immediately after fertilization and reach maximum length (Liu, 2004).

Table 2.2: Subdivision of soybean reproductive stages. Adapted from (Wright & Lenssen, 2013)

Reproductive stage	Description of stage
R1 - Beginning bloom	One flower open at any node on the main stem
R2 - Full bloom	Open flower at one of two uppermost nodes on the main stem with a fully developed leaf.
R3 - Beginning of pod	Pod 3/16 inch long at one of the four uppermost nodes on the main stem with a fully developed leaf
R4 - Full pod	Pod 3/4 inch long at one of the four uppermost nodes on the main stem with a fully developed leaf
R5 - Beginning seed	Seed 1/8 inch long in a pod at one of the four uppermost nodes on the main stem with a fully developed leaf
R6 - Full seed	The pod containing 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 of maturity	One normal pod on the main stem that has reached its mature color
R8 - Full maturity	95% of the pods have reached their mature color

2.2.4   Growth and water requirements

Temperature is a primary factor in the rate of plant growth; however, extreme temperatures and below optimum may affect crop growth (Hatfield & Prueger, 2015). In most cases, temperatures above 30˚C and below 13˚C have a negative impact on yields, especially during the flowering stage, which may result in low flowering and pod abortion (DAFF, 2010). Soybeans grow best under warm and humid conditions. For successful germination, the soil temperatures should be about 15˚C to stimulate growth, while the optimum temperature for other growth stages should be in a range of 20˚C to 25˚C (DAFF, 2010; Viana et al., 2013). Soybeans grow under a broad spectrum of soils but best on deep, well-drained soils, which are highly fertile and rich in nutrients such as calcium. Among legumes, soybeans grow best at lower soil pH (5.2); for optimum growth, pH should be around 5.6 to 7.0 (DAFF, 2010).

The soil must have an excellent water-holding capability for better yields, and soybeans adapt well to heavier soils (Li et al., 2020). They also perform exceptionally at altitudes between 0 to 2000 m above sea

level and take long as 180 days, approximately six months to mature (Maltsoglou & Khwaja, 2010). Soybeans are planted best in late winter in warmer-winter regions, and specifically, in South Africa, the best planting date is from early mid-November to end of December, since they prefer high temperatures for good yields. Plant spacing is an important consideration as it creates room to accommodate plant roots and maximum growth. Soybeans are grown in narrow row spacing of 50 cm or less; while for best results, the row spacing should be 75 to 100 cm (Zhou et al., 2012). The spacing between plants should be from 5 cm to 15 cm, noteworthy that the spacing also depends on the irrigation method to be used and water requirement (DAFF, 2010).

Water functions in almost all physiological and biochemical processes in plants and contributes approximately 90% of their mass (Souza et al., 2013). However, the plant's water requirement for daily functioning is influenced by various factors such as environmental weather conditions and cultivar growth stage. According to DAFF (2010), the total amount of water demand for soybean maximum productivity varies from 500 to 900 mm. Soybean's water requirement rate peaks at flowering to mid reproductive stages (7 to 8 mm per day). Approximately 45% of irrigation is required during flowering, 30% at the late flowering to pod development and 80% at late pod to maturity (Liu, 2004). In cases where soybean water demands are not met during reproductive stages, a significant yield reduction may occur (Smith et al., 1998).

The water demand during seedling is relatively low as too much water at this stage may result in prolonged vegetative stages and may delay flowering, increased plant height, and lodging. According to Kranz & Specht, (2012), approximately 65% of water use in soybean occurs from R1 to R8. It is noteworthy that soybeans are highly sensitive to water stress during R3 to R6. Therefore, the plant may require irrigation during flowering if the stress worsens, especially in soils with insufficient water holding capacity (Bodner et al., 2015; Liu, 2004). Irrigation at this stage will increase the number of seeds, which will result in greater yields. The best yield and most efficient water use are obtained when the available soil water in the plant's root system is not depleted by more than 50 to 60% (Smith et al., 1998). Hence, appropriate irrigation during early reproductive stages is crucial for soybean production under drought stress. Consequently, understanding of the plant's water use throughout the season will aid in efficient irrigation applications.

2.3 DROUGHT

Globally, climate extremes associated with climate change are increasing drastically, resulting in concerns on their effects on crop production (Kim et al., 2019). Drought is among the topmost severe environmental constraints to crop productivity (Farooq et al., 2009). Drought stress is referred to as multidimensional stress and results in changes in the plant's physiological, biochemical and molecular traits (Salehi-Lisar &

Bakhshayeshan-Agdam, 2016). The stress is caused by several factors, including lower than average rainfall in a given region and this was also found to be positively associated with increased salinity and high temperatures (Duba, 2017). In the case of low rainfall, less water is available in the plant rhizosphere than the required for efficient growth and biomass production (Osmolovskaya et al., 2018).

Drought stress in plants may also be a result of low water availability in the soil leading to poor water supply to the plant at the vegetative stage. Inadequate water in the soil causes impaired growth in plants, poor development and results in cell death and ultimately reduces crop production and yield. Drought stress depends on the severity and duration. The main challenge of the severity of water deficit is its unpredictability as it depends on various factors, including moisture content, soil capability to restore water and evaporation (Khan et al., 2018). Water deficit results in high risks to world food security with around 60% of the world's population (600 billion) affected by drought, especially those living in arid and semi-arid regions (Duba, 2017).

According to the drought index and crop yield data for the past 27 years, there has been close to three-quarters of cultivated land of major global crops, including maize, rice, soybean and wheat that has been affected by drought stress (Kim et al., 2019). The production losses due to drought are estimated at US$166 billion. Among the four top production crops, soybean is the most affected by droughts with losses of 0.67 billion hectares (ha) (91%), followed by maize with 124 million ha (82%), wheat with 161 million ha (75%), and rice with 102 million ha (62%) (Kim et al., 2019). Crop yield reduction due to drought stress has been estimated as 8% for wheat (0.29 tons per hectare), 7% (0.24 t/ha) for maize, 7% (0.15 t/ha) for soybeans and 3% (0.13 t/ha) for rice. Furthermore, Therefore, there is an urgent need for the improvement of well-adapted cultivars to drought stress with high yields.

2.4 PLANTS ADAPTATIONS TO DROUGHT STRESS

Apart from the adverse effects caused by water deficit, plants have evolved mechanisms to tolerate such conditions. Plant's adaptive mechanisms involve morphological, physiological and biochemical traits (Salehi-Lisar & Bakhshayeshan-Agdam, 2016). These adaptive mechanisms collectively contribute towards drought resistance (DR), which can be categorized into drought stress escape, avoidance and tolerance (Basu et al., 2016; Akinci & Losel., 2012).

Drought escape is reported as one of the crucial morphological mechanisms for plant survival under drought stress. Plants escape drought by shortening their life cycle or growing season prior to a future drought event. This increases the chances of reproducing before the soil is parched (Shavrukov et al., 2017). The plant's growth duration determines drought escape; however, this factor is also dependent on both the genotype

and environment. Early flowering and shorter vegetative growth stage are the main drought escape mechanisms (Krivosuda & Filova, 2017). The parameters of drought escape include soil moisture availability, successful phonological development, short growing season and terminal drought stress. These parameters are only crucial for DR but are positively correlated with high yields (Obidiegwu et al., 2015).

Drought avoidance is associated with a reduction in water loss within the plant tissues. This is observed through monitoring plant stomata during transpiration and maintaining the water uptake on the roots system (Basu et al., 2016). Plant biomass, length, density and depth are the most critical drought avoidance traits that also correlate with yield (Yang et al., 2019). Drought tolerance, on the other hand, is the ability of plants to survive under low moisture content through adaptive traits (Basu et al., 2016). Several studies have documented numerous physiological, morphological and biochemical adaptive mechanisms and drought-tolerance (Kamanga et al., 2018). Physiological traits and morphological traits are used for efficient selection for drought-tolerance-related traits in breeding programs.

2.5 THE EFFECTS OF DROUGHT STRESS ON SOYBEAN

2.5.1 The morpho-physiological responses of soybean to drought stress

The effects of drought stress have been studied on several crops, including maize (Abrokwah et al., 2017), wheat (Nezhadahmadi et al., 2013), rice (Lie et al., 2020), tomato (Khan et al., 2018) and cotton (Ali & Ahmadikhah, 2009), among others (Hussain, 2018). Studies have indicated that the sensitivity of crops to drought varies according to the growth stage (Liu, 2004). In soybean, the effects of drought stress on growth and yield depend on the degree of the stress and stage of growth at which it sets in (Tarumingkeng & Coto, 2003). Soybean is highly sensitive to drought stress during flowering and beginning of pod setting up to full seeding. The occurrence of drought at this stage induces several physiological and morphological changes, which may aid plants to adapt under such water conditions (Frederick et al., 2001). In a study by Ku et al. (2013), it was found that drought imposed at vegetative stages (V4) and at flowering (R1-R3) had adverse effects on soybean's morphological and physiological features than in any other stage.

2.5.2   Physiological responses

Under drought stress, soybean undergoes a series of physiological changes, including osmotic adjustment, decrease in stomatal conductance (G_s), photosynthetic rate (P_n) and chlorophyll content (Chowdhury et al., 2016). The first plant response to drought stress is stomatal closure, serving as an adaptive potential to some crops to reduce water loss (Basu et al., 2016). This process prevents water losses from the transpiration pathway and limits the CO_2 intake into the leaf for photosynthesis (Chowdhury et al., 2016). Some other crops have evolved xeromorphic features to adapt to drought to reduce the transpiration rate (T_r). These

features include leaf shedding, decrease in leaf number, size and branching off (Basu et al., 2016). Hence, the measurement of the transpiration rate is an excellent tool to access drought-tolerance.

Drought stress reduces photosynthesis, ultimately decreasing plant leaf area and the photosynthetic rate per unit leaf area (Hussain et al., 2018; Lawlor & Tezara, 2009). A reduction of photosynthesis is generally due to stomatal closure, as mentioned previously and other metabolic damage (Basu et al., 2016; Lawlor, 2002). Crop's adaptive responses to impairment of photosynthesis caused by induced-drought stress includes thermal dissipation of light energy, xanthophyll cycle and dissociation of the light-harvesting complexes from photosynthesis (Basu et al., 2016).

Another adaptation to drought stress is sclerophylly, that is, the formation of thick, hard leaves characterized by reinforcing tissues (thick-walled epidermal cell and sclereids) (Aroca, 2012). The reinforcing tissues prevent the structure from falling when there is no water available (Aroca, 2012; Basu et al., 2016). Under drought, the sclerophyllous leaves slightly reduce their volume through the thick cuticle and thick walls of epidermal cells, causing mesophyll cells to shrink, subsequently increasing intracellular spaces. By doing so, this maintains the photosynthetic rate to remain active while other leaves wilts (Aroca, 2012). Studies indicate that photosynthesis and stomatal conductance of leaves are known to be positively correlated among soybean genotypes (Chowdhury et al., 2016).

Osmotic adjustment is one of the physiological mechanisms that plants have developed during drought stress, accumulating solutes through passive dehydration (Ku et al., 2013; Sloane et al., 1990). This mechanism ultimately allows the regulation of cell turgor and turgor related traits, including photosynthesis during dehydration. The osmotic adjustment has been demonstrated by Souza et al., (2013); in soybean, it occurs in hypocotyls, and the leaf tissue, respectively, at low water potential. However, this is contrary to some studies which have not found any evidence showing the osmotic adjustment response in soybean (Sloane et al., 1990). The above adaptations of the plant through DR are due to several underlying morpho-physiological changes, which will be discussed in the following section.

2.5.3   Morphological responses

Drought stress effects on soybean physiology have been well documented in literature and many research papers. The fact that the effects of water deficit on growth and yield are highly dependent on both the degree of stress and the stage at which stress occurs, soybean is susceptible to drought stress at all growth stages (Sionit & Kramer, 1977). However, the most vulnerable stages of water stress in soybean are early flowering to full-pod filling (R1-R6). The same phenomena were reported by Marais and Bufé (2013), indicating that early to mid-pod filling and seed filling are critical stages that are susceptible to drought than any other stage in soybean growth.

Eck et al. (1987) reported soybean yield reduction of 9 to 13% under drought imposed during R1, and as the stress progressed to between R4 and R5, the yields were reduced by 46%. Stress imposed at the beginning of R5 decreased yield by 45% and R6 by 65% (Eck et al., 1987). Soybean seed yield decline is indicated by a reduction in the number of pods per plant, number of seeds per pod, total weight per pod and the process of symbiotic nitrogen fixation (Liu, 2004). Tarumingkeng et al. (2003) indicated that during the reproductive stage under water stress, flower abortion is likely to occur, resulting in a shorter flowering period, number of flowers, number of seeds and pods. Furthermore, Oqba (2017) reported that seedling height was reduced by 4.3% under drought stress at different stages.

A study conducted by Karam et al. (2005) here stress was applied at one of the growth stages and irrigated for the rest of the season reported maximum reduction in yield at full pod filling and less yield reduction when stress occurred from early flowering. Moreover, the effects of drought stress on the number of pods, date to maturity and seed size were also observed. A maximum decline in the number of pods was recorded from the stress that occurred during late flowering. However, stress occurring during early flowering and full pod filling had no significant effect on pod number. Another study by (Sionit & Kramer, 1977) reported similar findings; but the effect of drought in reducing the number of seeds per plant was observed at the flowering stage.

The seed weight of soybean is a yield component that is highly affected by drought stress (Karam et al., 2005) and this was supported by Demirtas et al. (2010), who observed a 15.2% reduction of 100 seed weight during drought stress Frederick et al. (2001) further showed a significant drought effect on soybean's number of branches, while Atti et al. (2004) demonstrated a 28% reduction of the mean number of branches during drought stress. A decline in the number of seeds per given plant under drought stress was also reported by (Li et al., 2020). An in-depth analysis of the effects of drought in soybean showed that the stress significantly reduced plant height at the V4 growth stage more than any other reproductive stage, including R2, R4 and R6 (Li et al., 2020).

2.6 PLANT BREEDING STRATEGIES TO IMPROVE CROPS PERFORMANCE UNDER WATER STRESS

Improving plants under drought stress is a challenging task faced by many plant breeders. Despite this challenge, in past decades, researchers have implemented and developed numerous methods to improve drought resistance through conventional breeding and field screening. The most efficient technique for improving drought-tolerance and crop production under water deficit is through selection of high yield under controlled drought stress and optimum environments (Rauf et al., 2016). The comparison among cultivars under these different environments increases genetic variation, genotype by environment

interaction (GEI) and makes the selection more efficient. The GEI allows plant breeders to define adaptations and yield of cultivars (Cleveland et al., 1999). The most commonly used strategies to improve yield can be categorized into empirical and analytical breeding.

Empirical breeding involves a direct selection for yield and yield components, giving rise to plant populations that are well adapted to drought-stressed environments (Rauf et al., 2016). This breeding strategy is efficient in regions where drought stress in permanent. However, analytical is an indirect selection involving crop improvement for yield under water stress based on secondary traits such as morphological, physiological and biochemical traits (Rauf et al., 2016). The second breeding strategy, the analytical selection, is commonly used to obtain information on several traits associated with yield components; in such a case, a high narrow-sense heritability for desired traits is required to give positive selection in breeding populations. The desired traits must be diverse, easily accessible and measurable.

2.6.1 Broad sense heritability

Heritability is the proportion of the observed phenotype attributed to genetic variation. It allows breeders to evaluate the genetic make-up of the trait that can be passed to the offspring. In the case of drought stress, broad-sense heritability is used to quantify the phenotypic response of the genotypes whether it is due to genetic or environmental viability. Traits such as flowering date, plant height and osmotic pressure has high heritability under drought stress. Tuberosa (2012) indicated that 50% flowering is the most crucial trait in phenotyping genotypes' performance under drought when heritability is low (0.3 to 0.4). A study by Bogale et al. (2012), involving maize lines (196 S_1 and A-511) indicated that the lines exhibited high broad-sense heritability based on flowering traits, which were significantly associated grain yield.

Heritability is a tool used to estimate the degree of variation in the phenotypic trait in a population considerably due to genetic variation among individuals in a given population. Broad sense heritability is the ratio of genetic variance to phenotypic variance while narrow sense heritability is the ratio of additive variance to phenotypic variance (Piepho & Möhring, 2007). The estimates of heritability typically range from 0.0, which indicates that genes are not part of all phenotypic expression of the populations to 1.0, which means genes account for all individual's differences (Hill & Mulder, 2010). Therefore, broad sense heritability (H^2) is estimated using the equation 1 below:

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} \qquad\qquad \text{Equation 1}$$

Where σ_g^2 is the total genetic variance and σ_p^2 is the phenotypic variance (Walker, 2012). However, for the genotypes' selection purposes, genotypes are normally selected based on means over the environment and replicates within the environment using broad sense heritability as shown in equation 2:

$$H = \frac{\sigma^2 g}{\sigma^2 g + \frac{\sigma^2 ge}{e} + \frac{\sigma^2 r}{re}} * 100 \qquad \text{Equation 2}$$

Where $\sigma^2 ge,$ is the genotype by environment interaction and $\sigma^2 r$ is the variance of residual error (Walker, 2012). Many previous studies have assessed heritability mainly for yield and related traits in soybean (Malek et al., 2014). These studies demonstrated high heritability for branch number, plant height, pod number and seed weight. In the current study, broad-sense heritability was estimated using variance components.

2.6.2 Yield based selection indices for drought-tolerance

Selection for superior promising varieties is a crucial step in plant breeding programs. It is widely exploited in studies to estimate genetic parameters, specifically for agronomic traits and is done through the selection indices method (Teixeira et al., 2017). Selection indices are an important selection method that provides researchers with information about genotype traits that are correlated, allows one to perform indirect selection particularly for characters that are difficult to select phenotypically and ultimately makes it possible for simultaneous selection of multiple traits (Michel et al., 2019). This technique has been explored in breeding programs, particularly for yield improvement studies and drought-tolerance in many crops including soybean.

The selection based indices combine all information of different desired traits for each unit to be selected and evaluates the expected indirect responses from the original traits, thereby improving selection efficiency and increasing chances of plant improvement in breeding programs (Lopez-Cruz et al., 2020). Astudy by Leite et al. (2018) used selection indices to evaluate F8 soybean lines and only five of the genotypes out the 27 different lines were found to be superior (Leite et al., 2018; Teixeira et al., 2017). In their study, Leite et al. (2018) used genetic and phenotypic correlation analysis to identify the traits to be used as surrogates for grain yield in order to apply indirect selection. High correlations were obtained between plant height at maturity stage with plant height at the beginning of pods and grain yield. These findings indicated that plant height at maturity stage has a tremendous direct influence on grain yield.

However, use of yield based selection indices for drought-tolerance in soybeans have not been well documented. Drought stress indices are quantitative measures of drought-tolerance based on yield losses under drought conditions in comparison to well-watered conditions and are typically computed in numerical

as representatives of drought diversity (Farshadfar & Elyasi, 2012). These numerical representations of drought stress are used to screen drought-tolerance to determine the severity, timing, duration and location of drought (Rad & Abbasian, 2011). This technique has been used in production breeding programs to quantify tolerance using relative yield responses as a selection criterion.

The relative yield responses can be determined and selected under drought stress using the following parameters: drought intensity index (DII), stress susceptibility index(SSI), stress tolerance index (STI), geometric mean of productivity (GMP), yield index (YI), yield stability index (YSI), Mean relative performance (MRP), relative efficiency index (REI), harmonic mean (HM), stress tolerance (TOL) and mean productivity (MP) (Mdluli, 2018; Mehraban et al., 2018). The above drought indices have been used to characterize drought-tolerance in different crops, including maize (Jafari et al., 2012), sunflower (Bahrami et al., 2014) wheat (Mehraban et al., 2018) and soybean (Bouslama & Schapaugh, 1984).

In a study conducted by Mdluli (2018), among 15 wheat genotypes that were evaluated for drought-tolerance using drought indices, the indices that showed significant differences for drought resistance (DR) were HM, MRP, STI, YI and YSI. In a different study by Mehraban et al. (2018), the yield responses for wheat cultivars at different growth stages showed significant and positive correlations under normal conditions with STI, MP, GMP and TOL, and negative correlations with YSI and DI, whilst under drought stress, yield had a positive and significant correlation with STI, GMP, MP and HM Therefore, the best drought indices to be used are STI, GMP, MP and HM. Bouslama and Schapaugh (1984), suggested that genotypes indicating low values of SSI and TOL should be considered as less drought sensitive. In another study Majidi et al. (2011) indicated that GMP and HM were the best yield-based selection drought indices for high yielding genotypes.

2.7 ANALYSIS TOOLS AND METHODS

2.7.1 Principal Components and Cluster Analysis

The genotype by trait in a given single environment, across environments and within the subset of environments can be computed and visualized for research studies through biplot analysis. Principal Components Analysis (PCA) biplot has been used by plant breeders to assess the differences among genotypes with respect to selected traits. Furthermore, the genotype by trait PCA biplot can help in understanding the relationships among traits; traits that are important in such can be used for the selection of parents and to identify the variables that are positively and negatively correlated. This statistical tool allows breeders to evaluate precisely the patterns of morphological and physiological traits and to avoid redundancy in a data set (Das et al., 2017). Primarily, it is regarded as a multivariate data analysis tool that

reduces a broad set of variables or components without losing important information of the original data (Espósito et al., 2007).

The PCA biplot does this by grouping components with robust inter-correlation that is expressed in percentages where each component contributes a certain percentage variation to the total variability (Das et al., 2017). The first quadrant (PC1) represents the largest contributors to the total variation in a population, followed by the second (PC2) and the third (PC3) (Espósito et al., 2007). The first three PCA are the most important in reflecting the variation patterns among accessions and character associations in differentiating the accessions. The traits of variables that form a small to 45˚ angle represent a positive and strong correlation, and the ones that meet at 90˚are less likely to be correlated or not associated at all while those that diverge forming a 180˚ angle are regarded to be negatively correlated (Rummel, 1976).

However, the synthetic dimensions are not much as meaningful cluster plot, the PCA biplot gives one reduced dimension with better graphics display of how variables are clustered. Clusters are assembled using all the variance in a dataset in comparison to the 60% to 90% represented by the first PCs in PCA analysis (Xue et al., 2011). Cluster plots allow breeders to explore relationships among genotypes and genotypes by trait. This statistical tool is used to find genotypes that perform similarly and in the case of drought stress studies, this aids to group individuals that are performing the same under optimum conditions and stressed conditions (Jangra et al., 2019).

2.7.2   Correlation and path coefficient analyses

Correlation analysis has been used by plant breeders as indirect selection criteria for advancing grain yield through understanding its association with secondary components. Path analysis, on the other hand, provides path coefficients, which partition the correlation coefficients into respective direct and indirect influences on yield (Chibanda, 2017). This method has been successfully used by researchers in selecting cultivars with various traits that are significant and highly contributing to yield (Li et al., 2020). In soybean, many studies have demonstrated that grain yield is highly influenced by 100 seed weight, number of pods per plant, number of pods per plots and number of seeds per pod (Salimi et al., 2013). Salimi et al. (2013) reported a significant, positive correlation between yield with weight, dry weight and number of seeds per plant. In the same study, the number of seeds per plant and 100 seed weight were the most important traits that can be used in breeding programs to select for yield under drought stress. In another study, Ramteke et al. (2010) reported that soybean grain yield is an integrated function of branches per plant, seeds per pod, 100 seed weight, plants per area, and pods per branch.

2.8 CONCLUSION

Among the various abiotic stresses, drought stress is the most destructive factor both economically and agronomically. On the other hand, climatic change is increasing rapidly, leading to extreme weather patterns, including droughts and floods globally. This ultimately causes instability in crop production and more farmers worldwide are struggling to make profits due to massive yield losses. Drought is the primary cause of impaired growth, development, crop yield and production. Soybeans are sensitive to drought stress during flowering and pod filling resulting in massive reductions in their secondary traits and ultimately reduced seed yield.

The screening of yield is a primary factor in improving yields under drought-stressed environments in plant breeding programs. Although the selection approach for yields in the water-stressed environment helps to develop and improve drought-tolerant genotypes, the use and understanding of morphological and physiological traits that are highly associated with yields in soybeans are important. Combined approaches can hasten the indirect selection process. It has been demonstrated that only a few of these traits contribute to yields under water stress. Together with yield selection, these known traits can improve the plant breeding process in either the parental selection or the screening of segregating material. Maximized genetic variance is vital for improving the yield under water-stressed conditions through indirect selection processes.

In addition, genetic characterization ensures an adequate determination of heterotic groups and the degree of genetic diversity among genotypes. The inclusion of this approach with phenotypic characterization using physiological and morphological traits with their correlation, using yield-based selection indices and estimating broad-sense heritability can improve accuracy and repeatability of selection for drought-tolerance in soybean genotypes under water stress. This can potentially be accomplished by examining the mean performance of traits, reduction rankings, Pearson's correlation, principal components, biplot analysis and cluster plots. Numerous research studies used this approach in breeding for drought-tolerance. However, there is still a large gap in research studies that focus on drought-tolerance of soybeans. Most of these studies mainly apply molecular approaches to improve drought-tolerance in soybeans. However, conventional techniques are still recognized, and are the most important first step and foundation of all breeding programs that need to be considered.

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Chapter Three

Screening soybean genotypes for drought-tolerance using morphological and physiological traits

ABSTRACT

Drought stress leads to significant reductions in soybean yields. Therefore, screening of genotypes under well-watered and water-stressed conditions is required to better understand and develop drought-tolerant soybean varieties. The objective of this study was to determine the morphological and physiological traits associated with drought stress under well-watered (WW) and water-stressed (WS) regimes in soybean lines at the reproductive stage. Thirty-six genotypes obtained from the International Institute of Tropical Agriculture (IITA) were screened in a 6x6 alpha lattice design with two replications in the field at Makhathini Research Station, Jozini and in the greenhouse under WW and WS conditions. Water stress was imposed two weeks after 50% flowering. The WW regime was used as a control and for comparison purposes. Data collected included plant height (PH), stem diameter (SD), leaf width (LW), leaf length (LL), moisture content (MC), stomatal conductance (Gs), chlorophyll content (CC), 100 seed weight (SW), grain weight per plot (GPP), and biomass yield (BMS). The morpho-physiological data were analyzed for descriptive analysis, combined analysis of variance, Pearson's correlation coefficient and principal components (PCA). Significant differences were detected for genotype-environment interaction for the traits PH, LW, STD and BM. Significant association under water-stressed regime was obtained between PH, LW, STD, SW, LL, MC and BM, while under well-watered regime it was observed for STD, LW, LL, PH, GY, MC, SW, CC, BM and FLW. The PCA and cluster-plot analyses identified high-yielding drought-tolerant genotypes, discriminated and grouped genotypes based on their responses to water stress. The genotypes TGX 2014-44FM (G10), TGX 2001-15DM (G12), TGX 2001-20FM (G22) and TGX 2014-4FM (G29) were identified as the best potential genotypes for improving drought-tolerance in soybean lines. Traits that could be utilized for selection in improving soybean yields under water-stressed conditions included LL, STD, FLW, BM, GY and MC.

Keywords: Soybean, drought-tolerance, morpho-physiological traits, reproductive stage, PCA

3.1 INTRODUCTION

Soybean (*Glycine max. L* Merrill) is one of the essential seed legumes worldwide that is a crucial source of oil (18% to 33%) and protein (35% to 40%) (Gupta, 2011). However, soybeans are the most sensitive to drought stress among legume crops and their water requirement is relatively high. Thus, drought is a major constraint to yield productivity. The occurrence of drought stress during reproductive growth, specifically in the mid-pod filling, causes a severe reduction in soybeans (Ku et al., 2013). Understanding the morpho-physiological processes responsible for drought-tolerance may help breeders to identify traits that can be incorporated into new cultivars that are better adapted to drought-prone environments.

The effects of drought stress on soybean have been well documented and studied, including morphological, physiological responses and seed production (Ku et al., 2013). Researchers have reported that drought stress reduces soybeans yields by 40%. The effects of drought stress depend on the duration, stage development and degree of stress. The most critical stage for water stress in soybeans is flowering to full maturity (C. Yan et al., 2020). The effects of drought stress during the reproductive stage result in fewer flowers, pod abortion and reduced seed number. During the seed filling, water stress reduces the seed filling rate and seed size, ultimately decreasing yields (Sehgal et al., 2018). The responses of soybeans to drought in the physiological state include changes in photosynthesis, stomatal conductance and the rate of transpiration (Bhatia et al., 2014). In addition, drought stress leads to decreased plant leaf area, plant height and 100 seed weight (Yan et al., 2020).

Despite the aforementioned effects of drought stress on soybeans, tremendous efforts have been made to develop drought-tolerant soybean varieties. These efforts have one main goal in common, which is to enhance yields under drought stress. The sole use of yield assessment as a selection criterion for drought-tolerance cannot be efficient due to the low variation and heritability of yield under drought conditions. Screening for secondary characteristics and yield-related traits is the most efficient strategy for improving drought-tolerance in soybean varieties. Stomatal conductance is considered one of the essential and reliable physiological indicators of drought-tolerance (Reddy, 2019). Under drought stress, soybean stomatal conductance is reduced due to a decrease in stomatal frequency, which is highly associated with a reduced rate of water loss (Bertolino et al., 2019). In the same study, it was reported that soybean lines vary in stomatal frequency. Genotypes with high frequency showed a significant correlation with high photosynthetic rate, stomatal conductance and plant height. Days from germination and flowering to maturity are used for early evaluation for drought stress tolerance.

The 100 seed weight is one of the parameters used to measure the degree of drought stress in soybeans, thereby eliminating significant measurement errors (Ku et al., 2013). A study conducted by Ayub et al.

(2000) demonstrated that most of the morphological traits have a positive correlation with seed yield. Li et al. (2020) illustrated that the traits with significant associations with seed yield include 100 grains and 50% flowering date. Seed yields are better understood by assessing the seed yield per plant and have shown a meaningful correlation with the highest direct impact on soybean yields (Ghanbari et al., 2018). Yield-based indices are important parameters used in selection programs for drought-tolerance of soybeans (Bahrami et al., 2014). In this study, soybean lines were assessed based on morphological and physiological traits to identify drought-tolerant lines.

3.2 MATERIALS AND METHODS

3.2.1 Plant material

Thirty-six soybean lines (F8) obtained from the International Institute of Tropical Agriculture (IITA), Lusaka, Zambia, were lines used in the study (Table 3.1).

Table 3.1: List of soybean lines to be investigated for drought-tolerance

Genotype	Genotype ID	Pedigree	Source
G1	47316	TGX 2001-10DM	IITA – Zambia
G2	47317	TGX 2001-13FM	IITA – Zambia
G3	47318	TGX 2001-24FM	IITA – Zambia
G4	47319	TGX 2001-5FM	IITA – Zambia
G5	47320	TGX 2001-8FM	IITA – Zambia
G6	47321	TGX 2002-6DM	IITA – Zambia
G7	47322	TGX 2014-21FM	IITA – Zambia
G8	47323	TGX 2014-23FM	IITA – Zambia
G9	47324	TGX 2014-43FM	IITA – Zambia
G10	47325	TGX 2014-44FM	IITA – Zambia
G11	47326	TGX 1987-62F	IITA – Zambia
G12	47327	TGX 2001-15DM	IITA – Zambia
G13	47328	TGX 2001-18DM	IITA – Zambia
G14	47329	TGX 2001-6FM	IITA – Zambia
G15	47330	TGX 2001-9DM	IITA – Zambia

Genotype	Genotype ID	Pedigree	Source
G16	47331	TGX 2002-17DM	IITA – Zambia
G17	47332	TGX 2002-23DM	IITA – Zambia
G18	47333	TGX 2002-4DM	IITA – Zambia
G19	47334	TGX 2002-5FM	IITA – Zambia
G20	47335	TGX 2001-16DM	IITA – Zambia
G21	47336	TGX 2001-18FM	IITA – Zambia
G22	47337	TGX 2001-20FM	IITA – Zambia
G23	47338	TGX 2002-6FM	IITA – Zambia
G24	47339	TGX 2014-34FM	IITA – Zambia
G25	47280	TGX 2001-11DM	IITA – Zambia
G26	47281	TGX 2001-5DM	IITA – Zambia
G27	47282	TGX 2002-35FM	IITA – Zambia
G28	47283	TGX 2014-27FM	IITA – Zambia
G29	47284	TGX 2014-4FM	IITA – Zambia
G31	47286	TGX 2002-14DM	IITA – Zambia
G32	47287	TGX 2014-15FM	IITA – Zambia
G33	47288	TGX 2014-24FM	IITA – Zambia
G34	47289	TGX 2014-31FM	IITA – Zambia
G35	47290	TGX 2014-33FM	IITA – Zambia
G36	47291	TGX 2014-9FM	IITA – Zambia

3.2.2   Study Site

The study was carried out under greenhouse and field conditions. The field experiment was conducted at Makhathini Research Station (S 27°23′42 45, E32°10′ 48 48, Elevation: 73 m), in Jozini, during the 2019 winter season under irrigation from May to September 2019. The mean annual rainfall is between 588 and 635 mm during the summer months. The greenhouse experiment was implemented at the Controlled Environment Research Unit (CERU) of the University of KwaZulu-Natal (22°27′32.9″S 30°24′18.8″E), Pietermaritzburg campus in South Africa from July to November 2019.

3.2.3 Experimental design and trial management

The 36 lines were evaluated using a 6×6 alpha-lattice design with two replications and two water regimes [well-watered (WW) and water-stressed (WS)]. The soybean lines were directly sown in row plots of 5 m length with the inter-row spacing of 10 cm and intra-row spacing of 5 cm at a target population of about 350, 000 plants per hectare. Fertilizer, nitrogen-phosphorus-potassium (NPK) at the rate of 65kg N, 65 kg P and 65 kg K was applied at planting. Weeds were controlled chemically by applying Roundup (by Efekto) herbicide at a rate of 5 L ha^{-1}immediately after planting and later mechanically through hand weeding. Pest management was done using recommended practice by using Coragen and karate insecticide at a rate of 1 l ha^{-1} (DAFF, 2010). The water-stressed treatment was implemented by withholding irrigation after 50% flowering corresponding to stage III as described by Cui et al. (2019).

Figure 3.1: The Jozini field layout for drought screening

The greenhouse experiment was laid out as 6×6 alpha-lattice, two replications and two water regimes WW and WS. The plots were arranged in 3 pots and randomly allocated per incomplete block to avoid the experimental error associated with water discharge from the drip irrigation (Mathew et al., 2019). Four seeds were sown directly in potting media in capacity plastic pots with a capacity of 5L and further thinned to one after one week of germination. The compound fertilizer was applied at the rate of 65kg N, 65 kg P and 65 kg K at planting time and every two-week time.

Before the experiment, the field capacity (FC) of the media was determined as described by Seremba et al. (2018) and Kesiime et al. (2016). At 60 days after planting, the water –stress treatment was carried out by

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withholding the drip irrigation. The pots were manually re-watered with tap water to keep the soil moisture as close as possible to the level of water stress. Fertilizer applications, and weed and pest management were done based on recommended practices (DAFF, 2010).

Figure 3.2: The greenhouse trial layout

3.2.4   Data collection

3.2.4.1 Morphological traits

Morphological data collected were number of days to 50% flowering (DF), plant height (PH), leaf width (LW), leaf length (LL) and stem diameter (STD) (Kangai Munene et al., 2018). Grain yield (GY) components were measured at maturity, including 100 seed weight (SW) and biomass (BM) to compute grain yield in tons per hectare. All data except the 50% flowering was collected on three randomly selected plants per genotype in the plot and on all the greenhouse pots. Plant height was measured in centimeters (cm) from the base of the plant to the tip of the top leaf (trifoliate) with an average of 3 randomly selected plants on each plot, two weeks after the flowering stage (Fried et al., 2018). Days to 50% flowering were estimated by counting days from the sowing date to the day in which at least 50% of the plants in a plot had fully opened flowers. The 100 seed weight was recorded in grams (g) by weighing 100 randomly selected

seeds of each genotype after threshing. Grain yield per plot in grams was measured by weighing all seeds harvested per plot (Chibanda, 2017).

3.2.4.2 Physiological parameters

Stomatal conductance was measured with an SC-1 leaf porometer (Decagon Devices®, Pullman, WA, USA) in mmol $m^{-2}s^{-1}$ (Pask et al., 2012). The measurements were recorded between 9:00 am and 13:00 pm on the central leaflet by clamping the top fully expanded leaf on a sensor head from three plants for each genotype under WS and WW conditions (Mashilo et al., 2018). The observation was collected at the R5 growth stage (beginning of seed fill) as described by (Buezo et al., 2019). The chlorophyll content was measured using a chlorophyll meter SPAD 502 in µmol m^{-2} (Konica Minoita Sensing, nc., Osaka, Japan) as described by Dong et al. (2019). This was achieved by measuring three plants selected at random and the value per plant was obtained by an average of three measurements on the three youngest fully developed leaves located at the top, center and bottom of the plant. The measurements were taken on the central leaflet at the same period with the physiological parameters.

3.2.5   Data analysis

3.2.5.1 Screening for drought-tolerance in soybean genotypes

Basic descriptive statistics were computed for all the quantitative traits. The Shapiro test was used to test for the normal distribution of the data. The data collected were analysed using ANOVA, multivariate analysis and generalized linear models with Pearson. A Tukey's honest significant difference test at P ≤ 0.05 was used to separate treatment means. Principal component analysis (PCA) was performed for traits and genotypes. All statistical analyses were carried out with R.3.6.0 software.

3.2.5.2 Analysis of variance

A combined and separate analysis of variance was performed for physiological, morphological traits, and grain yield, as shown in (Table 1.6) as a mixed model with genotypes as fixed effects models and water to determine the effects of genotype, water regimes, locations, and their interactions on the traits. The following model was used for ANOVA analysis:

$$Rijwkr = m + gi + lj + w + Br(Lj) + B(Lr)ik + Glj + Giw + wlj + eikrw$$

Where:

Rijwkr = response*; m* = grand mean; *g* = the genotype effects; *l*= the location effects; W = the water regime effects; *B* = block effects r= replication effects; e_{iekrw} = random error; *Br(Lj)* = the effect of *r* replication within *i* location; B(Lr)ik = the effects of *k* block in the replication and location; *GLj* = the interaction between the genotype and the location, *Giw* = the interaction between the genotype and the

water regime; *wlj* = the interaction between the; *eijwkr* = the random experimental error related with the mean of the *i* genotype in the location in the *k* block within *r* replication as shown in Table 2.2.

3.2.5.3 The genotype by trait correlation analysis

The genotype by trait model was used using R.3.6.0 software to determine if one trait can predict another (W. Yan & Rajcan, 2002). The model equation used is represented below as follows:

$$\frac{T_{ij-\beta_{ij}}}{S_j} = \Sigma_{n=1}^2 \lambda_n \xi_{in} n_{jn} + \Sigma_{n=1}^2 \xi_{in} n_{jn} + \varepsilon_{ij}$$

Where;

T_{ij} = is the mean value of the genotypes (*i*) and trait (*j*); β_{ij} = the overall value of all genotypes for traits *j*; S_j = The standard deviation of the trait between genotype means; λ_n = singular value of principal components (PCn); ξ_{in} = the PCn score for genotype *i*; n_{jn} = the PCn score for trait *j* ; ε_{ij} = the error of the as a result of genotype *i* on trait *j*. Noteworthy that the systematic scaling between genotype scores ξ_{in} and trait score n_{jn} was achieved through absorbing the singular value by the singular vector of the genotype *i* and traits *j*. This can be represented as $\xi_{in} = \lambda^{0.5}{}_n \xi_{in}$ and $n_{jn} = \lambda^{0.5}{}_n n_{jn}$. However, for a model to have at best for extracting pattern and to avoid any nose in the data PC1 and PC2 were retained in the model. The genotype by trait (GT) plot was biplot was computed by plotting $\xi^*{}_{i1}$ and $\xi^*{}_{i2}$ against $n^*{}_{j1\ and}\ n^*{}_{j2}$. In this way, genotype and trait were represented by a marker on the biplot. The GT biplot has a vector drawn at the origin of the biplot to each trait marker for the visualization of the correlation between and within genotypes.

3.3   RESULTS

3.3.1 Significance test

A boxplot was constructed to determine significant effect of water stress in soybean yields, this was achieved by mean comparison of grain yield under both well-watered and water-stressed regimes. A large grain yield variation between the water regime was observed across two water regime (Figure 3.3 a and b) and most genotypes showed higher yield under well-watered compared to water-stressed. The same trend was observed under each environment (figure 3.4), grain yield was higher in the field environment compared to the greenhouse under both water regimes. Therefore, this entails that drought stress contributes to low grain yields.
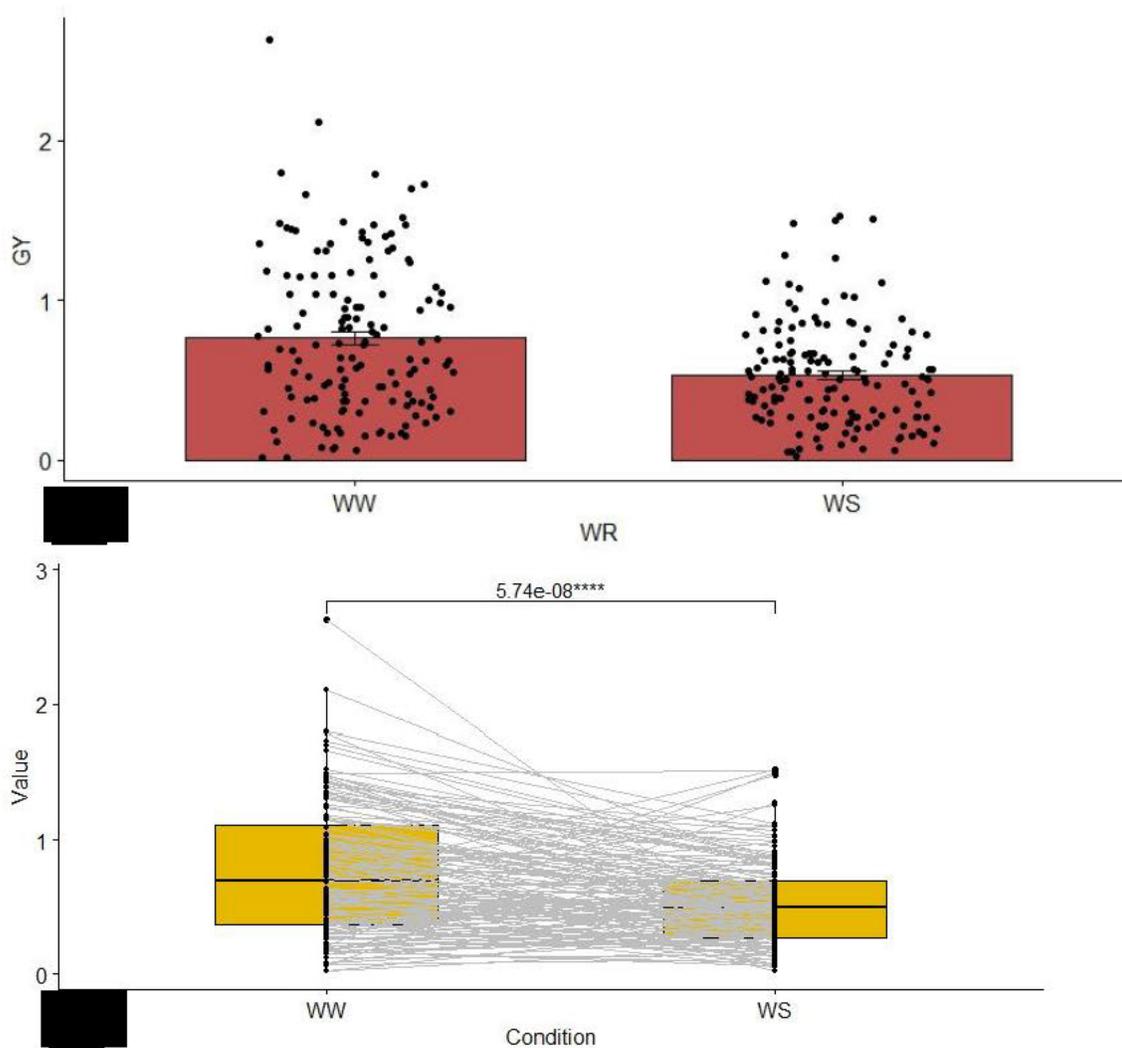
Figure 3.3: Box plot analysis of variation, showing the significant differences between the water regimes based on grain yield across environments
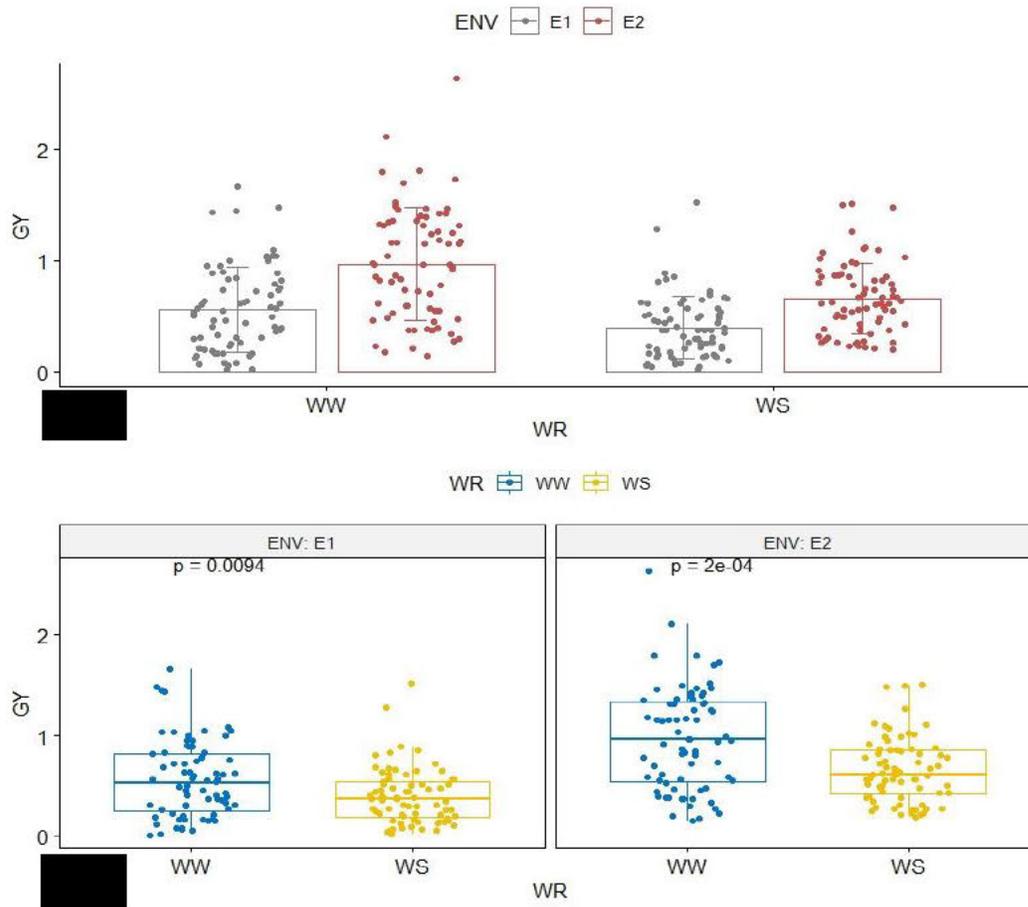
Figure 3.4: Box plot analysis of variation, showing the significant differences between the water regimes based on grain yield in the field and greenhouse environment.

The evaluation of the genotype using morphological traits

The analysis of variance was performed using R-software 3.6.6 by using the least of mean squares on the HSD test. The threshold level of the probabilities for the statistical significance was set at 0.05, where the genotype, water regime treated as fixed effect while the replicate and blocks were treated as a random effect. The combined analysis of variance across environments (field and greenhouse) showed genotypic differences that were significant for almost all morphological traits, including PH (P≤0.001), LL (P≤0.001), LW (P≤0.001), STD (P≤0.001), BM (P≤0.001), SW (P≤0.01) and GY(P≤0.05) (Table 2.3). The water regime indicated a significant effect for PH (P≤0.001), LL(P≤0.01), LW (P≤0.01), STD(P≤0.001), MC (P≤0.01), and GY(P≤0.001) (Table 3.2). The environment effect was significant for all morphological traits. The genotype by environment interaction showed a significant effect for PH (P≤0.01), LW (P≤0.01), STD (P≤0.01) and BM (P≤0.01). The environment by water regime interaction showed a significant effect for

PH (P≤0.001), FLW (P≤0.01), MC (P≤0.05), SW (P≤0.01). The environment by genotype and water regime effect was significant for SW (P≤0.05). The genotype by water regime interaction was not significant for all morphological traits.

Table 3.2: Combined analysis of variance showing mean squares for the morphological traits of soybean lines under WS and WW water regimes across the greenhouse and field environments.

SOV	DF	PH	LL	LW	STD	FLW	BM	MC	SW	GY
GEN	35	209***	4.4***	3.42***	4.9***	173 [NS]	0.90***	0.87 [NS]	20.6**	0.21*
WR	1	6523***	20.3**	7.75**	14.1***	853**	2.56***	0.23**	6.2 [NS]	4.02***
ENV	1	102846***	1184.3***	13.80***	824.1***	6791***	0.64 [NS]	45.49***	56.0*	7.90***
REP:ENV	2	792***	3.8 [NS]	2.36 [NS]	5.0**	205 [NS]	0.83**	0.46 [NS]	16.8 [NS]	0.01 [NS]
GEN:WR	35	63 [NS]	1.8 [NS]	0.10 [NS]	1.6 [NS]	89 [NS]	0.22 [NS]	0.80 [NS]	11.8 [NS]	0.14 [NS]
GEN:ENV	35	199**	2.6 [NS]	1.99**	2.2**	127 [NS]	0.43**	1.26 [NS]	17.4 [NS]	0.66 [NS]
ENV:WR	1	9408***	3.2 [NS]	0.91 [NS]	3.4*	9 [NS]	0.06 [NS]	4.23*	510***	0.42 [NS]
REP:ENV:BLK	20	173*	3.3 [NS]	1.65 [NS]	1.7 [NS]	235*	0.30 [NS]	1.26 [NS]	14.0 [NS]	0.18 [NS]
ENV:GEN:WR	35	83 [NS]	2.6 [NS]	1.72 [NS]	1.2 [NS]	124 [NS]	0.32 [NS]	0.60 [NS]	20.1*	0.10 [NS]
RESIDUALS	122	94	2.0	1.14	1.2	135	0.23	0.97	12.2	0.13

Note SOV-source of variation; DF-degree of freedom; PH-plant height; SD-stem diameter; LW-leaf width; LL-leaf length; MC-moisture content; STC-stomatal conductance; CC-chlorophyll content; SW-100 seed weight; BM-biomass Yield and GY-grain yield. ***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05 [NS] non-significant, respectively.

The evaluation of the genotypes using physiological traits

The combined analysis of variance indicated that the water regime, genotype by environment interaction, and water regime interaction effect discriminated genotypes for CC (P≤0.05), (P≤0.001), and (P≤0.05) only (Table 3.3). The environmental effect was significant for both studied physiological traits, STC (P≤0.001) and CC (P≤0.001). However, the genotype, replication by the environment, genotype by water regime, genotype-environment interaction, and water regime effect were not significant for all physiological traits examined.

Table 3.3: A combined analysis of variance showing mean squares and significant tests for physiological traits of soybean lines under WS and WW water regimes across the greenhouse and field environment.

SOV	DF	CC	STC
GEN	35	1414 [NS]	2662 [NS]
WR	1	13587*	1116 [NS]
ENV	1	262491***	1255170***
REP(ENV)	2	3350 [NS]	39519***
GEN*WR	35	734 [NS]	2816 [NS]
GEN*ENV	35	1326 [NS]	3009 [NS]
ENV*WR	1	1978 [NS]	1204 [NS]
BLK(ENV*REP)	20	1215 [NS]	2638 [NS]
ENV*GEN*WR	35	820 [NS]	2511 [NS]
RESIDUALS	122	1338	6028

Note: SOV-source of variation; DF-degree of freedom; PH-plant height; SD-stem diameter; LW-leaf width; LL-leaf length; MC-Moisture content; STC-stomatal conductance; CC-chlorophyll content; SW-100 seed weight; BM-biomass Yield and GY-grain yield. ***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05 [NS] non-significant, respectively.

Mean performance comparison for evaluated 36 soybean lines based on morphological and physiological traits

The mean comparison of the genotypes was performed across two environments and water regimes, using the Turkey test at a 5% level of significance (Tables 3.4 to 3.5). Under the water stress regime, the plant height (PH) in the field was between 28.67 cm and 73.67 cm, with G27 (genotype name) having the lowest value and G29 (genotype name) the highest value, while it was between 31.67 cm and 67 cm for the well-watered regime, with G27 (genotype name) having the lowest value and G15 (genotype name) the highest value. The stem diameter (STD) ranged from 5.84 cm to 12.50, with G30 having the lowest and G16 the highest value, while under WW, the values ranged from 4.67 cm to 7.84 cm, with G36 being the lowest and G31 being the highest (Table 2.5). Under WS, leaf width (LW) ranged from 3.67 cm to 6.67 cm, with G33 having the lowest and G29 being the highest, while under WW, it ranged from 7.33 cm to 11.67 cm, G3 being the lowest and G8 the highest value. The leaf length (LL) ranged from 4.17 cm to 9.00 cm under WS, with G24 having the lowest value and G16 the highest value, while under WW, it ranged from 4.17 cm to 7.34 cm, with G35 having the lowest value 35 had and G29 having the highest value.

The STC, under WS in the field, ranged from 32.82 mmol m^{-2}s^{-1} to 62.08 mmol m-2s-1, with G10 having the lowest value and G32 having the highest value whereas, under WW, it ranged from 30.29 mmol m^{-2}s to 61.52 mmol m^{-2}s, with G29 having the lowest value and G30 having the highest value (Table 2.6). The FLW under WS ranged from 49 days to 74 days, with G18 having the lowest value and G6 the highest value, whereas, under WW, FLW ranged from 44 days to 74 days, with G24 having the lowest value and G17 having the highest value. The CC under WS ranged from 29.98 µmol m^{-2} to 184.2 µmol m^{-2}, with G5 having the lowest value and G27 having the highest value, whereas, under WW, it ranged from 61.72 µmol m^{-2} to 215.64 µmol m^{-2}, with lowest value recorded for G29 and the highest for G33.

The MC, under WS in the field, ranged from 6.90 % to 8.90 %, with G29 having the lowest and G15 the highest, while under WW, it ranged from 6.55 % to 8.65 %, G3 had the lowest, and G4 the highest value (Table 2.7). The BM under WS ranged from 0.22 g to 1.37 g, with G22 having the lowest value and G19 the highest, while under WW, it ranged from 0.20 g to 2.43 g, with 19 being the lowest and G1 being the highest would have. The 100 seed weight (SW) under WS ranged from 13.00 g to 24.00 g, with G17 having the lowest value and G25 the highest value, while under WW, it ranged from 9.00 g to 19.00 g. with G14 having the lowest value and G22 the highest value. The GY under WS ranged from 0.28 t ha^{-1} to 1.05 t ha

[1], with G20 having the lowest and G3 the highest, while under WW, it ranged from 0.23 t ha^{-1} to 1.80 t ha^{-1}, with G22 the lowest and G29 had the highest value.

Table 3.4: The mean values of plant height, stem diameter, leaf length and leaf width among 36 soybean genotypes studied under water-stressed and well-watered environments in the field.

	Traits											
	Top 12 genotypes											
	PH (m)			STD (cm)			LL (cm)			LW (cm)		
Genotypes	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
29	63.50	73.67	-16.02	7.67	11.33	-47.72	9 17	6.67	27.28	7.34	7.00	4.57
31	53.83	56.83	-5.57	7.84	12.34	-57.43	7 34	6.00	18.20	6.00	8.17	-36.08
26	43.67	58.33	-33.57	6 17	9.34	-51.42	10.00	5.33	46.70	6.84	6.50	4.90
9	47.83	50.33	-5.23	6 17	8.50	-37.88	9.83	6.17	37.28	5.33	6.17	-15.67
36	33.00	41.50	-25.76	4.67	8.50	-82 21	8.84	4.67	47.20	4.67	5.34	-14.36
13	57.00	42.17	26.02	6 50	9.84	-51 31	9 17	4.50	50.90	6.00	7.17	-19.42
19	59.34	57.34	3.37	7 50	11.17	-48 93	8 34	6.00	28.01	6.17	7.50	-21.65
30	43.50	33.34	23.37	5.67	5.84	-3.00	9 17	5.00	45.44	5.17	4.50	12.88
2	51.50	67.33	-30.74	6 50	8.67	-33 31	8 33	4.67	44.00	5.33	6.34	-18.86
32	45.00	53.50	-18.89	5.84	9.33	-59 90	9 50	4.67	50.84	4.50	5.17	-14.78
	Bottom 6 genotypes											
8	49.00	47.00	4.08	5.67	8.50	-50.04	11.67	4.50	61.42	4.50	5.50	-22.22
22	37.33	50.33	-34.82	5 17	10.17	-96.81	9.84	6.34	35.59	5.67	7.00	-23.57
6	53.33	45.84	14.05	6 17	7.67	-24 31	10.67	5.17	51.57	5.00	5.50	-10.00
14	44.17	47.50	-7.55	6 34	9.84	-55 25	10.00	4.83	51.70	5.00	6.50	-30.00
23	48.33	59.50	-23.11	6.00	8.33	-38.83	10.00	5.00	50.00	4.84	5.67	-17.27
27	31.67	28.67	9.47	5.67	6.83	-20 56	10.17	4.50	55.73	4.67	4.17	10.72
Grand mean	48.45	50.22	-3.65	6 27	8.94	-42 58	9.21	5.12	44.39	5.44	6.06	-11.33
LSD	20.32	34.24		2 92	5.04		2 23	3.72		2.09	2.90	
SEM	103.71	147.13		2 14	3.19		1 25	1.74		1.10	1.06	
CV (%)	21.01	24.16		15.97	21.76		20.52	20.09		16.75	19.93	

Key: LSD-least significant differences, SEM-standard error mean, CV-coefficient of variance, WW-well-watered, WS-water-stressed, PH- plant height, STD-stem diameter; LW-leaf width and LL-leaf length.

Table 3.5: The mean values of Stomatal conductance, 50% days to flowering and chlorophyll content among 36 soybean genotypes studied under a water-stressed and well-watered environment.

	Traits								
	Top 12								
	STC (mmol m^{-2}s^{-1})			FLW (days)			CC (μmol m^2)		
Genotypes	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
29	30.29	43.24	-42.76	59.50	64.00	-7.56	61.72	88.22	-42 94
31	55.97	45.94	17.93	47.00	49.00	-4.26	67.95	96.77	-42.41
26	42.44	44.25	-4.28	49.50	50.00	-1.01	92.34	71.29	22.80
9	53.65	47.62	11.25	52.50	56.50	-7.62	133.45	98.97	25.84
36	43.89	50.45	-14.96	59.00	51.50	12.71	153.32	75.53	50.74
13	45.12	51.74	-14.67	61.00	62.50	-2.46	104.80	111.03	-5.94
19	49.22	47.23	4.03	58.00	57.00	1.72	131.50	115.85	11.90
30	61.52	52.64	14.44	48.50	58.50	-20.62	114.29	121.02	-5.89
2	43.12	43.83	-1.66	57.00	53.50	6 14	101.70	89.05	12.44
32	40.38	62.08	-53.74	51.00	54.50	-6.86	112.90	81.93	27.44
	Bottom 6								
8	45.30	44.47	1.84	58.50	72.50	-23.93	81.97	81.79	0.23
22	54.67	51.29	6.18	54.00	53.00	1.85	92.13	99.12	-7.58
6	52.04	48.17	7.44	56.00	73.50	-31.25	135.90	105.12	22.65
14	47.63	55.79	-17.12	53.00	56.50	-6.60	123.07	103.39	15.99
23	48.72	36.75	24.56	44.00	52.50	-19.32	68.74	64.76	5.78
27	56.25	40.87	27.35	54.50	56.00	-2.75	113.20	184.52	-63.00
Grand Mean	47.84	47.57	0.57	54.49	58.26	-6.93	112.57	94.14	16.37
LSD	18.07	27.55		16.52	26.19		94.96	109.00	
SEM	81.97	95.27		68.49	86.08		2263.43	1491.22	
CV	18.92	20.52		15.19	15.92		42.26	41.02	

Key: LSD-least significant differences; SEM-standard error mean, CV-coefficient of variance, WW-well-watered, WS-water-stressed, STC-stomatal conductance, 50% days to flowering and CC-chlorophyll content.

Table 3.6: The mean values of moisture content, seed biomass, 100 seed weight and grain yield in 36 soybean genotypes under water-stressed and well-watered regimes in the field environment.

Top 12												
	MC(%)			BM (g)			SW (g)			GY(t ha^{-1})		
Genotypes	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
29	8.05	6.90	14.29	1.21	0.51	58.26	16.00	19.68	-22.97	1.80	0.68	62.12
31	7.95	7.90	0.63	0.58	0.72	-24.35	16.00	22.00	-37.50	1.48	0.86	41.89
26	8.35	7.55	9.58	0.66	0.62	5.34	12.00	16.00	-33.33	1.48	0.59	60.47
9	7.70	7.90	-2.60	0.86	0.60	30.81	14.00	15.00	-7.14	1.43	0.95	33.92
36	7.85	8.35	-6.37	1.48	0.45	69.49	10.00	14.00	-40.00	1.42	0.59	58.80
13	7.85	7.70	1.91	1.04	0.73	29.47	13.00	15.00	-15.38	1.39	0.71	49.28
19	7.60	8.60	-13.16	0.20	1.37	-600.00	12.00	20.00	-66.67	1.32	0.99	25.00
30	8.00	8.30	-3.75	0.88	0.85	3.41	12.00	16.00	-33.33	1.27	0.58	54.15
2	7.80	7.55	3.21	0.58	0.56	3.45	11.00	18.00	-63.64	1.26	0.62	51.00
32	8.05	7.60	5.59	1.22	0.49	59.67	16.00	14.00	12.50	1.24	0.57	53.85
Bottom 6												
8	7.05	7.60	-7.80	0.83	1.31	-58.18	15.00	18.00	-20.00	0.40	0.67	-69.62
22	7.55	7.95	-5.30	0.83	0.22	73.49	19.00	16.00	15.79	0.23	0.40	-71.74
6	8.50	7.40	12.94	1.53	0.64	58.17	14.00	14.00	0.00	0.42	0.61	-45.78
14	7.65	7.80	-1.96	0.27	0.63	-133.33	9.00	18.00	-100.00	0.46	0.68	-47.83
23	7.55	7.45	1.32	0.60	0.50	17.50	14.00	18.00	-28.57	0.50	0.47	6.06
27	7.15	7.55	-5.59	0.82	0.36	55.83	13.00	13.00	0.00	0.50	0.88	-76.00
Grand Mean	7.73	7.68	0.63	0.87	0.65	25.03	13.50	16.44	-21.74	0.97	0.66	32.03
LSD	0.39	1.74		0.31	1.02		9.19	9.01		0.96	0.80	
SEM	1.24	0.37		1.12	0.13		6.05	10.19		0.23	0.08	
CV	8.03	8.01		64.17	55.54		22.45	19.43		49.54	43.30	

Key: LSD-least significant differences, SEM-standard error mean, CV-coefficient of variance, WW-well-watered, WS-water-stressed, MC-moisture content, SW-100 seed weight, BM-biomass Yield, and GY-grain yield.

Under WW in the greenhouse PH ranged from 31.67cm to 67 cm, with G27 having the lowest value and G15 the highest value, while in the WW, it ranged from 23.67 cm to 73.6 cm, G27 the lowest value, and G29 the highest Value. The STD ranged from 3.90 cm to 6.73 cm, with G24 being the lowest and G20 being the highest, while under WW, it ranged from 14.15 cm to 7.73 cm, with G35 being the lowest and G11 being had the highest value (Table 3.7). Under WS, LW ranged from 4.38 cm to 9.23 cm, with G18 being the lowest and G11 being the highest, while under WW, it ranged from 4.38 cm to 9.23 cm, with G34 being the lowest and G28 had the highest value. The LL ranged from 6.58 cm to 12.93 cm under WS, with G36 had the lowest value and G11 the highest value, while under WW, it ranged from 6.25 cm to 12.93 cm, G34 the lowest 27, and G28 the highest value.

The STC, under WS in the green-house, ranged from 88.18 mmol $m^{-2}s^{-1}$ to 330 mmol $m^{-2}s^{-1}$, with G35 having the lowest value and G15 having the highest value whereas, under WW, it ranged from 95.33 mmol $m^{-2}s$ to 282.28 mmol $m^{-2}s$, with G34 having the lowest value and G26 having highest value (Table 3.8). The CC, under WS, ranged from 31.75 µmol m^{-2} to 47.10 µmol m^{-2}, with G3 having the lowest value and G15 having the highest value, whereas, under WW, it ranged from 30.68 µmol m^{-2} to 71.58 µmol m^{-2}, with G24 having the lowest value and G3 having the highest value. The FLW under WS ranged from 32 to 77 days, with G1 having the lowest value and G3 having the highest value, whereas, under WW, it ranged from 29 to 60 days, with G4 having the lowest value and 29 having the highest value.

The MC under WS in the greenhouse ranged from 4.95 % to 8.25 %, with G2 having the lowest value and G34 the highest value, while under WW, it ranged from 4.60 % to 8.80 %, G10 the lowest value, and G10 the highest Value (Table 2.10). The BM under WS ranged from 0.18 g to 3.38 g, with G22 having the lowest value and G1 the highest, while under WW, it ranged from 0.18 g to 2.34 g, with G25 being the lowest and G1 being the highest. Under WS, BM ranged from 5.97 g to 26.7 g, with G3 having the lowest and G17 the highest, while under WW, it ranged from 12.10 g to 26.50 g, with G5 having the lowest and G29 the highest value. The GY under WS ranged from 0.08 t ha^{-1} to 1.00 t ha^{-1}, with G2 having the lowest value and G34 the highest value, while under WW, it ranged from 0.10 t ha^{-1} to 1.19 t ha^{-1}, with G4 having the lowest value and G1 had the highest value.

Table 3.7: The mean values of plant height, stem diameter, leaf length and leaf width, among 36 soybean genotypes studied under water-stressed and well-watered regimes in the greenhouse.

	Top 12											
	PH(m)			STD(cm)			LW(cm)			LL(cm)		
Genotypes	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	91.88	73.63	19.86	6.43	6.13	4.67	7.23	5.08	29.76	10.98	8.38	23.69
8	103.75	86.05	17.06	5.73	5.13	10.48	7.35	7.25	1.36	12.00	11.38	5.21
32	90.38	68.55	24.15	6.35	5.40	14.96	5.55	6.23	-12.16	8.85	8.10	8.47
36	89.00	87.10	2.13	6.05	4.90	19.01	7.48	4.65	37.79	10.65	6.58	38.26
34	90.00	70.63	21.53	4.68	5.38	-14.97	4.38	5.88	-34.29	7.63	8.00	-4.92
10	101.15	74.53	26.32	6.30	5.45	13.49	6.85	6.68	2.55	10.63	10.13	4.71
24	92.00	74.18	19.38	5.75	3.90	32.17	6.13	5.60	8.57	8.50	7.30	14.12
11	105.13	82.38	21.64	7.73	6.33	18.12	8.20	8.20	0.00	10.58	12.93	-22.22
31	101.25	77.10	23.85	5.98	5.65	5.44	5.98	6.48	-8.37	10.03	9.53	4.99
35	93.75	79.75	14.93	4.15	5.15	-24.10	4.58	6.10	-33.33	6.50	7.90	-21.54
13	98.50	76.93	21.90	6.70	5.68	15.30	6.90	5.13	25.72	9.35	9.00	3.74
	Bottom 6											
4	92.88	69.43	25.25	5.63	5.43	3.56	6.98	5.80	16.85	9.30	8.05	13.44
22	96.00	69.00	28.13	6.53	5.43	16.86	7.40	6.65	10.14	10.40	9.88	5.05
16	98.48	79.00	19.78	4.98	5.58	-12.06	7.78	7.05	9.32	9.55	10.23	-7.07
27	108.00	70.85	34.40	5.10	4.78	6.37	4.40	8.20	-86.36	6.25	10.08	-61.20
27	108.00	70.85	34.40	5.10	4.78	6.37	4.40	8.20	-86.36	6.25	10.08	-61.20
12	100.00	77.18	22.83	6.53	6.35	2.68	7.25	6.15	15.17	10.05	8.88	11.69
Gr and Mean	97.94	76.70	21.68	6.00	5.35	10.92	6.80	6.38	6.10	9.68	8.96	7.45
LSD	34.24	15.3		5.04	1.85		2.90	6.60		3.72	3.32	
SEM	147.14	60.58		9.12	0.86		3.38	10.93		1.61	2.77	
CV%	3.25	10.00		47.20	18.99		18.62	47.38		18.62	18.73	

Key: LSD-least significant differences, SEM-standard error mean, CV-coefficient of variance, WW-well-watered, WS-water-stressed, PH-plant height, SD-stem diameter, LW-leaf width and LL-leaf length.

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Table 3.8: The mean values of stomatal conductance, 50% days to flowering and chlorophyll content 36 soybean genotypes studied under water-stressed and well-watered regimes in the greenhouse environment.

	Top 12								
	STC(mmol m^{-2}s^{-1})			FLW(days)			CC(nm/cm^2)		
Genotypes	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	241.33	199.73	17.24	41.15	40.50	1.58	58.00	31.50	45.69
8	99.28	226.88	-128.53	43.98	41.68	5.23	42.50	44.00	-3.53
32	266.23	100.05	62.42	39.00	40.00	-2.56	47.00	35.50	24.47
36	95.33	194.15	-103.67	68.18	37.38	45.18	44.00	47.00	-6.82
34	174.25	187.65	-7.69	39.18	37.88	3.32	58.00	45.50	21.55
10	245.55	168.48	31.39	44.83	33.35	25.60	58.00	61.50	-6.03
24	168.70	223.68	-32.59	30.68	33.45	-9.05	38.50	42.50	-10.39
11	163.05	214.23	-31.39	55.65	38.50	30.82	37.00	64.50	-74.32
31	176.95	116.00	34.44	40.75	43.00	-5.52	45.50	47.50	-4.40
35	168.95	88.18	47.81	38.03	38.85	-2.17	42.50	37.00	12.94
13	201.60	119.88	40.54	54.28	34.68	36.11	47.00	42.50	9.57
4	205.28	172.75	15.84	37.33	35.35	5.29	28.50	33.00	-15.79
	Bottom 6								
22	146.15	194.18	-32.86	62.70	37.78	39.75	37.00	59.50	-60.81
16	192.55	291.00	-51.13	42.73	43.75	-2.40	38.50	41.00	-6.49
27	246.25	156.30	36.53	44.15	41.80	5.32	47.00	35.50	24.47
27	246.25	156.30	36.53	44.15	41.80	5.32	47.00	35.50	24.47
12	185.48	146.53	21.00	69.43	40.73	41.34	41.00	47.00	-14.63
Grand Mean	184.36	175.88	4.60	46.92	38.47	18.01	45.27	48.22	-6.51
LSD	27.55	120.80		26.19	11.69		109.00	28.56	
SEM	3834.22	3667.79		86.09	34.31		1491.2	204.83	
CV	34.67	33.67		29.43	15.07		42.07	29.68	

Key: LSD-least significant differences, SEM-standard error mean, CV-coefficient of variance, WW-well-watered, WS-water-stressed, STC-stomatal conductance, 50% days to flowering and CC-chlorophyll content

Table 3.9: The mean values of moisture content, biomass, 100 seed weight and grain yield in 36 soybean genotypes under water-stressed and well-watered regimes in the greenhouse environment.

							Top 12					
	MC			BM(g)			SW(g)			GY(t ha^{-1})		
Genotypes	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	8.30	7.20	13.25	2.34	3.38	-44.44	15.60	19.85	-27.24	1.19	0.76	36.13
8	7.50	7.80	-4.00	0.97	0.67	31.44	15.75	15.70	0.32	1.10	0.52	52.73
32	7.80	7.30	6.41	0.97	0.51	47.67	15.75	17.30	-9.84	0.97	0.62	36.08
36	7.10	6.45	9.15	1.00	0.44	56.50	16.65	13.75	17.42	0.96	0.39	59.90
34	8.50	8.25	2.94	1.59	0.79	50.63	12.35	14.25	-15.38	0.93	1.00	-8.11
10	4.60	5.55	-20.65	0.79	0.68	14.01	12.60	9.55	24.21	0.92	0.73	20.65
24	8.00	7.45	6.88	0.23	1.05	-356.52	14.65	20.70	-41.30	0.92	0.33	64.48
11	7.05	7.15	-1.42	1.83	2.35	-28.49	16.85	14.60	13.35	0.87	0.83	4.60
31	6.75	5.40	20.00	0.47	0.30	37.23	19.20	14.10	26.56	0.88	0.12	86.93
35	7.30	7.25	0.68	0.55	0.85	-55.05	12.65	13.50	-6.72	0.81	0.48	40.99
13	7.20	5.05	29.86	0.89	1.15	-29.94	17.10	11.95	30.12	0.71	0.36	49.65
							Bottom 6					
4	7.30	7.30	0.00	0.49	0.31	37.76	15.60	16.75	-7.37	0.10	0.25	-163.16
22	6.30	6.00	4.76	0.40	0.18	55.70	14.50	8.21	43.38	0.10	0.34	-235.00
16	7.30	6.90	5.48	0.32	0.88	-173.44	19.05	16.80	11.81	0.12	0.70	-483.33
27	6.10	6.45	-5.74	1.11	0.77	30.63	16.70	17.05	-2.10	0.15	0.29	-93.33
27	6.10	6.45	-5.74	1.11	0.77	30.63	16.70	17.05	-2.10	0.15	0.29	-93.33
12	7.35	7.05	4.08	0.36	0.26	27.78	17.80	17.35	2.53	0.21	0.42	-97.62
Grand Mean	7.17	6.64	7.40	0.93	0.78	16.69	17.06	14.67	13.98	0.56	0.40	28.38
LSD	1.53	2.43		0.38	1.39		14.34	10.15		0.13	0.54	
SEM	0.38	1.48		0.13	0.49		10.19	25.87		0.081	0.07	
CV%	17.22	18.30		65.70	89.94		22.25	34.66		65.11	67.48	

Key: LSD-least significant differences, SEM-standard error mean, CV-coefficient of variance, WW-well-watered, WS-water-stressed, MC-moisture content, SW-100 seed weight, BM-biomass Yield, and GY-grain yield.

3.3.2 Pearson's correlations among studied morphological and physiological traits in soybean genotype under water-stressed condition and water-stressed water regimes

Table 3.10 and table 3.11 represents the correlation coefficients(r) showing the magnitude of the relationships among morphological and physiological traits studied in the field and greenhouse environments. In the present study, correlation coefficients values of ± 1 were indicating perfect correlations, ±0.8 were high correlations, ±0.50 were moderate correlations, ±0.20 were weak correlations and 0.0 indicating no correlation. In the field under WS, GY showed significant (P≤0.01 and P≤0.05) and positive correlation with MC (r=0.33) and BM (r=0.59). However, GY under WW showed highly significant(P≤0.01) and weak positive correlation with LW(r=0.44). The FLW, under WS showed highly significant (P≤0.001) and weak negative correlation with FLW (-0.28) and under WW indicated similar trend with LW (-0.41) and LL (0.33). The analysis under both WS and WS, showed highly significant (P≤ 0.001) and positive correlation between PH with STD, LW and LL.

In the greenhouse under WS, GY showed significant (P≤0.01 and P≤0.05) and moderately, positive correlation with MC (r=0.56) and had a weakly positive correlation with BM (r=0.43). Similar association was observed between GY and BM (r=0.33; P≤0.05). The analysis under both WS and WS, showed highly significant (P≤ 0.001) and strongly positive correlation between LL with STD and had moderately positive association with LW. The STD, under WS showed a significant (P≤0.05) and weakly negative correlation with MC(r=-0.05)

Table 3.10: The Pearson's correlations among studied morphological and physiological and in soybean genotype under water-stressed condition (lower diagonal) and water-stressed water regime (upper diagonal) in the field environment

		Well-watered										
		PH	STD	LW	LL	STC	CC	FLW	MC	BM	SW	GY
Water-stressed	PH	**1.00**	0.80***	0.66***	0.76***	-0.44**	0.10	-0.22	0.13	0.01	0.23	0.30
	STD	0.69***	**1.00**	0.69***	0.94***	-0.29	0.04	-0.27	0.06	-0.15	0.13	0.29
	LW	0.62***	0.59***	**1.00**	0.64***	-0.29	0.11	-0.41**	0.24	-0.07	0.20	0.44***
	LL	0.66***	0.93***	0.57***	**1.00**	-0.25	-0.02	-0.33*	0.02	-0.14	0.14	0.25
	STC	-0.01	0.09	0.05	0.05	**1.00**	-0.22	0.09	-0.22	-0.24	-0.19	-0.36*
	CC	0.03	-0.02	0.12	0.04	-0.08	**1.00**	0.46***	-0.13	0.27	0.05	0.23
	FLW	-0.28***	-0.07	0.01	-0.07	-0.01	0.11	**1.00**	-0.14	0.13	-0.32*	-0.02
	MC	-0.19	0.07	-0.01	0.21	0.23	-0.24	0.27	**1.00**	0.43***	0.17	0.21
	BM	0.18	0.33*	0.03	0.43***	-0.07	0.26	0.00	0.45*	**1.00**	0.31	0.09
	SW	0.55***	0.59***	0.41**	0.58***	0.03	0.11	0.04	0.00	0.36*	**1.00**	0.00
	GY	-0.21	-0.10	-0.14	-0.03	-0.16	-0.05	0.15	0.39**	0.36*	-0.07	**1.00**

Note: PH-plant height; SD-stem diameter; LW-leaf width; LL-leaf length; MC-Moisture content; STC-stomatal conductance; CC-chlorophyll content; SW-100 seed weight; BM-biomass Yield and GY-grain yield; *Significant at P≤0.05, perfect correlation ± 1; strong correlation ± 0.80; Moderate correlation ± 0.50; weak correlation ±0.20; No correlation ±0.0; ***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05.

Table 3.11: The Pearson's correlations among studied morphological and physiological soybean genotype under water-stressed condition (lower diagonal) and water-stressed water regime (upper diagonal) in the greenhouse.

		Well-Watered										
		PH	STD	LL	LW	STC	CC	FLW	MC	BM	SW	GY
	PH	**1.00**	0.22	0.28	0.18	0.01	-0.16	0.14	-0.25	-0.22	0.12	-0.07
	STD	-0.12	**1.00**	0.68***	0.59***	-0.07	0.25	0.06	-0.17	0.06	0.43**	0.04
	LL	-0.06	0.31	**1.00**	0.82***	-0.21	0.34*	0.01	-0.20	-0.01	-0.37*	-0.19
Water-stressed	LW	-0.10	0.52***	0.75***	**1.00**	-0.30	0.28	0.21	-0.16	0.06	0.28	0.07
	STC	0.15	0.14	0.23	0.28	**1.00**	-0.07	-0.11	-0.16	0.16	-0.03	-0.03
	CC	0.05	0.39	0.40**	0.39**	0.44**	1.00	-0.04	-0.07	-0.14	0.18	-0.25
	FLW	0.00	0.27	0.23	0.30	0.14	-0.09	1.00	-0.04	0.15	-0.05	0.28
	MC	0.29	-0.05*	0.12	-0.07	0.16	0.11	-0.01	**1.00**	0.24	0.05	0.20
	BM	0.09	-0.05	0.05	0.25	0.20	0.11	-0.04	0.13	**1.00**	-0.25	0.33*
	SW	0.07	-0.02	-0.01	-0.15	0.18	0.28**	-0.40**	0.80	0.22	**1.00**	-0.26
	GY	0.08	0.24	0.09	0.17	0.16	0.08	-0.01	0.56***	0.43**	-0.04	**1.00**

Note: PH-plant height; SD-stem diameter; LW-leaf width; LL-leaf length; MC-Moisture content; STD-stomatal conductance; CC-chlorophyll content; SW-100 seed weight; BM-biomass Yield and GY-grain yield; *Significant at P≤0.05, Perfect correlation ± 1; Strong correlation ± 0.80; Moderate correlation ± 0.50; weak correlation ±0.20; No correlation ±0.0; ***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05

3.3.3   Principal component analysis

The eigenvalues from the principal component analysis were used to determine the proportion of the total variation of traits retained by each principal component (Table 3.12). The PCA showed that the first five eigenvalues contributed to the total percentage of the variation among studied traits across the environments under WS and WW regimes. In both environments and water regimes, the first five main PCAs obtained were extracted from a large dataset with a total percentage in the field of 75.59% under WS and 82.11% under WW conditions), and in the greenhouse of 74.75% under WS and 74.98% under WW. Almost all five components had eigenvalues >1 except for the last components. The first PCA showed the traits that contributed the most to the total variation.

The PCA grouped the morphological and physiological traits into five main components where in the field under water-stressed (FWS), the first DIM1 accounted for 26.31%; DIM2 17.32%; DIM3 13.46%; DIM4 10.67% and DIM5 8.14% and under well-watered (FWW), DIM1 accounted for 35.10%, DIM2 17.13%, DIM3 14.10%, DIM4 9.11% and DIM5 6.77%. However, in the greenhouse under water-stress (GWS) the first DIM1 accounted for 22.36%; DIM2 19.09%; DIM3 12.90%; DIM4 11.93% and DIM5 8.44%, whereas under well-watered (GWW), DIM1 accounted 27.31%, DIM2 15.09%, DIM3 13.20%, DIM4 10.71% and DIM5 8.67%. The first dimension in the FWS was more related to STD, LL, PH, SW and LW, whereas in FWW it was more related were STD, PH, LL and LW.

 In the GWS, DIM1 was more associated with LW, LL, STD, CC, FLW and STC, while in GWW it was more associated with LW, LL, STD and SW (Table 3.13). The second dimension for the FWS was more associated with MC, GY and BM, while in FWW it was more associated with BM, CC, STC and MC. In the GWS, DIM2 was more associated with MC, SW, FLW, GY and PH, while in GWW, it was more associated with GY, BM and FLW. The most strongly correlated traits with DIM1 in FWS were STD, LL, PH, SW and LW, while in FWW were PH, LL and LW. In the GWS, the characteristics most strongly correlated with DIM1 were LW, LL, STD, FLW, CC and STC, while in the GWW LW, LL, STD and SW were. The traits most strongly correlated with DIM1 were MC, GY and BM, while in FWW STC and MC were more closely related.

The traits most strongly correlated with DIM2 in GWS were MC and SW, while GWW were GY, BM and FLW. The most positive and DIM3-correlated traits in FWS were STC, while in FWW were STC and CC. The traits most positively correlated with DIM3 in GWS were MC and GY, while GWW were PH and CC. The most positive and most strongly correlated traits with DIM4 in FWS were CC, while they were SW in FWW. The traits most positively correlated with DIM4 in GWS were PH, while GWW was STC. The most positive trait in FWS and strongly correlated with DIM5 was FLW. The traits correlated most positively with DIM4 in GWW were SW.

Table 3.12: The eigenvalues and principal components from the correction matrix of morphological and physiological traits contribution on the total variation under a well-watered (WW) and water-stressed (WS) regime in the field environment.

TRAITS	Water regime	Eigenvector				
		DIM 1	DIM2	DIM3	DIM4	DIM5
PH	WS	0.83	-031	0.005	-0.16	0.031
	WW	0.88	-0.01	0.13	0.13	1.8
LL	WS	0.92	0.14	-0.12	-0.03	-0.07
	WS	0.89	-0.22	0.19	0.01	0.22
LW	WS	0.72	-0.18	-0.036	0.22	-0.26
	WW	0.85	-0.28	0.14	0.12	0.23
STD	WS	0.92	0.13	-0.07	-0.05	-0.30
	WW	-0.51	-0.46	-0.08	0.06	0.25
FLW	WS	0.07	0.04	-0.70	0.43	0.54
	WW	-0.51	-0.46	-0.04	0.054	0.25
STC	WS	0.11	-0.05	0.75	0.43	0.41
	WW	0.07	0.59	0.62	0.29	-0.10
CC	WS	-0.11	0.41	0.11	0.71	-0.49
	WW	-0.43	0.39	0.64	-0.00	0.33
BM	WS	0.07	0.82	-038	0.04	0.37
	WW	0.25	0.46	-0.53	-0.45	0.34
MC	WS	0.43	0.66	0.31	-0.17	0.37
	WW	0.01	0.80	-0.29	0.14	0.26
SW	WS	0.74	0.04	0.12	0.12	-0.01
	WW	0.31	0.27	-0.51	0.61	-0.25
GY	WS	-0.11	0.72	0.12	0.17	0.37
	WW	0.48	0.35	0.22	-0.51	-0.42
Explained variance (eigenvalue)	WS	2.89	1.91	1.48	1.17	0.89
	WW	3.86	1.88	1.55	1.00	0.75
Proportion of total variance (%)	WS	26.31	17.32	13.46	10.67	8.14
	WW	35.10	17.13	14.10	9.11	6.77
Cumulative variance (%)	WS	33.45	51.43	63.26	73.17	81.58
	WW	35.10	52.22	66.33	75.49	82.21

Key: PH-plant height, SD-stem diameter, LW-leaf width, LL-leaf length, MC-Moisture content, STC-stomatal conductance, CC-chlorophyll content, SW-100 seed weight, BM-biomass Yield and GY-grain yield.

Table 3.13: The eigenvalues and principal components from the correction matrix of morphological and physiological traits contribute to the total variation under a well-watered (WW) and water-stressed (WS) regime in the greenhouse environment.

TRAITS	Water regime	Eigenvector				
		DIM 1	DIM2	DIM3	DIM4	DIM5
PH	WS	0.35	0.46	0.21	0.54	0.39
	WW	0.35	-0.15	0.67	0.08	0.17
LL	WS	0.72	-0.28	-0.09	0.23	-0.08
	WS	0.80	0.18	0.02	0.15	0.23
LW	WS	0.81	-0.40	-0.22	-0.02	-0.08
	WW	0.91	0.07	0.05	-0.03	-0.04
STD	WS	0.68	-0.22	-0.01	-0.40	0.19
	WW	0.83	0.32	-0.02	-0.03	-0.14
FLW	WS	0.64	0.13	-0.49	0.20	-0.10
	WW	0.08	0.54	-0.48	-0.04	-0.81
STC	WS	0.54	0.23	-0.17	0.38	0.31
	WW	-0.26	-0.19	-0.00	0.78	0.44
CC	WS	0.64	0.13	-0.49	0.20	-0.10
	WW	0.43	-0.06	0.64	0.19	-0.30
BM	WS	0.46	0.39	0.081	-0.54	0.39
	WW	-0.12	0.68	0.36	0.42	0.06
MC	WS	0.25	0.63	0.50	0.23	-0.37
	WW	-0.30	0.35	0.40	-0.57	0.44
SW	WS	0.07	0.55	-0.65	-0.08	0.04
	WW	0.55	-0.28	0.13	-0.23	0.59
GY	WS	0.45	0.48	0.53	-0.08	0.04
	WW	-0.23	0.76	-0.20	0.01	0.14
Explained variance(eigenvalue)	WS	2.46	2.09	1.42	1.31	0.92
	WW	3.00	1.74	1.45	1.17	0.95
Proportion of total variation (%)	WS	22.36	19.09	12.90	11.93	8.44
	WW	27.31	15.09	13.20	10.71	8.67
Cumulative variance (%)	WS	22.36	41.45	54.34	66.26	74.72
	WW	27.31	43.21	56.42	67.12	75.79

Key: PH-plant height, SD-stem diameter, LW-leaf width, LL-leaf length, MC-Moisture content, STC-stomatal conductance, CC-chlorophyll content, SW-100 seed weight, BM-biomass Yield and GY-grain yield.

Separate biplot displays for principal components were constructed using morphological and physiological traits across two sites (field and greenhouse) to determine which among the studied traits discriminate most genotypes respectively under each water regime and environment (Figure 3.5). This was achieved by plotting the first two principal components as they are referred to be the most important. A cosine angle

between vectors approximates the correlation coefficient between any two traits. A strong positive correlation between PH, LW, STD, SW and LL; GY with MC and FLW; MC and BM in FWS, whereas in FWW were formed between STD, LW, LL and PH; LW and GY; MC, SW, CC and BM as indicated by the small acute angle between their vectors(r=cos0=+1) (Figure 3a and b). In GWS, the same angle was formed between STD, LW, LL, FLW; GY, BM, STC, CC; PH, SW and MC, whereas in GWW were between STD, LL and LW; LL and CC; STD and SW; BM and GY; MC, FLW, GY and BM (Figure 3c and d).
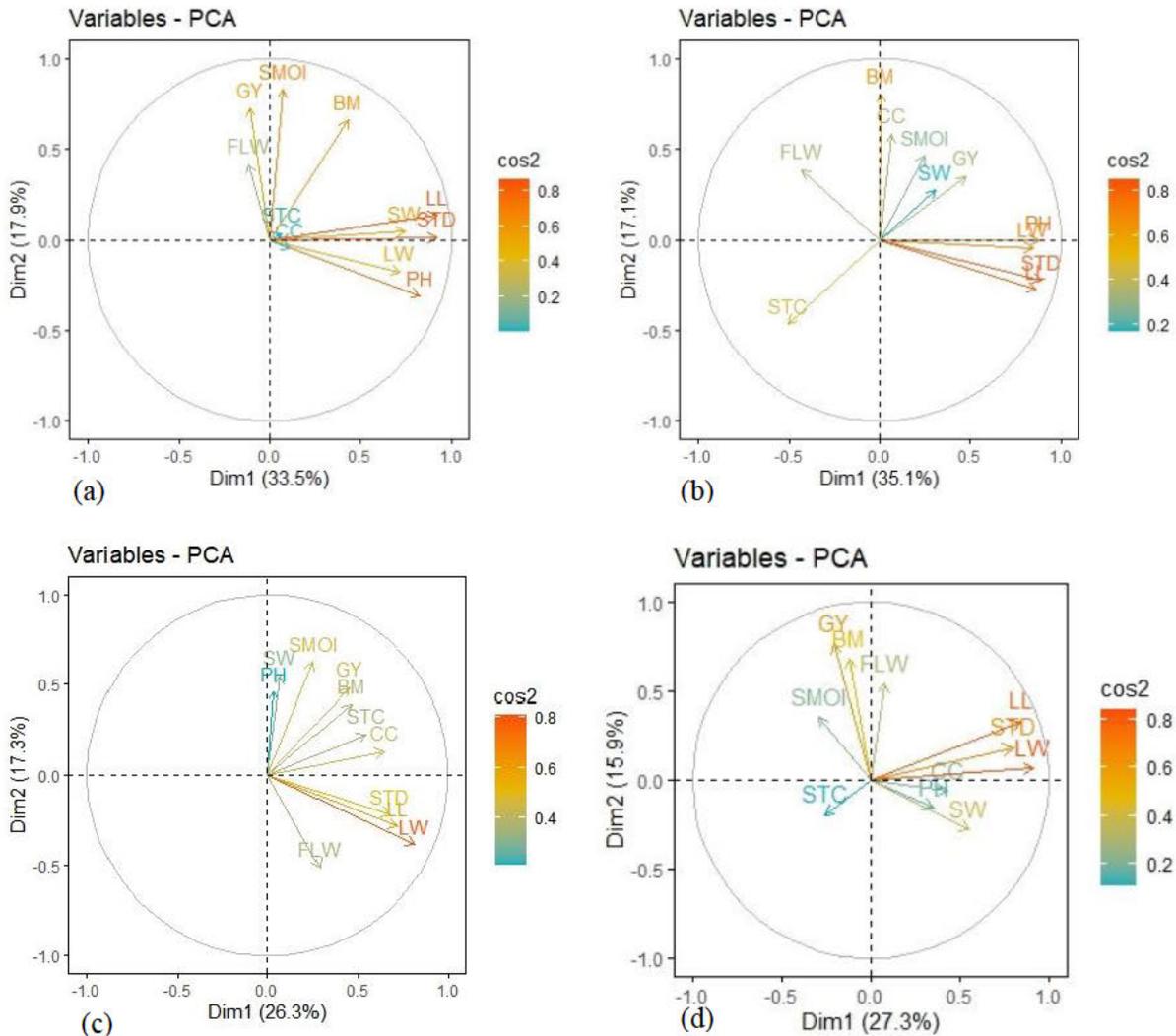
Figure 3.5: (a) Loadings plot of the first two PCAs showing the relationship among various traits in soybean lines (Correlation circle) for water-stressed in the field environment (FWS), (b) for the well-watered regime in the field environment (FWW), (c) for water-stressed in the greenhouse environment (GWS) and (d) for the well-watered regime in the greenhouse environment (GWW). PH-plant height, SD-stem diameter, LW-

leaf width, LL-leaf length, MC-Moisture content, STC-stomatal conductance, CC-chlorophyll content, SW-100 seed weight, BM-biomass Yield and GY-grain yield.

A nearly zero correlation in FWS was observed between BM with PH and LW; GY with LL, SW and STD; FLW and LL; MC with STD and SW as indicated by a perpendicular angle between their vectors (r=cos90=0), whereas in FWW it was observed between FLW with STC, SW, GY and MC; BM with LL, LW and PH (Figure 3.5). There was nearly zero correlation between MC with STD, LL and LW; FLW with STC in the GWS, whereas in GWW was formed between MC and STC; GY with LL, LW and STD; BM with LL, LW and STD; FLW with PH and CC (Figure 3.5). There was a negative correlation in FWS between PH and STC, FLW and CC, as indicated by an obtuse angle that is approximately 180 degrees (r=cos180=-1) (Figure 3a). In FWW the obtuse angle was observed between STC with GY and SW; FLW with STD and LL (Figure 3b). In GWS, a negative correlation was observed between PH and FLW whereas in GWW, MC with FLW and PH, STC with LL and STD (Figure 3.5).

3.3.4   Cluster analysis

The clusters were delineated by k-means clustering for the 36 soybean genotypes in the field and in the greenhouse environment under WW and WS regimes (Figure 3.6). The analysis showed that cluster I in FWS consisted of 25 genotypes, cluster II of 7 and cluster III of 4 (Table 3.14), whereas in FWW, cluster I, II and IV consisted of the same number of genotypes (11) and cluster III had 3. In GWS, cluster I had 15 genotypes, cluster II had 13 and cluster III had 8 genotypes, whereas in GWW, cluster I consisted of 8 Genotypes, Cluster II had 17 and Cluster III had 11 members (Table 3.14).

The soybean lines grouped into cluster II consisted of high mean values for PH (59.03 cm), LW (11.75 cm) and CC (62.93 µmol m^2) (Table 3.14). Cluster III consisted of high means for most traits studied in FWS, including STD (11.75 cm), LL (8.17 cm), STC (48.66 mmol m^{-2}s$^-$), FLW (110 days), MC (8.29), BM (1.15 g), SW (20.25 g) and GY (0.82 t ha^{-1}). Cluster I in the FWW had high mean values for STC (52.38 mmol m^{-2}s$^-$). Cluster II consisted of a high mean value of GY (1.13 t ha^{-1}) (Table 2.17). Cluster III consisted of high mean values for CC (62.50 µmol m^2), FLW (138.12 days), MC (8.17 %), BM (1.97 g) and SW (15.67 g). Cluster IV consisted of high mean values for PH (54.51 cm), LL (10.50 cm) and LW (7.05 cm).

In the GWS, cluster I consisted of high mean values for MC (7.17 %) (Table 3.14). Cluster II consisted of high mean values for almost all traits including, PH (82.06 cm), STD (5.91 cm), LW (6.84 cm), LL (10.25 cm), STC (245.33 mmol m^{-2}s), CC (43.33 µmol m^2), BM (1.45 g), SW (16.59 g) and GY (0.56 t ha^{-1}). Cluster III consisted of high mean values for FLW (51.19). In the GWW, cluster I consisted of high mean values for STD (196.01 cm). Cluster II consisted of high mean values for FLW (51.45 days) and SW (17.72

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g). Cluster III consisted of high mean values for PH (102.7 cm), LL (6.55 cm), LW (7.58 cm), CC (11.26 μmol m^2), STC (50.73 mmol m^{-2}s) and MC (1.05 %).
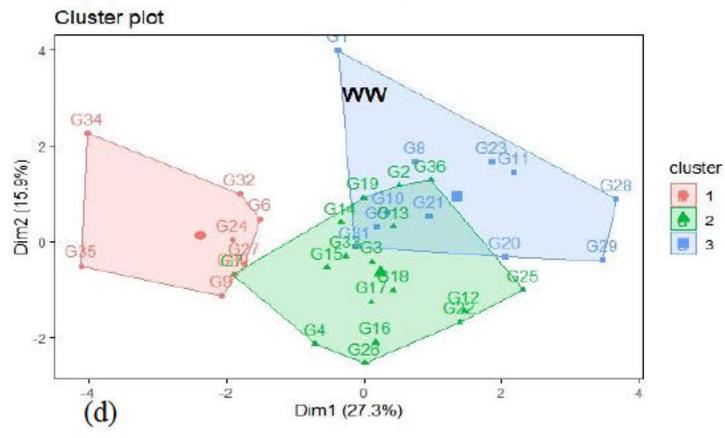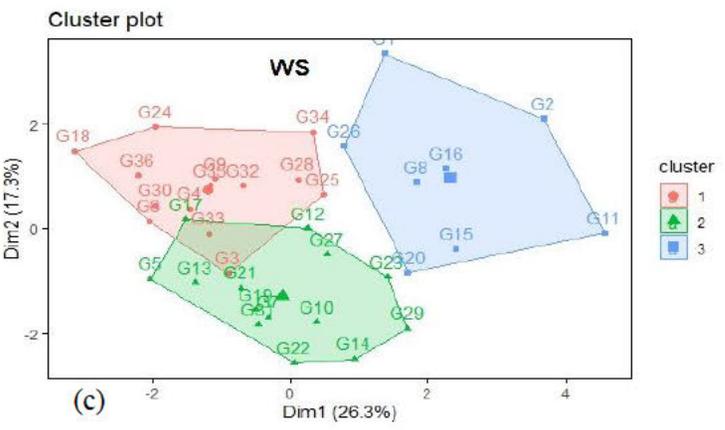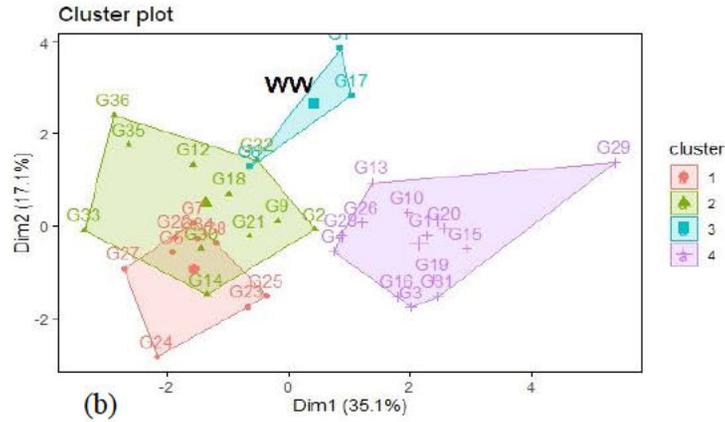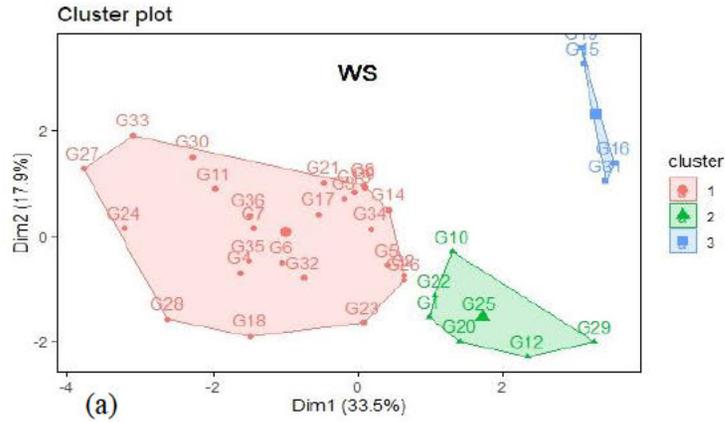
Figure 3.6: Cluster plot of the first two PCAs showing the relationship among 36 soybean lines (a) for water-stressed in the field environment, (b) for the well-watered regime in the field environment, (c) for water-stressed in the greenhouse environment and (d) for the well-watered regime in the greenhouse environment.

Table 3.14: Cluster mean for the morphological and physiological means studied under WS and WW in the field environment

Cluster	Water Regime	Number of genotypes	Genotypes included
Field environment			
I	WS	25	G2, G3, G4, G5, 56, G7, G8, G9, 11, G13, G14, G17, G21, G23, G24, G26, G27, G28, G30, G32, G33, G34, G35, G18 and G36
I	WW	11	G5, G7, G8, G13, G22, G23, G24, G25, G27, G31 and G34
II	WS	7	G1, G10, G12, G20, G22, G25 and G29
II	WW	11	G2, G9, G12, G14, G18, G21, G30, G32, G33, G35 and G36
III	WS	4	G15, G16, G19 and G31
III	WW	3	G1, G6 and G17
IV	WW	11	G3, G4, G10, G11, G15, G16, G19, G20, G26, G28 and G29
Greenhouse environment			
I	WS	15	G3, G4, G6, G9, G18, G19, G24, G25, G28, G30, G32, G33, G34, G35 and G36
I	WW	8	G6, G9, G24, G27, G30, G32, G34 and 35
II	WS	13	G5, G7, G10, G12, G13, G14, G17, G21, G22, G23, G27, G29 and G31
II	WW	17	G2, G3, G4, G7, G12, G13, G14, G15, G16, G17, G18, G19, G22, G25, G26, G33 and G34
III	WS	8	G1, G2, G8, G11, G15, G16, G20 and G26
III	WW	11	G1, G5, G8 G10, G11, G20, G21, G23, G28, G29 and G31

3.4 DISCUSSION

Among environmental stresses, water stress negatively affects morphological, physiological, biochemical and molecular processes of plants in all growth stages. Breeding for drought-tolerance is a primary goal of plant breeding programmes focused on improving grain yield and maintaining production under environmental stress. Hence, identifying and selecting promising genotypes with morphological and physiological traits stable under drought environment will help in improvement of drought tolerance in soybean line. The present study was carried out to identify drought-tolerant soybean genotypes by examining morphological and physiological traits under well-watered, water-stressed regimes in the field and greenhouse environment flowering stage.

3.4.1 Genotypic variation in grain yield and mean performance of morphological and physiological traits

Screening of morphological and physiological traits was carried out under well-watered and water-stressed conditions in the field and greenhouse environment during the flowering phase. The mean square of the ANOVA showed significant differences between genotypes in almost all traits, including PH, LL, LW, STD, BM, SW. This indicated genetic variability amongst the genotypes that can be utilized for drought-tolerance selection. This observation agrees with the study by Mimi et al. (2016) that reported a significant genotypic variation among soybean genotypes for drought-tolerance. However, FLW, CC and STC were not significantly different among genotypes, meaning non-existence of genetic variability for these traits. In the present study, water stress reduced morphological and studied physiological traits to different extents depending on the environment, since the recorded values of the traits was lower under water-stressed than under well-watered conditions. A similar observation was reported by Oqba (2017), Mdluli (2018) and Ngalamu et al. (2013).

The mean performance for grain yield showed high-yielding genotypes in both environments and under both water regimes, were TGX 2001-10DM (G1), TGX 1987-62F (G11) and TGX 2014-15FM (G32). This means that these genotypes had a strong recovery capacity, hence they were able to maintain relatively high grain production under both water regimes (Yang et al., 2019). Therefore, these three genotypes could be helpful for future drought breeding programs, including crosses for better yielding genotypes under drought stressed environments. The cluster plot grouped the 36 soybean lines into groups based on their morpho-physiological similarities. These phenotypic differences are important as they simultaneously reflect the influence of genes and environmental factors. The large genotypic variation in genotypes allows for soybean improvement through selection of genotypes from different clusters for crosses to preserve genetic diversity that is critical for breeding (Govindaraj et al., 2015).

Under WS in the field experiment, genotypes TGX 2001-9DM (G15), TGX 2002-17DM (G16), TGX 2002-5FM (G19) and TGX 2002-14DM (G31), represented as cluster (III) out yielded all studied genotypes in the field environment and in the greenhouse were represented as cluster TGX 2001-8FM (G5), TGX 2014-21FM (G7), TGX 2014-44FM (G10), TGX 2001-15DM (G12), TGX 2001-18DM (G13), TGX 2001-6FM G14, TGX 2002-23DM G17, TGX 2001-18FM (G21), TGX 2001-20FM (G22), TGX 2002-6FM(G23), TGX 2002-35FM (G27), TGX 2014-4FM (G29) and TGX 2002-14DM (G31). The possible reason for these genotypes to produce high seed yields is that they exhibited wide stem diameter, longer leaves, high seed moisture, high biomass and these genotypes took longer to flower, ultimately contributing to high yields. Leaf size, profoundly affects a number of biological processes including carbon-dioxide, water and energy (Wang, 2019). The wider and longer the leaves, the longer water can be restored, whereas the smaller the leaves faster the water losses. Therefore, leaf area is one of the important parameters in plant breeding for understanding the adaptation mechanisms to water stress. Moreover, these genotypes maintained a relatively higher chlorophyll content which may directly induced photosynthesis rate thereby promoting higher yields and which indicate higher drought tolerance (Monteoliva et al., 2021). Suggesting that increasing photosynthetic efficiency may improve soybean yield production under water stress. Therefore, these four genotypes could be recommended for soybeans production under drought in the field environment and can be helpful in crosses targeting to improve high yield in soybeans with high biomass, seed weight, wide stem diameter, long leaf length and genotype taking fewer days to flower. Noteworthy that high yielding genotypes had a moderate plant height,

Whistle, soybean genotypes represented as cluster I, in the field environment and as cluster III in the greenhouse environment, have a poor capacity to maintain their physiological functions at low water levels and couldn't recover after the stress was removed which led to yields reduction. In the field environment low yielding genotypes indicated that water stress in soybeans induces a gradual reduction in plant height, biomass, 100 seed weight. The stomatal conductance and chlorophyll content of these genotypes decreased, the sensitivity of these genotypes to water stress was attributed to reduction in stomatal conductance, which decreased the rate of carbon dioxide and thereby limiting the rate of photosynthesis of which are important components for soybean yields (Abid et al., 2018). The leaves of these genotypes were smaller hence they exhibited short leaf length and width. This was due to water loss, nutrient availability and nutrient uptake in the leaves. Leaf reduction was an indication that the genotypes were under water stress since soybeans reduces their leaf area as a mechanism to maintain water loss during drought stress (H. Wu et al., 2004).

3.4.2 The impact of water regime and environments on morphological and physiological traits

The combined analysis of variance of variance of soybean yields and agronomic traits under water stress across studied two environment revealed water stress has high influence on soybean morphology,

physiology, growth and yield production, hence water regime showed significant differences in PH, LL, LW, STD, FLW, BM, MC, GY and CC (Table 2.1). The highest grain yield GY, under WS in the field, was 1.05 t ha^{-1}and the lowest was 0.28 t ha-^{1}whereas, under WW, the highest grain yield was 1.80 t ha^{-1} and lowest was 0.23 t ha^{-1}. Moreover, the means reduction analysis indicated that the water stress under field environment significantly reduced soybean yield by of 32 % when compared to yields under well-watered regime. This means that during water stress some soybean cultivars were able to maintain their yields under water stress and some couldn't withstand water stress and couldn't recover quickly from stress which led to poor performance in producing good seed yields. However, under WS in the greenhouse, GY the highest was 1.00 t ha^{-1} and lowest was 0.08 t ha^{-1}, which was slightly lower than the grain yield obtained in the field whereas, under WW, the highest yield obtained was 1.19 t ha^{-1} and lowest was 0.10 t ha^{-1}. In the greenhouse GY was reduced by 28%. Having slight differences in yield means that the studied soybean lines are stable in various environment and stress conditions. Therefore, from these finding it was clear that water stress influence on based in yields may vary with genotypes and environment or sites in which the stress is imposed and these two factors plays crucial role in breeding drought tolerant soybean genotypes. These findings agree with the results of Atti et al. (2004), Bajaj et al. (2008) and He et al. (2017). Mathew (2018) reported a 40% reduction in grain yield in wheat lines. A significant yield reduction of 27.5% in soybean genotype due to drought stress was reported by Zhang et al. (2020). BM showed a 25% reduction in the field environment and a 17 % reduction in the greenhouse. These findings corresponded with the results by Prudent et al. (2015), who demonstrated 22 % of the shoot biomass losses. Contrary to the study by Demirtas et al. (2010), which reported that biomass was significantly reduced when drought stress imposed in pod filling than in the flowering stage. In addition, Jumrani and Bhatia (2018) reported a total biomass reduction of 23 and 53% at vegetative and reproductive stages, respectively, in soybean genotypes.

The water stress significantly reduced seed moisture content by 18%, in the greenhouse, this indicated that the soybean genotypes had were experiencing great water losses, therefore this clearly indicated that the strategy used in the study was effective and can be recommended in screening for drought stress in soybean genotypes. The 50% days to flowering also revealed that water stress delayed soybeans genotype to flower under water stress when compared genotypes under control genotypes since the FLW was ranging range of 32 to 77 days under the water stress regime and 29 to 60 days with good irrigation. The pH in the field environment showed a 4% increase due to water stress; however, it was reduced by 22% in the greenhouse. This is in line with previous studies. Contrary to the findings of Yan et al. (2020), which disapproves of the fact that the pH value in the flowering phase is significantly reduced by water stress than in any other growth phase. This reduction was due soybeans water stress defense mechanisms including cell swelling, cell wall and reduction in enzymes synthesis, which ultimately reduces growth and plant height (Oqba, 2017). The same study reported that plant height was reduced by 20.13% in the vegetative stage and 8.49%

in the reproductive stage. A reduction in STD was observed in the field trial by 44% and in the field trial by 11%. LW showed a 44% reduction due to water stress in the field environment and a 6% reduction in the greenhouse.

The CC was reduced by 18 % in the field environment and increased by 7% in the greenhouse environment. Szabo (2020) achieved similar results, showing an 11.8% reduction in CC in soybean lines due to drought stress. In addition, a reduction in CC has been reported by (Lamacque et al., 2019). In the greenhouse, the STD was reduced by water stress by 11 %. STC revealed non-significant, low reduction in both environments, respectively (5%) and greenhouse (1%). The same findings were also obtained for 100 SW, showing a 14% reduction due to water stress in the greenhouse environment and an increase of 22% in the field environment Yan et al. (2020) recorded a low 100 seed weight reduction of 0.16 in diverse soybean genotypes. The significant environmental variation among traits was observed for almost all traits (PH, LL, LW, STD, CC, STC, SW, MC, FLW and GY) except for BM. The environment by water regime interaction was significant for PH, STD, MC and SW. These findings indicate that genotypes may not be stable across environments. Such behavior was demonstrated in a study conducted Mathew (2018). Therefore, PH, LL, LW, STD, FLW, BM, MC, GY and CC could be considered as criteria for drought-tolerance evaluation in soybean genotypes.

3.4.3 Correlation between grain yield and morpho-physiological traits under well-watered and water-stressed regimes

Grain yield under water stress is the primary trait in breeding programs for drought-prone environments. However, for effective and successful drought-tolerance, screening for secondary traits is important, as these are related to grain yield to a large extent (Lafitte et al., 2003). Previous research has consequently illustrated a positive correlation between morpho-physiological traits such as biomass, 100 seed weight, seed yield, plant height and 50% days to flowering. These findings are in line with the present study results. This linear association means that's when one these traits increases so as the other and decreases as other trait decreases, simply meaning these traits can predict one another. A positive and significant association in grain yield was observed between MC and BM under water-stressed and well-watered regimes. This suggests that the selection of high-yielding soybean genotypes can be successful through indirect selection of MC and BM under both water regimes. Under WW, a significant negative correlation of GY with STC was recorded, implying that reduced stomatal conductance promotes high yield. These findings are contrary to those of Yu et al. (2020). The possible reason to the contrary findings is that most researches have demonstrated that not all soybean genotypes experience stomatal conductance degradation under drought stress, which simply means the ability to maintain stomatal conductant under drought may vary with soybean cultivars and depending on stress duration and intensity.

PCAs also revealed a strong positive correlation between PH, LW, STD, SW, LL, MC and BM was observed among genotypes under a water-stressed environment. This means that this linear association among these traits were highly influenced by water stress, implying that soybean genotypes with reduced plant high tend to have smaller leaf width and stem diameter, short leaf length, reduced biomass and low seed moisture content. Under a well-watered regime, STD, LW, LL, PH, GY, MC, SW, CC, BM and FLW showed significant associations. This implies that the selection aimed at targeting high soybean seed yields would invariably select for high biomass, 100 seed weight, chlorophyll content, plant height, seed moisture content, leaf width, leaf length and longer to flower.

3.5 CONCLUSION

In this study, the results illustrated that the morpho-physiological traits used to screen the 36 soybean lines were effective in discriminating genotypes for drought-tolerance. High genetic variation was observed in grain yield and the morpho-physiological traits under both well-watered and water-stressed regimes. Significant reduction in agronomic traits was observed for GY, BM, MC, STC, LL, LW, STD and PH in G10, G12, G22 and G29 clearly water stress has significant negative impact on soybeans morphology, physiology, growth and seed yield production. Additionally, this also means the approach used in the study for imposing stress in soybean lines was effective and can be highly to be recommended in plant breeding for the screening of water stress in soybean lines. The genotypes, TGX 2001-10DM (G1), TGX 1987-62F (G11) and TGX 2014-15FM (G32) were the best potential genotypes for improving drought-tolerance in soybean lines. The LL, STD, FLW, BM, GY and MC could be effectively used for indirect selection in improving yields in soybeans under water-stressed conditions. Based on the results, it was concluded that the approach of PCAs and cluster plot analysis was effective in identifying high-yielding drought-tolerant genotypes, discriminating and grouping genotypes based on their water stress responses.

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Chapter Four

Asessment of drought-tolerance in soybean (*Glycine Max* L. Merr.) genotypes using drought-tolerance indices

ABSTRACT

Thirty-six soybean genotypes were tested for grain yield performance under water-stress (WS) and well-watered (WW) conditions across field and greenhouse environments. The genotypes were screened in a 6x6 alpha-lattice design with two replications for each water regime. Eleven drought indices, which included Drought intensity index (DII), Stress susceptibility index (SSI), yield index (YI), Stress tolerance index (STI), Mean relative performance (MRP), Geometric means of productivity (GMP), Yield stability index (YSI), Mean productivity (MP), Stress tolerance (TOL), Harmonic mean (HM) and Relative efficiency index (REI) were calculated based on grain yield for each genotype. Combined analysis of variance (ANOVA) for grain yield and indices, Pearson's correlation, principal component analysis (PCA), mean ranking, biplot and cluster plot analysis were performed using R.3.6.0 software on the data collected. Significant differences for grain yield were detected for environment ($P \leq 0.05$), genotype ($P \leq 0.001$) and water regime ($P \leq 0.001$). Further, significant differences among soybean genotypes for indices were detected for SSI, STI, YI, YSI, HM and GMP. The mean reduction ranking revealed that genotypes G26, G32, G9, G29 and G12 were moderately susceptible to water stress. Correlation analysis results, showed that MRP, GMP, MP, MRP, HM and REI could distinguish drought-tolerance in soybean genotypes with high yield performance under both environments. The PCA showed two important principal components (PC1 and PC2), which explained 66.7% variation among genotypes. Based on biplot and cluster plot, G1, G9, G10, G12, G13, G19, G21, G26, G29, G31 and G32 had a high correlation for SSI, STI, MRP, GMP, MP, TOL, HM and REI, hence were identified as the most tolerant genotypes that can be considered as potential to be further improved for drought-tolerance in soybean breeding programs.

Key words: Soybean genotypes, drought-tolerance indices, principal component analysis, correlation, biplot, cluster analysis.

4.1 INTRODUCTION

Drought is one of the major environmental constraints globally, restraining crop production. Thus, improving drought-tolerance is a priority in most plant breeding programs, especially in the marginal areas (Abdi et al., 2013). Due to the present global climate changes, drought stress occurrence is expected to rise. This may lead to high yield losses in economically important crops worldwide (Tint et al., 2011). Numerous research studies for the evaluation of drought-tolerance have been undertaken using various techniques, including molecular approaches. However, breeding for drought-tolerance is challenging because drought stress implemented in experimental layout and field conditions gives invariability and poor correspondence to implemented level of stress (Sofi et al., 2018). Even in the case of screening similar traits through different systems.

Drought-tolerance in plants is described as the relative yield of one genotype to another, subject to the same drought stress conditions (Farshadfar et al., 2012). Yield has been the primary objective in plant breeding, especially under drought stress and this has been achieved by evaluating yield and yield-related traits based on correlated responses. However, screening genotypes for drought tolerance based on yield alone under optimum and drought stress for tolerance has been challenging. Over the past few decades, plant breeding programs have relied on selecting a genotype that, under optimal conditions, resembles high yield and further examining its performance under different degrees of drought stress and at different stages of stress (early drought, intermittent and terminal stress). This is done with the assumption that high-yielding genotypes will perform less the same across environments.

Pantuwan et al. (2002) reported that high yield potential under well-watered conditions is accompanied by mild stress, while yield and drought tolerance can be obtained under severe stress. Therefore, to avoid the above challenges, it is advisable to evaluate drought-tolerance based on the stress stage, stress severity and duration. Drought-tolerance indices, intrinsically, are potential indicators of various biological drought responses (Sofi et al., 2018). Although indices are estimated using the exact yield, the selection of drought-tolerant genotypes based on different indices may provide a more helpful criterion for improving yields under water stress. Yield-based indices, including SSI, TOL, MP, GMP, and STI are the most reliable for ranking genotypes and discriminating the resilience and productivity of the crop. These indices can be categorized into two groups, SSI and TOL discriminate between tolerance and susceptible, whereas MP, GMP and STI distinguish tolerance with high mean yielding (Thiry et al., 2016).

Fischer and Maurer (1978), reported SSI as a suitable index for estimating yield stability and has a significant influence on both potential and actual yield in a wide spectrum of environments. A study by Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield under drought-

stressed (Y_s) environment and optimum environment (Y_p), whilst mean productivity (MP) is the average of (Y_s and Y_p). The stress tolerance index (STI) was developed in the early 90s and is referred to as the advance index, which can be used to classify genotypes high yielding both under Ys and Y_p (Fernandez, 1992). In the same study genotypes were grouped into four distinct groups based on their performance under both drought stress and optimum conditions as follows: genotypes with good performance under both water conditions (Group a), genotypes with good performance under water-stressed conditions only, genotypes with good performance under or in well-watered (Group b) and those genotypes with poor performance under both conditions (Fernandez, 1992). Yield index (YI) (Gavuzzi et al., 1993), Mean relative performance (MRP) (Raman et al., 2012), -Geometric mean of productivity (GMP) (Fernandez, 1992), Yield stability index (YSI) (M. Bouslama & Schapaugh, 1984) and Relative efficiency index (REI) (Raman et al., 2012) is a good indicator of drought-tolerance and yield stability under water stress. The evaluation of these selection drought-tolerance indices has been reported in several studies and several crops. The objective of this study was to identify and estimate the most suitable selection drought-tolerance index and drought-tolerant soybean genotypes.

4.2 MATERIALS AND METHODS

4.2.1 Plant material, study sites, experimental design and trial management

Thirty-six soybean lines selected in chapter 2, were screened under well-watered and water-stressed conditions across the field and greenhouse environment at Jozini and the University of KwaZulu-Natal (Pietermaritzburg campus). The lines were evaluated using a 6×6 alpha-lattice design with two replications under the two water regimes. Detailed crop management, growing conditions, pest management and the imposed stress are described in chapter 2 under materials and methods.

4.2.2 Data collection and data analysis

4.2.2.1 Grain Yield

Grain yield was collected and recorded as 100 seed weight (g) and grain yield of plants per plot and further converted into tons per hectare. Grain yield data were analyzed using two-way ANOVA at a 5% level of significance. The mean performance, grain yield range, LSD and CV (%) were computed using the package variability in R.3.6.0 software. Reduction percentage was calculated between grain yield under WS and WW conditions and presented as Y_S and Y_P.

4.2.2.2 Yield-based indices of drought stress tolerance

The yield-based selection indices of drought stress tolerance were determined using the grain yield (Table 4.1). Multivariate analysis, including ANOVA were performed on means and separated for significant

difference at 5% level of probability. Principal component analysis (PCAs), biplot display of grain yield under WW and WS using 11 drought-tolerance indices were performed across the environment. The correlation between grain yield and drought-tolerance indices was done per water regime. All data analyses were computed using R.3.6.0 software.

Table 4.1: Yield-based indices of stress tolerance.

Indices	Formula	References
Drought intensity index (DII)	$DII = 1 - (X_{DS}/X_{NS})$	Fischer and Maurer (1978)
Stress susceptibility index (SSI)	$SSI = (Y_{NS} - Y_{DS})/(Y_{NS} \times DSI)$	Fischer and Maurer (1978)
Stress tolerance index (STI)	$STI = (Y_{NS} \times Y_{DS})/(X_{NS}^{2})$	Fernandez (1992)
Geometric mean of productivity (GMP)	$GMP = \sqrt{Y_{NS} \times Y_{DS}}$	Ramirez-Vallejo and Kelly (1998)
Yield index (YI)	$YI = Y_{DS}/X_{DS}$;9	M. Bouslama and Schapaugh (1984)
Yield stability index (YSI)	$YSI = Y_{DS}/Y_{NS}$	M. Bouslama and Schapaugh (1984)
Mean relative performance (MRP)	$MRP = (Y_{DS}/X_{DS}) + (Y_{NS}/X_{NS})$,	Raman et al. (2012)
Relative efficiency index (REI)	$REI = (Y_{DS}/X_{DS}) \times (Y_{NS}/X_{NS})$;	Raman et al. (2012)
Harmonic mean (HM)	$HM = 2 (Y_{DS} \times Y_{NS})/(Y_{DS} + Y_{NS})$	Jafari et al. (2012)
Stress tolerance (TOL)	$TOL = (Y_{NS} - Y_{DS})$;	Rossielle and Hamblin (1981)
Mean productivity (MP)	$MP = (Y_{NS} + Y_{DS})/2$	Rosielle and Hamblin (1981)

Where:

X_{DS} = mean grain/biomass yield of all genotypes under WS;

X_{NS} = mean grain/biomass yield of all the genotypes WW (Biju et al., 2018);

Y_{DS} = grain/biomass yield of a genotype under drought stress conditions and

Y_{NS} = grain yield /biomass yield of a genotype under WW (Sayyah, 2012)

The DII varies at a range of 0 and 1 and the larger the value of DII, the more severe is the stress. When SSI values < 1, genotypes are relatively drought-tolerant, while the genotypes with SSI > 1 are relatively drought susceptible.

4.2.2.3 Ranking of genotypes using grain yield reduction

The genotypes were ranked based on yield reduction due to water stress. Cluster analysis was performed to classify 36 soybean genotypes imposed to water tress and computed using the K-means based on grain yield;

Rank sum (RS)= Rank Mean (R) + St, the standard deviation of rank (SDR) and SDR=$(S^2i)^{05}$ as described by Studnicki et al. (2019).

4.3 RESULTS

4.3.1 Significant tests

The combined analysis of variance and mean squares for grain yield of 36 soybean genotypes and studied indices across environments is shown in table 4.2. The main effects due to the environment, genotype and water regime were significant for GY.

Table 4.2: Combined analysis of variance showing the mean squares and significant tests for grain yield under well-watered (YP) and water-stressed (Ys) regimes and different drought-tolerance indices in 36 soybean genotypes

SOV	DF	Y_P	Y_S	STI	GMP	HM	MP	MRP	REI	SSI	TOL	YI	YSI
						MEAN SQUARES							
REP	1	0.001 NS	0.38*	66.00 NS	0.008 NS	0.29**	0.15 NS	7.21 NS	0.11 NS	100.0 NS	0.20 NS	0.18 NS	7.20 NS
GEN	35	0.24 NS	0.09 NS	54.00 NS	0.145*	0.06 NS	0.08 NS	0.01 NS	0.82 NS	92.4 NS	0.28 NS	5.58 NS	6.83 NS
RESIDUALS	50	0.17	0.08	55.00	0.079	0.05	0.06	6.36	0.98	84.7	0.25	5.70	5.62

Note: SOV-source of variation; DF-degree of freedom; YP- yield of genotypes under well-watered, YS-yield of genotypes; SSI-Stress susceptibility index; YI- yield index; STI-Stress tolerance index; MRP-Mean relative performance; GMP-Geometric mean of productivity; YSI-Yield stability index; MP-Mean productivity; TOL-Stress tolerance; HM-Harmonic mean and REI-Relative efficiency index.***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05 NS non-significant and respectively.

The table 4.3, shows the significant and mean squares of grain yield of 36 soybean genotypes under well-atered and well-watered (Y_P) and water-stressed (Ys) water regime. The significant differences were observed among genotypes, water regimes and across environments.

Table 4.3: Analysis of variance showing the mean squares and significance for grain yield under well-watered (Y_P) and water-stressed (Ys).

SOV	DF	MEAN SQUARES
GEN	35	0.21*
WR	1	4.02***
ENV	1	7.90***
REP	1	0.18 NS
REP(ENV)	2	0.01 NS
GEN*WR	35	0.14 NS
GEN*ENV	35	0.66 NS
ENV*WR	1	0.42 NS
REP(BLK*WR)	20	0.18 NS
ENV*GEN*WR	35	0.10 NS
RESIDUALS	122	0.13

Note: SOV-source of variation; DF-degree of freedom; YP- yield of genotypes under well-watered, YS-yield of genotypes; SSI-Stress susceptibility index; YI- yield index; STI-Stress tolerance index; MRP-Mean relative performance; GMP-Geometric mean of productivity; YSI-Yield stability index; MP-Mean productivity; TOL-Stress tolerance; HM-Harmonic mean and REI-Relative efficiency index***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05 NS non-significant and respectively

4.3.2 The mean responses of genotypes for grain yield and drought indices

The values of 11 drought-tolerance indices were computed using corresponding grain yield responses of the 36 soybean genotypes under WW and WS conditions (Table 4.4).

Table 4.4: Mean performance values of 36 soybean genotypes evaluated using grain yield and 11 drought-tolerance indices under a well-watered and water-stressed regimes in the field environment.

GEN	Y_P	Y_S	SSI	YI	STI	MRP	GMP	YSI	MP	TOL	HM	REI
G1	0.91	0.86	-0.15	1.56	14.81	7.36	0.90	1.64	0.86	0.10	0.71	2.38
G2	0.71	0.56	0.55	1.53	9.63	4.66	0.54	1.32	0.59	0.24	0.49	0.70
G3	0.66	0.36	-4.45	2.49	16.19	5.12	0.69	2.12	0.72	-0.13	0.66	1.13
G4	0.60	0.63	0.19	3.71	5.97	3.84	0.53	0.86	0.56	0.09	0.51	1.00
G5	0.67	0.62	0.05	1.71	6.46	4.57	0.63	1.08	0.65	0.05	0.61	1.39
G6	0.67	0.43	-3.14	1.63	3.38	3.82	0.39	1.37	0.51	0.21	0.31	0.33
G7	0.41	0.29	-2.09	1.88	7.07	3.12	0.41	1.67	0.44	-0.05	0.38	0.53
G8	0.35	0.38	-0.97	7.68	3.49	2.61	0.37	1.32	0.39	-0.08	0.35	0.36
G9	1.18	0.54	1.39	1.02	18.06	1.73	0.84	0.53	0.92	0.52	0.61	1.89
G10	0.93	0.66	2.11	1.97	8.52	5.03	1.36	1.40	0.64	0.56	0.49	0.80
G11	0.78	0.58	0.66	1.09	12.79	4.97	0.66	0.80	0.67	0.20	0.65	1.17
G12	1.07	0.42	1.78	1.46	7.31	4.37	0.59	0.49	0.71	0.71	0.49	1.05
G13	1.13	0.63	1.82	1.06	14.23	6.34	0.74	0.43	0.83	0.59	0.68	1.52
G14	0.45	0.33	0.02	1.48	4.24	3.09	0.39	1.10	0.46	-0.02	0.42	0.47
G15	0.83	0.54	0.91	1.45	7.84	4.78	0.64	0.61	0.69	0.29	0.60	1.15
G16	0.75	0.64	-0.87	2.15	9.30	4.13	0.62	2.28	0.67	0.15	0.57	1.26
G17	0.75	0.43	1.27	2.00	11.45	5.20	0.65	0.66	0.66	0.18	0.63	1.05
G18	0.74	0.38	1.65	1.84	6.56	4.19	0.46	0.34	0.52	0.43	0.41	0.53
G19	0.86	0.56	-0.47	2.46	16.38	5.73	0.73	1.10	0.77	0.18	0.70	1.28
G20	0.65	0.71	-2.24	2.14	4.58	4.40	0.46	1.69	0.54	0.22	0.39	0.86
G21	0.91	0.59	0.83	2.89	9.24	5.40	0.65	0.66	0.81	0.20	0.75	1.56
G22	0.28	0.28	-0.78	1.08	1.47	1.37	0.27	1.18	0.28	-0.02	0.26	0.23
G23	0.61	0.55	0.18	1.35	5.36	3.11	0.56	0.94	0.57	0.09	0.55	1.11
G24	0.44	0.36	0.55	1.39	9.79	3.32	0.48	1.10	0.50	-0.11	0.47	0.85
G25	0.79	0.88	-0.03	0.97	6.90	5.36	0.64	0.97	0.71	0.15	0.59	1.44
G26	1.32	0.70	2.52	1.81	12.25	6.65	0.71	0.28	0.86	0.91	0.60	1.32

	YP	YS	SSI	YI	STI	MRP	GMP	YSI	MP	TOL	HM	REI
G27	0.52	0.43	-3.37	1.00	6.16	4.18	0.57	1.62	0.62	-0.20	0.52	1.08
G28	0.81	0.64	-0.15	0.76	6.40	4.59	0.54	0.99	0.62	0.36	0.48	0.94
G29	1.14	0.50	2.03	2.17	13.13	5.60	0.66	0.46	0.77	0.73	0.70	1.17
G30	1.03	0.75	1.25	1.78	11.79	6.81	0.79	1.28	0.56	-0.12	0.37	1.23
G31	0.94	0.61	-0.72	1.65	17.00	6.57	0.81	1.27	0.86	0.15	0.77	1.83
G32	1.12	0.49	2.24	3.08	11.87	6.11	0.69	0.37	0.74	0.75	0.59	0.93
G33	0.90	0.52	-4.36	1.41	11.60	5.31	0.71	0.65	0.74	0.31	0.69	1.29
G34	0.53	0.28	-2.21	0.99	5.56	3.55	0.48	1.39	0.51	0.04	0.45	0.71
G35	0.68	0.28	1.52	1.12	6.63	3.98	0.49	0.62	0.58	0.21	0.54	1.09
G36	0.97	0.66	-3.21	1.03	8.62	5.05	0.59	1.81	0.74	0.46	0.48	0.99
Mean	1.85	0.77	0.53	1.85	9.03	4.55	0.61	1.35	0.63	0.22	0.53	1.06
SEM	0.18	0.08	84.73	5.70	54.67	6.35	0.08	5.62	0.06	0.26	0.05	0.80
LSD	0.56	0.40	13.07	3.39	10.5	3.58	0.38	3.69	0.37	0.71	0.33	1.28
CV%	55.05	53.90	-782.81	129.35	81.89	55.37	46.08	175.89	40.52	228.63	43.65	84.33

Note: LSD-least significant differences, SEM-standard error mean, CV-coefficient of variance, GEN-genotype; Y_P- yield of genotypes under well-watered, Y_S-yield of genotypes; SSI-Stress susceptibility index; YI- yield index; STI-Stress tolerance index; MRP-Mean relative performance; GMP-Geometric mean of productivity; YSI-Yield stability index; MP-Mean productivity; TOL-Stress tolerance; HM-Harmonic mean and REI-Relative efficiency index.

The analysis of variance of grain yield (Y_P and Y_S) showed significant differences (P<0.05) among the 36 soybean genotypes (table 4.4). Across environments, the average grain yield under well-watered (Y_P) was 0.78, while under water-stressed (Y_P) was 0.52, with a decline of 25.97%. The Y_p ranged from 0.27 to 1.32, with G22 having the lowest value and G26 with the highest value (Table 1.2). The Y_s ranged from 0.28 to 0.87, with the corresponding lowest value for G35, with G25 having the highest value.

The DII varies at a range of 0 and 1 and the larger the value of DII, the more severe the stress. In the study, according to the calculation using grain yield, DII was below the range (-1.23). The overall mean for SSI was 0.53, ranging from -4.36 to 2.52, with G33 having the lowest value and G26 having the highest value. The overall mean for YI was 1.85 and ranged from 0.76 to 7.6, with G7 being the lowest and G10 being the highest. High YI mean values were observed for G8, G4 and G2 and the low values for G28, G25 and G34. The overall STI mean was 9.09 with a minimum of 1.46 and a maximum of 18.05. Taking the STI index into account, the genotypes with high indices were G9, G31 and G19, which were considered prone to drought, while low values of STI were observed for G22, G6 and G8 were considered drought-tolerant genotypes (Table 4.2). The overall mean for MRP was 4.55 with a minimum of 1.36 and a maximum of 7.36. The MRP showed the high mean values for G1, G30 and G26 and the low values for G22, G9 and G8

The overall mean for GMP was 0.61, with a minimum value of 0.27 and a maximum of 1.35. High mean values for GMP were obtained for G10, G1 and G9, while low GMP were obtained for G22, G8 and G6, indicating drought-tolerant genotypes. The overall mean for YSI was 1.35, with a minimum value of 0.16 and a maximum of 3.53. The highest YSI was attained for G32, G3 and G16 and the lowest mean values for G26, G18 and G13. The overall mean for MP was 0.63, with a minimum value of 0.28 and a maximum of 0.91. The highest MP were attained for G9, G26 and G31, lowest mean values for G22, G8 and G7. The overall mean for GMP was 0.61, with a minimum of 0.27 and a maximum of 1.35. The highest mean values for GMP were obtained for G10, G1 and G9, while the lowest GMP values were obtained for G22, G8, G6 and G14 indicating drought-tolerant genotypes. The overall mean for YSI was 1.35, with a minimum of 0.16 and a maximum of 3.53. The highest YSI values were achieved for G32, G3 and G16 and the lowest mean values for G26, G18 and G13. The overall mean for MP was 0.63, with a minimum of 0.28 and a maximum of 0.91. The highest MPs were achieved for G9, G26 and G31and the 33 lowest mean values for G22, G8 and G7.

The overall mean for TOL was 0.22, with a minimum of -0.20 and a maximum mean value of 0.91. Based on the TOL index, the highest indices were obtained for G26, G29 and G12 indicating susceptibility to water stress, and the lowest values were obtained for G27, G3 and G30 as an indication of being more tolerant to water stress. Rossielle and Hamblin (1981) indicated that genotypes with a high TOL index are

most susceptible to drought stress, whereas genotypes with the lowest values of the TOL index represent drought-tolerance.

The overall mean value of HM was 0.53, with a minimum of 0.29 and a maximum of 1.16. The highest HM index was obtained for G31, G21 and G1 as a representation of genotypes that are more susceptible to water stress were as genotype showed least means for G22, G6 and G8. The overall mean value for REI was 1.56, with a minimum mean value of 0.24 and a maximum of 2.38. For the REI, G1, G9 and G31 exhibited the highest means. The least means for REI were obtained for G22, G6 and G8.

4.3.4 Correlation among grain yield and 11 studied drought-tolerant indices

Table 4.5: The Pearson's correlation for grain yield and 11 studied, drought-tolerant indices of soybean lines under well-watered and water-stressed regimes across environments.

	Y_P	Y_S	SSI	YI	STI	MRP	GMP	YSI	MP	TOL	HM	REI
YP	**1.00**											
YS	0.54***	**1.00**										
SSI	0.46**	0.18	**1.00**									
YI	-0.20	-0.10	0.00	**1.00**								
STI	0.66***	0.32	0.18	-0.11	**1.00**							
MRP	0.66***	0.62***	0.19	-0.09	0.57***	**1.00**						
GMP	0.64***	0.55***	0.31***	-0.12	0.63***	0.57***	**1.00**					
YSI	-0.46*	0.05	-0.68	0.06	-0.13	-0.11	-0.05	**1.00**				
MP	0.85***	0.55***	0.23	-0.20	0.81***	0.65***	0.66***	-0.28	**1.00**			
TOL	0.80***	0.24	0.53***	-0.13	0.29	0.33*	0.37	-0.60***	0.54***	**1.00**		
HM	0.56***	0.38*	0.17	-0.11	0.76***	0.60***	0.55***	-0.26	0.56***	0.27	**1.00**	
REI	0.61***	0.63***	0.18	-0.22	0.72***	0.58***	0.60***	-0.12	0.86***	0.18	0.81***	**1.00**

YP- yield of genotypes under well-watered, YS-yield of genotypes; SSI-Stress susceptibility index; YI- yield index; STI-Stress tolerance index; MRP-Mean relative performance; GMP-Geometric mean of productivity; YSI-Yield stability index; MP-Mean productivity; TOL-Stress tolerance; HM-Harmonic mean and REI-Relative efficiency index Perfect correlation ± 1; High correlation ± 0.50-1; Moderate correlation ± 0.30-0.49; Low correlation ±0.29. ***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05 NS non-significant, respectively.

The correlation Coefficients explaining, the magnitude of overall linear relationship for grain yield and studied drought-tolerance across environments, respectively are presented in Table 4.5. The YP, showed highly significant(P≤0.001) and positive correlation with YS for almost all studies indices, including (r=0.54), SSI (r=0.46), STI (r=0.66), MRP (r=0.66), GMP (r=0.64) and MP (r=0.85), TOL (r=0.80), HM (r=0.64) and REI (r=0.85) and had a significant (P≤0.05) negatively correlation with YSI(r=-0.46). The YS,

showed highly significant (P≤0.001) and positive correlation with MRP (r=0.62), GMP (r=0.55) and MP (r=0.55), HM (r=0.38) and REI (r=0.61).

The SSI, indicated a significant(P≤0.001) and positive correlation with GMP (r=0.31) and TOL (r=0.80). The STI, showed a significant (P≤0.001) and positive correlation MRP (r=0.57), GMP (r=0.63) and MP (r=0.81), HM (r=0.76) and REI (r=0.72). The MRP, showed a significant (P≤0.001) and positive correlation GMP (r=0.57) and MP (r=0.65), TOL (r=0.33) HM (r=0.60) and REI (r=0.58). The GMP, showed a significant and positive correlation with MP (r=0.66; P≤0.01) REI (r=0.60; P≤0.001), HM (r=0.55; P≤0.001). The YSI, showed a significant negative correlation with TOL (r=-0.60; P≤0.001). The MP, showed a significant and weakly positive correlation with TOL (r=0.53; P≤0.001), HM (r=0.56; P≤0.001) and moderately positive correlation with REI (r=0.86; P≤0.001). The HM, showed a significant (P≤0.05) and weakly positive correlation with REI (r=0.53).

4.3.5 Principal components analysis (PCA)

The PCA grouped the grain yield and 11 drought tolerance indices into four main principal components with a total percentage of 74.97%.

The first DIM1 accounted for 50.07%; DIM2 for 16.56% and DIM3 for 8.34% (Table 4.6). The drought-tolerance indices that contributed positively and had high factor loadings to DIM 1 were MP (0.95), YP (0.90), REI (0.85), HM (0.81), STI (0.80), GMP (0.76) and MRP (0.76) and YS (0.64). For DIM 2, YSI (0.81), SSI (-0.71), and TOL (-0.63) contributed positively and recorded high values. For DIM 3, YI (0.93) contributed positively and had a high factor loading. YS was the index that had a high value for DIM 4.

Table 4.6: The eigenvalues and principal components from the correction matrix of grain yield under the well-watered and water-stressed regime and drought-tolerance indices in the greenhouse and field environment.

Indices	Eigenvector			
	DIM 1	DIM 2	DIM 3	DIM 4
YP	0.90	-0.25	-0.01	0.13
YS	0.64	0.27	0.20	0.57
SSI	0.42	-0.71	0.16	0.08
YI	-0.20	-0.03	0.93	-0.25
STI	0.80	0.22	-0.01	-0.36
MRP	0.76	0.20	0.17	0.19
GMP	0.76	0.16	0.12	0.18

| | Eigenvector | | | |
Indices	DIM 1	DIM 2	DIM 3	DIM 4
YSI	-0.36	0.81	0.07	0.23
MP	0.95	0.12	-0.07	-0.14
TOL	0.59	-0.63	-0.03	-0.17
HM	0.81	0.21	-0.03	-0.42
REI	0.85	0.33	-0.08	0.21
Explained variance (eigenvalue)	6.01	1.99	1.00	0.91
Proportion of total variation (%)	50.07	16.56	8.34	7.66
Cumulative variance (%)	50.08	66.46	74.97	82.63

Note: YP- yield of genotypes under well-watered, YS-yield of genotypes; SSI-Stress susceptibility index; YI- yield index; STI-Stress tolerance index; MRP-Mean relative performance; GMP-Geometric mean of productivity; YSI-Yield stability index; MP-Mean productivity; TOL-Stress tolerance; HM-Harmonic mean and REI-Relative efficiency index.

4.3.6 Biplot analysis

Biplot analysis was computed for using the PC analysis for the 36 soybean lines studied using grain yield under well-watered and water-stressed regimes and 11 drought-tolerance indices (Figure 3.2). The first two dimensions, Dim1 and Dim 2, accounted for 66.7% of the total variation among yields and indices and each component accounted for 50.1% and 16.6% (Figure 3.2).

Figure 3.2: Principal components biplot of 36 soybean genotypes for grain yield and 11 drought-tolerance selection indices studied across environments, showing the first two principal components axes (Dim 1 and Dim2).

A strong positive correlation between drought-tolerance indices and grain yield was observed between YP, YS, GM, HM, GMP, STI, REI, MP and MRP; YP with TOL and SSI, as indicated by the small acute angle between their vectors(r=cos0=+1). A nearly zero correlation was observed between YS, REI, TOL, and SSI; YI with YSI and SSI as indicated by a perpendicular angle between their vectors(r=cos90=0). There was a negative correlation between YSI with SSI and TOL; YI YP, YS, GM, HM, GMP, STI, REI, MP and MRP as indicated by an obtuse angle, that is approximately 180 degrees (r=cos180=-1).

4.3.7 Ranking of genotypes

The drought-tolerant soybean genotypes were determined by ranking the genotypes based on their yield reduction due to water stress (Table 4.7). A hypothetical scale was made to group the genotypes based on yield reduction. According to the yield reduction, the genotypes were ranked into four respective groups; tolerant (less than 50.00 %), moderately tolerant (50.01-60.00 %), moderately susceptible (60-70.00 %), and susceptible (above 70.01%). The results revealed 31 tolerant genotypes to water stress. This had high yields under both well-watered and water-stressed conditions resulting in low yield reduction. One genotype was moderately tolerant to water stress (G13) and five genotypes (G26, G32, G9, G29, G12) were ranked as moderately susceptible to water stress. No genotypes were susceptible to water stress.

Table 4.7: The ranking of 36 soybean genotypes based on yield reduction due to water stress

Rank Order	Yield reduction (%)	Genotypes (36)
Tolerant	Less than 50.00	G22, G4, G8, G1, G23, G5, G20, G24, G27, G25, G16, G14, G7, G2, G28, G11, G6, G34, G10, G30, G3, G15, G19, G36, G17, G21, G31, G18, G33, G35 and G13
Moderately Tolerant	50.01-60.00	G13
Moderately Susceptible	60-70.00	G26, G32, G9, G29 and G12

Rank Order	Yield reduction (%)	Genotypes (36)
Susceptible	Above 70.01	-

4.3.8   Cluster plot analysis

The clusters were delineated by k-means clustering for the 36 soybean genotypes based on grain yield and drought-tolerance indices across environments for water tolerance (Figure 4.5). Cluster I exhibited the least genotypes (8), cluster II had the most genotypes (17), and cluster III had 11 genotypes (Table 4.8). The soybean genotypes grouped into cluster I, were characterized with the least means for YP (0.48), YS (0.34), SSI (-0.87), STI (5.19), MRP (3.13), GMP (0.41), MP (0.45), TOL (0.05), HM (0.38), REI (0.50) and high mean values for YI (2.25) (Table 3.9).

Cluster II was characterized by moderate mean values for grain yield under both conditions and almost all indices. This cluster had a low mean value for YSI (1.19). Cluster III had high mean values for grain yield under both well-watered and water-stressed conditions and for almost all drought-tolerant indices. Cluster III consisted of high mean value of YP (1.04), YS (0.59), SSI (4.23), MRP (12.98), GMP (5.54), MP (0.80), TOL (0.49), HM (0.64) and REI (1.43). However, cluster III had the least values for YI (1.92) and YSI (0.79).
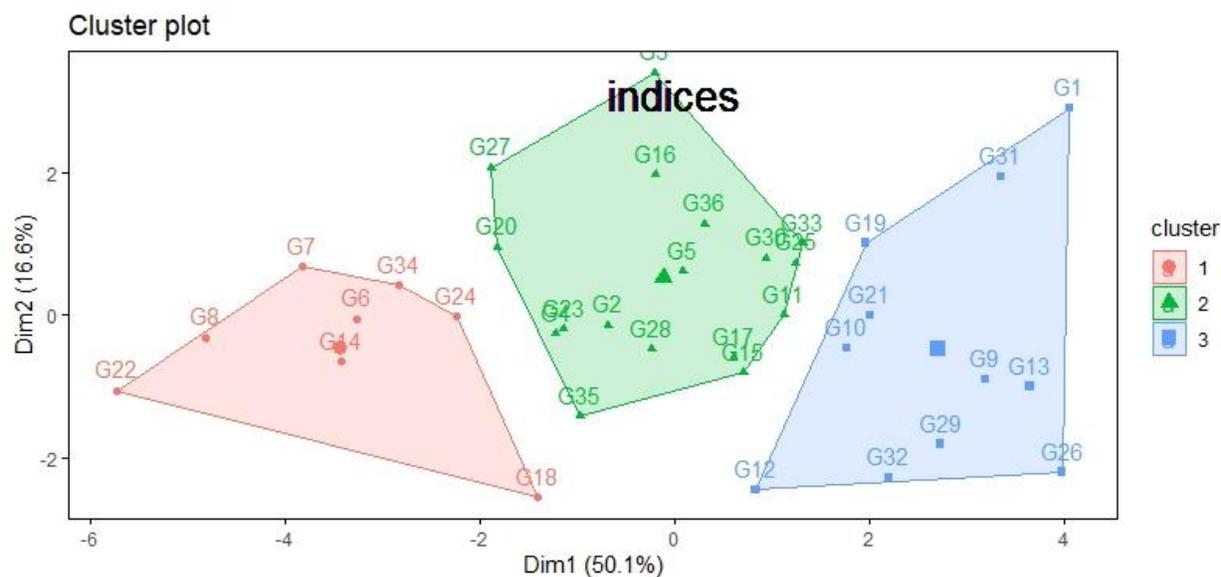
Figure 4.1: Cluster Plot of the first two PCAs showing the relationship among 36 soybean lines for grain yield well-watered and water-stressed and drought-tolerance indices across the environment.

4.4 DISCUSSION

4.4.1 Yield performance

Analysis of variance for grain yield under both water regimes revealed significant genotypic variation, suggesting that selection of better performing genotypes under both water regimes would be possible. These findings are in accordance with the study reported by Mdluli (2018). The water stress reduced the grain yield by 29%, with the highest reduction observed for G12 and least for G22. In addition, a significant positive correlation was obtained for grain yield under well-watered and water-stressed regimes (0.54**) across the two environments, suggesting that superior genotypes selected under optimum conditions could give relatively high yields under drought stress. Abdi et al. (2013) reported that the variability in yield under optimum conditions and water stress suggests that it would be possible to select genotypes that are drought tolerant through traditional breeding techniques.

According to Fischer and Maurer (1978), the SSI index showed that G33, G36, G6, G20 G34, G7, G8, G16, G22 and G31 were most susceptible to drought, whereas G26, G10, G29, G13, G12, G35, G9, G17, G30, G15 and G21 were tolerant to drought stress (Table 4.4). In addition, the lowest values of SSI indicate genotypes that are most likely to be less susceptible to drought stress; therefore, the SSI index can be estimated to identify genotypes that susceptible and ones tolerant to drought stress (Shiranirad and Abbasian, 2011).

4.4.2   Relationships among drought-tolerant indices

The ANOVA showed significant differences among soybean genotypes for SSI, STI, YI, YSI, HM and GMP. The correlation coefficients were used to identify the best criterion for selecting drought-tolerance genotypes. The drought-tolerance indices that had a significant correlation with yield under both under well-watered and water-stressed are suitable for use in selection for drought-tolerance in breeding programs (Abdi et al., 2013). The drought-tolerant selection indices that revealed significant positive correlations with gran yield under well-watered and water-stressed regimes were MRP, GMP, MP, MRP, HM and REI. These six indices were comparably effective in selecting and predicting better grain yielding genotypes under well-watered and water-stressed regimes than SSI, YI, STI, YSI and TOL. Similar observations were made in a study by Anwar et al. (2011) for wheat genotypes.

Grain yield under a well-watered regime (Y_P) was significantly and positively correlated with all selection indices YI except for and YSI. A negative correlation between YP and YS was observed for sorghum lines tested for drought tolerance under two contrasting environments (Abebe et al., 2020). The YS was significantly and positively correlated with MRP, GMP, MP, HM and REI. The SSI and TOL indicated a

significant positive correlation, suggesting that these indices can predict one another (Zare, 2012). Eyni Nargeseh et al. (2020) and Arif et al. (2021) also reported a positive association between SSI and TOL.

In the present study, the principal components grouped the grain yield and 11 drought tolerance into four main components in both environments. The PCs separated SSI, YI, STI, MPR, GMP, MP, TOL, HM and REI, which were referred to as group 1, MRP, YSI as group 2 and YI as a group, 3 and group 4 as YS. The selection of variables with high values in DIM was considered favorable in the present study. This component revealed the most relationship with indices and grain yield under both water regimes. These findings corroborate the study conducted by (Mdluli, 2018). In the same study PC1, revealed high values for HM, MP, STI, and SSI whilst, DIM 2, and 3 did not contribute to the selection of high-yielding genotypes under well-watered and water-stressed conditions. However, DIM 2 and 3 had high loading values of SSI, YSI and TOL. Zare (2012) demonstrated high loading values in PC2 that were positive for and negatively associated with SSI and TOL. These findings suggested a water stress tolerant dimension that can possibly discriminate water stress-tolerant and well-watered tolerant genotypes. Therefore, genotypes with high values in DIM 1 and 2 can be successfully used for the selection of high-yielding genotypes under both well-watered and water-stressed regimes.

4.4.3   Screening for drought-tolerance and high yielding soybean genotypes

According to Fernandez (1992), the genotypes that were classified as group 1 were G2, G3, G4, G5, G11, G15, G16, G17, G20, G23, G25 G27, G28, G30, G35, G36 and G33; thus, they had high yield under both water conditions. These genotypes showed high significant differences according to ANOVA results (Table and were highly associated with SSI, STI, MRP, GMP, MP, TOL, HM and REI based on principal components (Figure 4.5 and Table 4.2) and cluster plots, presented as cluster III (Figure 4.3, Table 4.8 and 4.9). Whilst G6, G7, G8, G14, G18, G22, G24 and G34 were identified as poor yield performers under both water regimes as indicated by the least values of Y_S and Y_P and poor relationship with almost all indices except for YI.

Based on the genotype rankings of yield reduction as described by Chowdhury et al. (2016), 31 genotypes were relatively tolerant to water stress (Table 4.7) as shown by high yield potential under both water regimes and had low yield reduction. Only one genotype showed moderate tolerance to water stress which demonstrated a lower yield than tolerant genotypes. Four of the genotypes were identified as moderately susceptible to water stress. For mean performance of yield indices (Table 4.3), genotypes G34, G22, G8, G14, G7, G3 and G6 had high mean values, and G31, G19, G1, G13, G29, G26, G33 and G9 revealed low index mean values. Based on the SSI, G26 was tolerant to water stress and G7 prone to water stress.

4.5 Conclusion

In conclusion, MRP, GMP, MP, MRP, HM, and REI were the drought-tolerant indices with significant positive correlations for gran yield under well irrigated and water-stressed regimes. These indices were comparably effective in selecting and predicting better grain yielding soybean genotypes under well-watered and water-stressed regimes. Most of the studied soybean genotypes were tolerant to water stress based on yield reduction rankings. Among these genotypes, G1, G19, G13, G33, G31 had high mean performance, tolerance, and association with SSI, STI, MRP, GMP, MP, TOL, HM and REI. However, G26, G32, G9, G29, G12 were considered as moderately susceptible to water stress, and G7, G8, G14, G22, G34 had low mean performance values and low association with indices. Therefore, based on these findings, the sole use of grain yield and yield reduction is not suitable for the genetic improvement of water stress in soybean genotypes and can lead to incorrect conclusions when selecting drought-tolerant genotypes. Consequently, the use of these approaches combined with drought-tolerant indices guarantees a better yield in the event of water stress. In addition, PCA can be used to select genotypes for numerous indices, and clustering genotypes based on grain yield and drought tolerance indices as independent variables can potentially identify the Fernandez classifications mentioned in the introduction section. These analytical techniques also provided informative and simplified results with clear relationships between grain yields under both water regimes and indices.

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Chapter Five

Estimation of variance components, the heritability of yield and agronomic traits in soybean under well-watered and water-stressed

ABSTRACT

Thirty-six soybean genotypes obtained from the International Institute of Tropical Agriculture (IITA) in Zambia were evaluated using a 6 x 6 alpha lattice design with two replications at Makhathini Research Station, Jozini and in the greenhouse at the University of KwaZulu-Natal (UKZN), Pietermaritzburg Campus; (i) to estimate broad-sense heritability of the morpho-physiological traits under well-watered and water-stressed conditions and (ii) to determine the phenotypic and genotypic correlations of grain yield and agronomic traits. Significant differences among the soybean genotypes were observed for grain yield and studied traits demonstrating the presence of genetic variability among the genotypes for these traits. The phenotypic coefficient of variation (PCV) ranged from 7.03 cm for plant height(PH) to 92.84 t ha^{-1}grain yield (GY) whereas, the genotypic coefficient of variation (GCV) values ranged from 0.07 for chlorophyll content (CC) to 60.77 grain yield (GY). Grain yield had a positive and significant genotypic and phenotypic correlation with plant height (PH), leaf length (LL), leaf width (LW), Stomatal conductance (STD) and seed moisture content MC. This implies that an increase in these traits would ultimately increase grain yield. However, there was no clear trend in studied traits for heritability across both environments and water regimes, due to reduced effects by environmental stress. In the field environment high PH heritability were obtained both under WS (0.41) and WW (0.41), suggesting that it was less likely to be affected by the environment. Overall most traits had low heritability in both water regimes, therefore one should be careful in selection for drought-tolerance using these traits.

Keywords: Soybean, yield, Genetic viability, broad sense-heritability and Correlation

5.1 INTRODUCTION

Soybean (*Glycine Max* L. Merr.) is an important oilseed and affordable source of protein worldwide. It is commonly consumed as human food, processed oil and meal for livestock feeding Hartman et al. (2011). Soybean seed oil is rated as the second most widely used vegetable oil and its meal accounts for 70% of world protein meal consumption (Birthal et al., 20210; Jamet et al., 2016). Currently, the estimated world soybean production is 340.47 million metric tons (WASDE, 2020). The United States of America, followed by Brazil and Argentina, are the top three leading producers of soybean worldwide and contribute about 83% of the world output (Lee et al., 2016). Moreover, China is also a competitor of the world big exporters of soybeans including as USA, Brazil and Argentina, due to an increase of high soybeans consumers since 2000's more than ever (Jamet et al., 2016).

Soybean production is threatened by several abiotic stresses, with drought stress constituting the major environmental factor limiting soybean production. Moreover, the average yield of soybean in South Africa over the last 20 years is 40% lower than the average yield of the three top producers worldwide. Consequently, South Africa is consistently failing to meet the national demand for soybean (FAO, 2017). In the 2015/2016 season, South Africa experienced a decline in soybean production of 27% and the season ended with a very minimal export value between 2014 to 2016, which was a consequence of severe drought conditions in the major producing regions (Nortjé, 2017). Despite this setback, the soybean production industry managed to reach a peak and is still growing. The recent highest soybean production in South Africa was 1 540 000 tons and was cultivated over 787 200 hectares (Nortjé, 2017). Nevertheless, to maintain these figures, in addition to soybean remaining a profitable enterprise with high production efficiency, there is a need to increase the grain yield per hectare. The effects of drought stress on soybean yields can be combated by introducing new drought-tolerant varieties with superior yielding capabilities.

The development of new soybean varieties that are tolerant to drought is a primary key to meeting the South African demand for food and oil resources. Soybean grain yield, as in any other crop, is regarded as a complex quantitative trait dependent on several variables and is generally characterized by a low heritability, ultimately causing low selection responses under drought conditions (Hill, 2010; X. Wu et al., 2012). Hence plant breeding programs should understand and utilize relationships between yield and its related traits for effective grain yield selection. The correlation research studies, direct and indirect influence of the secondary traits on grain yield, are essential for successful breeding programs (Ojha et al., 2018).

Several statistical tools, including correlation and path coefficient analysis, have been utilized to understand the association between yield and its secondary components. The correlation analysis studies of direct and

indirect influence of the secondary traits on grain yield are essential for successful breeding programs. Correlation analysis provides essential knowledge on the degree of relationships between traits (Cyprien & Kumar, 2011). Plant height, number of stems, pods per plant, biological yield, harvest index, 100 seed weight, days to flowering and maturity are the most important characters for soybean improvement for increasing seed yield due to their direct and indirect correlation (Toker, 2004). Many studies have demonstrated that grain yield is highly influenced by 100 seed weight, number of pods per plant, number of pods per plots, number of seeds per pod (Kumagai & Takahashi, 2020; R. Souza et al., 2016). In this study, variance components, heritability and correlations among grain yield and secondary traits were determined under well-watered and water-stressed conditions, with the aim of identifying traits that can be used for indirect selection under water-stress conditions.

1.2 MATERIALS AND METHODS

5.2.1 Plant material, study sites, experimental design, and crop establishment

The 36 soybean genotypes that were used in chapter 2 were evaluated under well-watered and water-stressed conditions under controlled conditions in Jozini and at the University KwaZulu-Natal (PMB campus). The study was conducted using a 6 x 6 alpha lattice design with two replications. The details of the crop establishment, growing conditions, and treatments were discussed in chapter 2.

5.2.2 Data collection and data analysis

All data were collected and recorded, as described in chapter 2, and computed using R.3.6.0 software. The analysis of ANOVA was done for individual sites and across environments.

5.2.3 The estimation of genetic parameters

The expected mean squares under the assumption of the mixed model were computed using the linear combinations of mean squares. The phenotypic and genotypic variances were computed as described by (Falconer, 1952) as follows;

$$\text{Genotypic variance:} \quad \sigma^2 g = \frac{Mg - Me}{r}$$

$$\text{Phenotypic variance:} \quad \sigma^2 = \sigma^2 e - \sigma^2 g$$

Where;

Mg represents the mean sum of squares for the genotypes, and Me represents the random error mean squares from the ANOVA.

5.2.4 The genotypic and phenotypic coefficient of variation

The genotypic and phenotypic coefficient of variation were estimated for all quantitative traits, as a relative indicator of trait variability according to Singh and Chaudhary (2010), using the equations:

$$GCV\ (\%) = \frac{\sqrt{\sigma^2 g}}{\bar{x}} * 100$$

$$PCV\ (\%) = \frac{\sqrt{\sigma^2 p}}{\bar{x}} * 100$$

Where;

$\sigma^2 g$ = genotypic variance; $\sigma^2 p$ = phenotypic variance and $\bar{x}$ = grand mean

5.2.5 Estimation of broad-sense heritability

The broad-sense heritability (H^2) was estimated as a percentage (%) for both water regimes using the variance components mentioned above; phenotypic variance and genotypic variance obtained from the ANOVA as described by Allard (1999). The environment and water regimes were considered as fixed models and genotype effects as random.

$$H = \frac{\sigma^2 g}{\sigma^2 g + \dfrac{\sigma^2 ge}{e} + \dfrac{\sigma^2 e}{re}} * 100$$

Where; $\sigma^2 g$ = total genotypic variance, e = environment, r = replication, $\sigma^2 ge$ = genotype by location variance and the heritability was estimated on mean entry basis (Chibanda, 2017).

5.2.6 The Pearson correlation and path coefficient analysis

Genetic correlations under each water regime were estimated (Ribeiro, 2004) and their significance was tested using the formula in R.3.6.0 software:

$$r = \frac{\Sigma(x - m_x)(y - m_y)}{\sqrt{\Sigma(x - m_x)^2\ \Sigma(y - m_x)^2}}$$

5.3 RESULTS

5.3.1 Estimates of variance components and broad-sense heritability

Combined analysis of variance of results are presented in chapter 3, Table 3.3 and Table 3.4. The estimates of the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), and components of variances (σ^2 g, σ^2p and σ^2e) for the 11 studied traits of 36 soybean lines across field and greenhouse environment are represented in Table 5.1 and 5.2. According to the results, the genotypic variance was lower than the phenotypic and environmental variances in almost all traits in each environment and under the different water regimes, except for PH, LL FLW and STC in the field environment under WS and FLW in the field the greenhouse under the same environment. The genotypic variance in the field environment was negative under the WW regime for LW, FLW and CC.

While in the greenhouse under WW, the genetic variance was negative for FLW and SMOI, whereas, under WS, it was negative for PH, LL, STD and CC. Furthermore, the PCV values were generally higher than the GCV in each environment and under the respective water regime for all studied traits. The PCV and GCV are considered to have high values when above 20%, moderate at a range of 10% to 20% and lower when less than 10% (Chandrawat et al., 2017). The GY and BM showed high phenotypic and genotypic variance in each environment and under each water regime. High PCV values in the field under WW were obtained for PH, LW, BM and SW, and GY whereas, under WS high values were recorded for LW, FLW, STC, BM, SW and GY.

Moderate PCV under WW was obtained for LL, LW and SMOI, whereas under WS was recorded for PH, LL, STD and SMOI. Low PCV under WW was recorded for PH. High GCV in the field under WW was obtained for BM and GY, whereas under WS it was recorded for BM and GY. Moderate GCV under WW was obtained for PH whereas, under WS it was recorded for PH, LL, STD and SW. Low PCV under WW was obtained for LL, LW, STD, CC, FLW, SMOI and SW, whereas WS was recorded for LW, CC, FLW, STC and SMOI. High PCV in the greenhouse under WW was obtained for STD, CC, FLW, STC and BM whereas, under WS was recorded for PH, LL, LW, CC, STC, BM and GY. Moderate PCV under WW was obtained for LL, CC, FLW and STC whereas, under WS it was recorded for STD, FLW and SW.

 Low PCV under WW and WS was recorded for SMOI. High GCV in the field under WW was obtained for BM and GY, whereas under WS was recorded for the same two traits. Moderate GCV under WW was obtained for STD, CC, FLW and SMOI whereas, under WS was recorded for FLW, STC and SW. Low PCV under WW was obtained for PH, LL, LW, STC and SMOI, whereas in WS was recorded for PH, LL, LW, STD CC and SMOI. The estimates of broad-see heritability under WW ranged from 0.00 for SMOI to 0.41 for PH whereas, under WS it was 0.29 GY for FLW to 0.55 for STD. The estimates of broad-sense

heritability under WW in the field ranged from 0.00 for LW to 0.41 for PH whereas, under WS, it ranged from 0.00 for GY to 0.55 STD. In the greenhouse environment under WW, the estimates of broad sense heritability ranged from 0.00 for FLW to 0.34 for BM, whereas under it ranged from 0.00 for STD to 0.43 BM.

Table 5.1: Range, mean, variance, broad see heritability, genotypic coefficient of variation, and critical difference at 5% and 1% of 26 soybean genotypes under WW and WS in the field environment.

Traits	Range	Mean ± S.E Mean	$\sigma^2 g$	$\sigma^2 p$	$\sigma^2 e$	GCV (%)	PCV (%)	H^2
Water-stressed								
PH	23.33-73	73.07±5.81	47.79	115.28	67.49	14.27	22.16	0.41
LL	5-13.33	9.17±0.86	0.68	2.16.	1.49	8.96	16.05	0.31
LW	3.33-8.67	5.44±0.85	0.00	1.38	1.14	5.36	20.56	0.00
STD	3-8.67	5.44±085	0.00	1.37	1.46	5.36	21.56	0.062
CC	28.53-67.2	47.84±5.49	22.50	82.84	60.32	9.96	19.02	0.27
FLW	31-87	54.48±6.49	0.00	81.38	16.83	3.07	16.56	0.00
STC	28.53-67.2	47.84±.49	22.50	82.82	60.32	9.92	19.02	0.22
BM	0.05-2.71	0.87±0.33	0.11	0.321	53.17	37.39	64.99	0.33
SMOI	5.4-8.8	7.73±0.41	0.05	0.40	0.35	2.87	8.19	0.12
SW	8-22	13.5±1.95	1.47	9.10	7.63	8.98	22.34	0.16
GY	0.15-2.63	0.97±0.33	0.04	0.26	0.27	21.54	52.65	0.17
Well-watered								
Traits	Range	Mean ± S.E Mean	$\sigma^2 g$	$\sigma^2 p$	$\sigma^2 e$	GCV (%)	PCV (%)	H^2
PH	25.00-79.00	50.21±6.09	69.21	143.59	17.17	16.58	23.86	0.48
LL	3.0-9.67	6.06±0.65	0.86	1.72	0.85	15.29	21.66	0.50
LW	3.33-9.9	5.12±0.75	0.00	1.13	1.13	1.60	20.74	0.00
STD	4.47-13.67	8.95±0.83	1.74	3.13	13.18	14.74	19.78	0.55
CC	10.17-212.83	94.14±28.51	0.00	1572.37	1626.37	7.76	42.13	0.00
FLW	41-90	58.26-6.67	0.00	83.8	13	3.96	15.71	0.00
STC	27.27-81.43	47.56±6.82	3.47	96.49	93.03	3.91	20.65	0.04
BM	0.12-1.78	0.65±0.24	0.01	0.13	0.12	20.47	56.47	0.13
SMOI	6-9.1	7.69±0.48	0.00	0.40	0.47	3.13	8.26	0.00
SW	10-26	16.40±1.73	4.06	10.02	5.95	12.29	19.29	0.45
GY	0.2-1.51	0.66±0.24	0.00	0.09	0.12	25.12	46.73	0.00

Key: PH-plant height, SD-stem diameter, LW-leaf width, LL-leaf length, SMOI-Moisture content, STC-stomatal conductance, CC-chlorophyll content, SW-100 seed weight, BM-biomass Yield and GY-grain yield, S.E. Mean= Stand and error of the mean, σ 2 g= Genotypic variance, σ 2 e = Environmental variance, σ 2 p= Phenotypic variance, H2 (%) = Broad see heritability, GCV (%) = Genotypic coefficient of variation, PCV (%) = Phenotypic coefficient of variation, (%). Note: GV is negative, GCV calculated by using absolute GV.

Table 5.2: Range, mean, variance, broad see heritability, genotypic coefficient of variation and critical difference at 5% and 1% of 26 soybean genotypes under WS in the greenhouse

					Well-watered			
Traits	Range	Mean ± S.E Mean	σ^2g	σ^2p	σ^2e	GCV (%)	PCV (%)	H^2
PH	73.38-103.18	95.80±4.65	2.05	45.36	43.30	1.49	7.03	0.05
LL	4.33-9.77	6.68±0.83	0.27	1.65	1.38	7.66	18.93	0.16
LW	6.37-13.4	9.87±1.15	0.79	3.44	2.65	9.01	18.80	0.22
STD	3.33-29.7	6.40±1.99	0.99	8.99	7.99	15.59	46.85	0.11
CC	9.2-180.2	49.17±13.81	46.28	427.62	381.33	13.86	42.05	0.10
FLW	10.8-80	44.67±11.16	0.00	185.91	249.19	17.77	30.46	0.00
STC	9.2-327.7	178.1±43.28	96.45	3842.81	3747.36	5.50	37.71	0.025
BM	0.12-2.73	0.94±0.38	0.14	0.43	0.28	40.76	69.70	0.34
SMOI	2-10.3	7.18±0.91	0.00	1.48	1.67	6.10	16.94	0.00
SW	8.8-28.6	17±0.22	4.18	14.71	19.07	12.15	22.54	0.28
GY	0.02-1.66	0.56±0.25	0.02	0.15	0.11	29.09	67.73	0.18
					Water-stressed			
Traits	Range	Mean ± S.E Mean	σ^2g	σ^2p	σ^2e	GCV (%)	PCV (%)	H^2
PH	56.13-101.13	77.83±6.68	0.00	74.40	89.28	4.95	11.08	0.00
LL	5.53-13.3	8.92±1.23	0.00	2.73	2.92	4.81	18.54	0.00
LW	4.1-24.37	6.98±2.32	0.05	10.82	10.77	3.24	47.14	0.01
STD	3.53-7.8	5.45±70	0.00	0.87	1.00	6.59	17.57	0.00
CC	28.57-55.27	38.89±4.18	0.00	34.42	34.42	0.07	15.08	0.00
FLW	30-85	48.22±23	34.17	203.77	27.00	12.12	29.60	0.17
STC	41.73-401.13	17±9.97	567.21	3875.84	3308.62	13.23	34.59	0.15
BM	0.02-3.1	0.77±0.39	0.2.20	0.52	0.29	60.77	92.84	0.43
SMOI	4.1-8.9	6.64±0.85	1.5	0.07	1.54	3.85	18.47	0.04
SW	0.02-30.1	14.67±3.29	3.72	25.40	21.68	13.14	34.37	0.14
GY	0.02-3.91	0.77±0.38	0.22	0.52	0.29	60.77	92.84	0.42

Key: PH-plant height, SD-stem diameter, LW-leaf width, LL-leaf length, SMOI-Moisture content, STC-stomatal conductance, CC-chlorophyll content, SW-100 seed weight, BM-biomass Yield and GY-grain yield, S.E. Mean= Standard error of the mean, σ^2 g= Genotypic variance, σ^2e = Environmental variance, σ^2p= Phenotypic variance, H^2 (%) = Broad see heritability, GCV (%) = Genotypic coefficient of variation, PCV (%) = Phenotypic coefficient of variation (%).

5.3.2 Phenotypic and genotypic correlations among soybean genotypes per environment

The phenotypic and genotypic correlation coefficients explaining the magnitude of the relationship among morphological and physiological traits studied in the field and greenhouse environment respectively are presented in Table 5.3 and 5.4. Under WS in the field environment, PH showed a significant and positive correlation with STD (r=0.77; P≤0.01), LW (r=0.51; P≤0.01), LL (r=0.73; P≤0.01), GY (r=0.26; P≤0.05) and had significant negative correlation with STC (r=-0.31; P≤0.01). The STD, indicated a significant and positive correlation with LW (r=0.56; P≤0.01), LL (r=0.89; P≤0.01) and GY (r=0.27; P≤0.01). The LW, showed a significant and positive correlation with LL (r=0.55; P≤0.01) and GY (r=0.36; P≤0.01). The LL, showed a positive significant correlation with GY (r=0.30; P≤0.01).

However, under WW for the field environment, PH showed a significant and positive correlation with STD (r=0.66; P≤0.01), LW (r=0.45; P≤0.01), LL (r=0.65; P≤0.01), SW (r=0.43; P≤0.05). The STD, indicated a significant and positive correlation with LW (r=0.46; P≤0.01), BM (r=0.36; P≤0.01), SW (r=0.58; P≤0.01) and had a significant and negative correlation with LL (r=-0.83; P≤0.91). The LW, showed a significant and positive correlation with LL (r=0.45; P≤0.01). The LW, showed a significant and positive with SMOI (r=0.28; P≤0.01, BM (r=0.49; P≤0.01) and SW (r=0.56; P≤0.01). The CC, showed a positive significant correlation with SMOI (r=0.30; P≤0.01). The SMOI, showed a positive significant correlation with BM (r=0.51; P≤0.01).

Under WS in the greenhouse environment, CC showed a significant and positive correlation with PH (r=0.24; P≤0.05), STD (r=0.33; P≤0.01), LW (r=0.36; P≤0.01), LL (r=0.55; P≤0.01). The FLW, indicated a significant and positive correlation with STC (r=0.56; P≤0.01). The BM, showed a significant and positive correlation with STD (r=0.31; P≤0.01). The SW, showed a positive significant correlation with SMOI (r=0.29; P≤0.01) and BM (r=0.21; P≤0.01). The GY, showed a positive significant correlation with SMOI (r=0.47; P≤0.01) and 37 (r=0.21; P≤0.01). However, under WW for the field environment, PH showed a significant and positive correlation with SW (r=0.; P≤0.01) and had significant negative correlation with FLW (r=-0.25; P≤0.01). The STD, indicated a significant and positive correlation with SW (r=0.35; P≤0.01). The LW, showed a significant and positive correlation with CC (r=0.40; P≤0.01). The LW, showed a significant and positive with SMOI (r=0.28; P≤0.01, BM (r=0.49; P≤0.01) and SW (r=0.56; P≤0.01). The LL, showed a significant and positive with SW (r=0.35; P≤0.01). The SMOI, showed a positive significant correlation with GY (r=0.34; P≤0.01). The BMI, showed a positive significant correlation with SMOI (r=0.38; P≤0.01).

Table 5.3: The phenotypic correlation for soybean lines under well-watered and water-stressed regime in the field environment.

		Well-watered										
		PH	STD	LW	LL	STC	FLW	CC	SMOI	BM	SW	GY
	PH	**1.00**	0.65**	0.45**	0.65**	-0.02	-0.01	-0.12	0.05	0.19	0.43**	-0.00
	STD	0.77**	**1.00**	0.46**	-0.91**	-0.02	-0.01	0.02	0.16	0.36**	0.58**	0.12
Water-stressed	LW	0.51**	0.56**	**1.00**	0.45**	-0.01	0.14	0.10	0.14	0.02	0.25	0.20
	LL	0.73**	0.89**	0.55**	**1.00**	0.01	-0.04	0.02	0.28*	0.49**	0.56**	0.14
	STC	-0.31**	-0.14	-0.17	-0.24	**1.00**	-0.20	0.04	0.02	-0.02	0.00	-0.23
	FLW	0.14	0.10	0.19	0.05	-0.05	**1.00**	-0.08	-0.09	0.11	0.00	0.06
	CC	-0.18	-0.12	-0.19	-0.30*	0.20	0.30	**1.00**	0.30**	6e04	0.14	0.18
	SMOI	0.06	-0.01	-015	0.01	0.16	0.03	-0.10	**1.00**	0.51**	0.11	0.53
	BM	-0.0	-0.12	0.02	-0.12	-0.08	0.16	0.16	**0.39**	**1.00**	0.41	0.39
	SW	0.05	-0.00	0.04	0.08	-0.06	0.09	-0.14	0.19	0.21	**1.00**	-0.03
	GY	0.26 *	0.27 *	036 **	0.30 *	-0.16	0.20	-0.09	0.23	-014	-0.01	**1.00**

Note: PH-plant height; SD-stem diameter; LW-leaf width; LL-leaf length; SMOI-Moisture content; STC-stomatal conductance; CC-chlorophyll content; SW-100 seed weight; BM-biomass Yield and GY-grain yield; *Significant at P≤0.05, perfect correlation ± 1; High correlation ± 0.50-1; Moderate correlation ± 0.30-0.49; Low correlation ±0.29; ***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05.

Under WS in the field environment, the genotypic correlation for STD showed a significant and positive correlation with PH (r=0.90; P≤0.01. The STD, indicated a significant and positive correlation with LW (r=0.56; P≤0.01), LL (r=0.89; P≤0.01) and GY (r=0.27; P≤0.01). The LL, showed a significant and positive correlation with PH (r=0.91; P≤0.01) and STD (r=0.36; P≤0.01). The STC, showed a negative and significant correlation with PH (r=-0.89; P≤0.01) and LL (r=-0.53; P≤0.01). The FLW, showed a significant and negative correlation with LW (r=-0.97; P≤0.01). The CC, showed a significant and negative correlation with LW (r=-0.97; P≤0.01). The SMOI, showed a positive significant correlation with PH (r=0.40; P≤0.01) and LW (r=0.40; P≤0.01) and a significant and negative correlation with STC (r=-0.59; P≤0.01). The BM, showed a significant and negative correlation with STC (r=-0.74; P≤0.01).

Table 5.4: The phenotypic correlation for soybean lines under the well-watered and water-stressed regimes in the greenhouse.

		Well-Watered										
		PH	STD	LW	LL	STC	CC	FLW	SMOI	BM	SW	GY
	PH	**1.00**	0.19	0.13	0.12	0.10	-0.12	-0.25**	0.03	-0.14	0.03**	-0.15
	STD	-0.04	**1.00**	0.36**	0.31**	0.05	0.09	0.20	-0.22	-0.03	0.35**	0.03
Water stressed	LW	0.10	0.54	**1.00**	0.83**	-0.20	0.40**	-9e04	-0.16	0.06	0.20	0.04
	LL	0.03	-0.04	0.03	**1.00**	-0.13	0.35**	-0.06	0.18	0.02	0.22	-0.06
	STC	0.16	0.01	-0.04	-0.08	**1.00**	-0.09	0.09	0.02	0.16	0.15	0.03
	CC	0.24*	0.33**	0.36**	-0.04	0.14	**1.00**	0.06	-0.012	0.23	-0.03	-0.03
	FLW	-0.16	0.09	0.13	-0.01	0.31**	0.06	**1.00**	-0.13	0.21	0.02	0 23
	SMOI	0.19	0.05	-0.18	0.20	0.00	-0.15	-0.13	**1.00**	0.21	0.04	0 24*
	BM	0.05	0.31**	-0.12	-0.08	-0.02	0.09	-0.01	0.12	**1.00**	-0.20	0 38**
	SW	0.02	0.05	-0.21	0.14	0.15	0.12	-0.19	0.29**	0.21*	**1.00**	-0.17
	GY	-0.02	0.12	0.08	-0.11	-7e-04	-0.08	0.10	0.47**	0.37**	0.04	**1.00**

Note: PH-plant height; SD-stem diameter; LW-leaf width; LL-leaf length; SMOI-Moisture content; STC-stomatal conductance; CC-chlorophyll content; SW-100 seed weight; BM-biomass Yield and GY-grain yield; *Significant at $P \leq 0.05$, perfect correlation $\pm$ 1; High correlation $\pm$ 0.50-1; Moderate correlation $\pm$ 0.30-0.49; Low correlation ± 0.29; ***Significant at $P \leq 0.001$, ** significant at $P \leq 0.01$, *Significant at $P \leq 0.05$.

The BM, showed a significant and negative correlation with STC (r=-0.74; $P \leq 0.01$). The SW, showed a positive significant correlation with PH (r=0.93; $P \leq 0.01$), LW (r=0.70; $P \leq 0.01$), LL (r=0.45; $P \leq 0.01$) and had a negative correlation with STC (r=-0.75; $P \leq 0.01$). The GY, showed a positive significant correlation with PH (r=0.47; $P \leq 0.01$), LW (r=0.36; $P \leq 0.01$) and had a negative correlation with STC (r=-0.27; $P \leq 0.01$).

However, under WW for the field environment, PH showed a significant and positive correlation with STD (r=0.77; $P \leq 0.01$), LL (r=0.68; $P \leq 0.01$), SW (r=0.81; $P \leq 0.05$). The STD, indicated a significant and positive correlation with LL (r=0.98; $P \leq 0.01$), STC (r=0.91; $P \leq 0.01$), SW (r=0.81; $P \leq 0.01$). The STC, showed a

negative and significant correlation with PH (r=-0.89; P≤0.01) and LL (r=-0.53; P≤0.01). The LW, showed a significant and negative correlation with FLW (r=-1.15; P≤0.01), SMOI (r=-1.15; P≤0.01), CC (r=-0.82; P≤0.01) and GY (r=-0.67; P≤0.01). The LL, showed a significant and positive correlation with SW (r=0.62; P≤0.01). The FLW showed a significant and positive with CC (r=0.07; P≤0.01) and a negative correlation with SMOI (r=-0.1.41; P≤0.01) and GY (r=-0.72; P≤0.01). The CC, showed a negative correlation with SMOI (r=-0.88; P≤0.01) and GY (r=-0.54; P≤0.01). The SMOI, showed a significant negative correlation with GY (r=-0.51; P≤0.01).

Under WS in the greenhouse environment, STD showed a significant negative correlation with PH (r=-0.85; P≤0.01). The LL showed a negative correlation with PH (r=-0.45; P≤0.01). The CC showed a significant and a negative correlation with PH (r=-0.38; P≤0.01) and STD (r=-0.03; P≤0.01) and LL (r=-0.03; P≤0.01). The FLW showed a significant negative correlation with LL (r=-0.61 P≤0.01) and GY (r=-0.38; P≤0.01). The SMOI showed a significant and positive with STC (r=0.38; P≤0.01) and a negative correlation with LL (r=-0.72; P≤0.01). The BM showed a significant positive with LL (r=0.51; P≤0.01). The SW showed a significant positive with LL (r=0.70; P≤0.01). The GY showed a significant positive with STC (r=0.38; P≤0.01) and a negative correlation with LL (r=-0.72; P≤0.01).

Table 5.5: The genotypic correlations among studied morphological and physiological soybean genotype under water-stressed condition (lower diagonal) and water-stressed water regime (upper diagonal) in the field environment.

		WS										
		PH	STD	LW	LL	STC	FLW	CC	SMOI	BM	SW	GY
WW	PH	**1.00**	0.77**	NA	0.68**	0.089	NA	NA	NA	0.13	0.81**	NA
	STD	0 90**	**1.00**	NA	0.98**	0.91**	NA	NA	NA	0.29	0.63**	NA
	LW	NA	NA	**1.00**	NA	NA	-1.15**	-3.82**	-5.46**	NA	NA	-6.67**
	LL	0 91**	1.12**	NA	**1.00**	0.43	NA	NA	NA	-0.07	0.618**	NA
	STC	-0.89**	-0.53**	NA	-0.31	**1.00**	NA	NA	NA	-0.07	0.27	NA
	FLW	NA	NA	-1.97**	NA	NA	**1.00**	4.07**	-1.41**	NA	NA	-0.72**
	CC	NA	NA	-5.62**	NA	NA	6.38	**1.00**	-0.88**	NA	NA	-0.54**
	SMOI	0.40**	0.40**	NA	0.09	-0.59**	NA	NA	**1.00**	NA	NA	-0.16**
	BM	0.05	-0.28	NA	-0.22	-0.74**	NA	NA	0.70	**1.00**	0.23	NA
	SW	0 93**	0.70**	NA	0.45**	-0.75**	NA	NA	0.03	0.80	**1.00**	NA
	GY	0.47**	0.36*	NA	-0.05	-0.27**	NA	NA	0.14	-0.11	0.07	**1.00**

Note: PH-plant height; SD-stem diameter; LW-leaf width; LL-leaf length; SMOI-Moisture content; STC-stomatal conductance; CC-chlorophyll content; SW-100 seed weight; BM-biomass Yield and GY-grain yield; Perfect correlation ± 1; Strong correlation ± 0.80; Moderate correlation ± 0.50; weak correlation

±0.20; No correlation ±0.0; *Significant at P≤0.05***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05.

Under WW in the greenhouse environment, PH showed a significant and positive genotypic correlation with STD (r=0.54; P≤0.01), LW (r=0.22; P≤0.01), LL (r=0.42; P≤0.01), STC (r=0.14; P≤0.01), CC (r=0.62; P≤0.0), FLW (r=0.65; P≤0.01), SW (r=0.40; P≤0.01) and GY (r=0.26; P≤0.50). The STD, indicated a significant and positive correlation with, LW (r=0.89; P≤0.01), LL (r=0.48; P≤0.01), STC (r=0.63; P≤0.01), CC (r=0.09; P≤0.0), FLW (r=0.98; P≤0.01), SW (r=0.24; P≤0.01). The LW, showed a significant and positive correlation with LL (r=0.48; P≤0.01), STC (r=0.61; P≤0.01), CC (r=0.18; P≤0.0), FLW (r=0.98; P≤0.01), SW (r=0.24; P≤0.01) and had a significant negative correlation with GY (r=-0.58; P≤0.50). The LL, showed a positive significant correlation with, STC (r=0.30; P≤0.01), CC (r=0.31; P≤0.0), FLW (r=0.51; P≤0.01) and SW (r=0.14; P≤0.01). The STC showed a significant and positive correlation with CC (r=0.47; P≤0.0), FLW (r=0.98; P≤0.01), SW (r=0.67; P≤0.01) and GY (r=0.34; P≤0.50). The CC, indicated a significant and positive correlation with FLW (r=0.55; P≤0.01), SW (r=0.63; P≤0.01) and GY (r=0.56; P≤0.50). The BM, showed a significant and positive correlation with SW (r=0.57; P≤0.01) and GY (r=0.58; P≤0.01).

Table 5.6: The genotypic correlations among studied morphological and physiological soybean genotype under water-stressed condition (lower diagonal) and water-stressed water regime (upper diagonal) in the greenhouse environment.

		Well-Watered										
		PH	STD	LW	LL	STC	CC	FLW	SMOI	BM	SW	GY
	PH	1.00	0.54**	0 22**	0.86**	0.14**	0.62**	0.65**	NA	NA	0.40**	0.50**
	STD	-0.85**	1.00	0.89**	0.61**	0.63**	0.09**	0.98**	NA	NA	0.24**	0.11
	LW	-0.45**	-0.24	1.00	0.36**	0.61**	0.18**	0.86**	NA	NA	0.94**	-0.58**
	LL	NA	NA	NA	1.00	0.30**	0.31**	0.51**	NA	NA	0.67**	0.34*
Water-stressed	STC	NA	NA	NA	-7.76*	1.00	0.47**	0.98**	NA	NA	0.14**	-0.11
	CC	-0.38**	-0.03**	-0.08**	NA	NA	NA	0.05**	NA	NA	0.63**	0.56**
	FLW	NA	NA	NA	-0.61**	-0.38**	NA	1.00	NA	NA	NA	NA
	SMOI	NA	NA	NA	-0.72**	2.38**	NA	0.02**	1.00	NA	NA	NA
	BM	NA	NA	NA	0.51**	0.13	NA	-0.06	0.14	1.00	0.57**	0.58**
	SW	NA	NA	NA	0.70**	0.06	NA	-0.36**	-0.58**	0.33*	1.00	-0.12
	GY	NA	NA	NA	-0.27**	0.95**	NA	-0.49**	2.20**	0.71**	-0.09	1.00

Note: PH-plant height; SD-stem diameter; LW-leaf width; LL-leaf length; SMOI-Moisture content; STC-stomatal conductance; CC-chlorophyll content; SW-100 seed weight; BM-biomass Yield and GY-grain yield; Perfect correlation ± 1; Strong correlation ± 0.80; Moderate correlation ± 0.50; weak correlation ±0.20; No correlation ±0.0*Significant at P≤0.05***Significant at P≤0.001, ** significant at P≤0.01, *Significant at P≤0.05

5.4 DISCUSSION

5.4.1 Variance components and heritability

The PCV alone provides the measure of the total relative variation in a given trait, whereas GCV provides one with an estimate of the genetic variation in a particular trait (Oppong-Sekyere et al., 2019). In the present study, the PCV was higher than GCV for all traits across environments and water regimes, thus suggesting the importance of the environmental expression for all traits. The PCV values ranged from 7.03% to 92.84 % whereas, the GCV values ranged from 0.07 % to 60.77%. The traits that revealed low PCV and GCV indicate their sensitivity to the respective environment and water regimes (Chandrawat et al., 2017). The PH and BM showed high values of PCV and GCV in each environment and under water respective water regime, which indicates a wide genotypic variation for these traits. Phenotypic expression of these traits would be a good indicator of the genetic potential and thus phenotypic selection would be efficient in the improvement of the traits. The same phenomena as reported by Getnet (2018), where high values of PCV and GCV were observed for grain yield, biomass yield, number of pods per plant, plant height, total nodes per plant, effective nodules per plant and harvest index.

Heritability estimates are important in breeding programs for quantitative traits and thus provide insight into the extent to which a character can be passed to the next-generation (Hill, 2010). In studies evaluating drought tolerance, broad-sense heritability is used by breeders to predict the genetic gain that would be realized when breeding for the particular trait. In this case, traits with high environmental effects are considered to be susceptible to water stress. In the present study, the heritability estimates under WW ranged from 0.0 to 55, while under WS ranged from 0.00 to 43. The increase of traits heritability under a water-stressed regime compared to well-watered may lead to the suitability of respective traits for genetic selection (Sanad et al., 2019).

There was no clear trend for heritability in the different traits in both environments and water regimes due to reduced effects by environmental stress, as reported by (Bogale et al., 2012). However, in the greenhouse environment, PH exhibited high heritability values under both water regimes and recorded the highest heritability across environments, indicating high genetic variation and less influence from the environment. Therefore, PH can be used for selection purposes under both well-watered and water-stressed regimes. Mathew et al. (2019), reported high genetic control and heritability for PH, respectively 65% and 79%. Under WS, GY showed relatively low heritability and these findings are in accordance with the study by(Talebi & Fayyaz, 2012).

5.4.2   Phenotypic and genotypic correlation among traits

In almost all cases of genetic correlations were higher compared to phenotypic correlations across environments and water regimes. This indicates that studied traits responses were highly contributed by genotypic effect. The grain yield had positive and significant genotypic and phenotypic correlations with PH, LL, LW, STD and SMOI. This suggests that the soybean genotypes with high mean values for these traits will result in grain yield increases. Therefore, these traits can be utilized for indirect selection to improve grain yield in soybean under a wide spectrum of water regimes. These findings are in accordance with the report by Malik et al. (2007). In contrast, grain yield showed negative genotypic correlation with FLW under WS, suggesting the possibility of developing short-duration, high yielding soybean lines that can be incorporated various cropping methods (Arshad et al., 2006).

5.5 CONCLUSION

The present study demonstrated the presence of genetic variability among 36 studied soybean genotypes, indicating that selection for drought tolerance would be possible under both environments and water-regimes. There was no clear trend in studied traits for heritability across both environments and water regimes, due to reduced effects by environmental stress. High heritability for PH was obtained, suggesting that it is less likely to be affected by the environment. However, overall most traits had low heritability in both water regimes, therefore, one should be careful in selection for drought-tolerance using the studied traits. In future, further evaluation of more yield-related traits across more field environments and over more seasons is essential to obtain reliable results.

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Chapter 6

An overview of the study

6.1 INTRODUCTION

The Soybean (*Glycine Max* L. Merr.) is one of the most important oilseeds, an affordable source of protein and accounts for 57% of the world's total oilseeds. South Africa is considered to be the second largest soybean producer in Sub-Saharan Africa (SSA) and its socio-economic value is important in the country as it is grown in all nine provinces. As with any other crop, the sustainability of its production is severely affected by drought, with the reduction in yield increasing by up to 40% annually. South Africa is one of the countries facing drought stress challenges. Over 80% of the irrigated arable land suffers from high water stress and 20% is on dry land. To date, 50% of the Agri Northern Cape are still affected by extreme drought stress. As a result, this province is one of the provinces that are still under-producing soybeans due to drought stress.

There are a number of commercially available varieties that are well adapted to a wide variety of climatic conditions in different provinces. However, as the country's drought worsens in the northern and western regions, there is still an urgent need to develop drought tolerant soybean genotypes. Hence, enhancing high-yielding with drought tolerance genotypes would sustain farmers' profits in both large-scale and small-scale farming. The present study was conducted from 2019 and 2020 in early summer growing seasons in the field and greenhouse trials to achieve the following objectives:

i)   To screen 36 soybean genotypes for drought-tolerance using morphological and physiological traits.
ii) Assess drought-tolerance in soybean genotypes using drought-tolerance indices.
iii) Estimate the variance components and heritability of yield and yield components of soybean under well-watered and water-stressed.

6.2 SUMMARY OF THE MAJOR FINDINGS

In the present study, each objective is presented separately as they were mitigated as an independent chapter.

6.2.1 Morph-physiological responses of soybean (*Glycine Ma*x L. Merr.) genotypes under well-watered and water-stressed conditions

The 36 imported from the International Institute of Tropical Agriculture (IITA) were evaluated in the field and greenhouse environment, using a 6×6 alpha-lattice design with two replications. The water stress was imposed to weeks after 50% flowering for each respective genotype and a well-watered regime was used as a control treatment. The genotypes were screened using morphological, physiological traits including; plant height (PH), stem diameter (STD), leaf width (LW), leaf length (LL), seed moisture content (SMOI), stomatal conductance (STC), chlorophyll content (CC), 100 seed weight(SW) and biomass Yield (BM).

➢ The ANOVA showed significant differences among soybean genotypes for PH, LL, LW, STD, BM, SW and GY. The water regime indicated a significant effect for PH, LL, LW, STD, SMOI and GY. The environment effect was significant for all morphological traits PH, LL, LW, STD, FLW, SMOI, SW and GY. The environment by water regime interaction showed a significant effect for PH, FLW, SMOI, SW.

➢ Significant reduction in agronomic traits was observed in G10, G12, G22 and G29 were the best potential genotypes for improving drought-tolerance in soybean lines.

➢ The PH, LL, LW, STD; GY, SMOI, BM and SW could be effectively used for selection in improving yields in soybeans under water-stressed conditions since they were positively correlated with GY.

➢ The approach of PCAs and cluster plot analysis was beneficial in identifying high-yielding drought-tolerant genotypes, discriminating and grouping genotypes based on their water stress responses. The principal components revealed that Dim1 was consistently correlated with PH, LL, LW and STD.

➢ The SW, CC, FLW, STC, SMOI, BM and GY were either associated with Dim2 or Dim3.

➢ The cluster plot revealed that under WS in the field experiment, G1, G10, G12, G20, G22, G25 and G29 had high means values and similar associated for STD, LL, STC, FLW, SMOI, BM, SW and GY based on principal components and cluster plot, represented as cluster II. Whistle, G5, G7, G10, G12, G13, G14, G17, G21, G22, G23, G27, G29 and G31, showed significant high mean values and association for PH, STD, LW, LL, STC, CC, BM, SW and GY in the greenhouse environment.

6.2.2 Assessment of drought-tolerance in soybean (*Glycine max* L. Merr.) genotypes using drought-tolerance indices

The selection for drought-tolerance in 36 soybean lines under well-watered and water-stressed regime was performed using yield based selection indices, including; Drought intensity index (DII), Stress

susceptibility index (SSI), yield index (YI), Stress tolerance index (STI), Mean relative performance(MRP), GMP-Geometric mean of productivity (GMP), Yield stability index (YSI), Mean productivity(MP), TOL-Stress tolerance (TOL), Harmonic mean (HM) and Relative efficiency index (REI).

- ➢ The ANOVA indicated that the main effects due to the environment, genotype and water regime were found to be significant for GY at the level of significance of (P≤0.05), (P≤0.001) and (P≤0.001).

- ➢ The drought-tolerant indices that revealed significant positive correlations for gran yield under well-watered and water-stressed regimes were MRP, GMP, MP, MRP, HM and REI (P<0.001-P<0.05). These indices were comparably effective for the selection and prediction of better grain yielding soybean genotypes under well-watered and water-stressed regimes than SSI, YI, STI, YSI and TOL.

- ➢ Most of the studied soybean genotypes resembled water stress tolerance, including G22, G4, G8, G1, G23, G5, G20, G24, G27, G25, G16, G14, G7, G2, G28, G11, G6, G34, G10, G30, G3, G15, G19, G36, G17, G21, G31, G18, G33, G35, G13, based yield reduction rankings.

- ➢ However, G26, G32, G9, G29, G12 were considered as moderately susceptible to water stress and G7, G8, G14, G22, G34 had low mean performance values and low association with indices.

- ➢ The PCA grouped the grain yield and 11 drought-tolerance into three main components, which were extracted from a large dataset with a total percentage of 74.97% where first DIM1 accounted for 50.07%; DIM2 for 16.56% and DIM3 for 8.34%.

- ➢ Based on the PC, biplot and cluster plot G1, G19, G13, G33, G31 revealed high yield means performance, tolerance and association with SSI, STI, MRP, GMP, MP, TOL, HM and REI, whereas G7, G8, G14, G22, G34 had low mean yield performance and low association with indices.

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6.2.3 The estimation of variance components and heritability of yield and secondary traits in (*Glycine max* L. Merr.) genotypes under well-watered and water-stressed

The 36 imported from the International Institute of Tropical Agriculture (IITA) were evaluated in the field and greenhouse environment, using a 6×6 alpha-lattice design with two replications. The water stress was imposed to weeks after 50% flowering for each respective genotype and a well-watered regime was used as a control treatment. The genotypes were screened using using morphological, physiological traits including; PH, STD, LW, SMOI, STC, CC, SW, BM and GY to estimate variance components and broad-sense heritability.

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- ➢ The present study demonstrated the presence of genetic variability among 36 studied soybean genotypes. Hence one can explore the presence of variability in these soybean lines for crop improvement programs through indirect selection.

- ➢ According to the results, a higher genotypic coefficient was in accordance with broad-sense heritability was observed for grain yield under both water regimes. The PCV was higher than GCV for all traits across environment s and water regimes, thus suggesting the importance of the environmental expression for all traits. The PCV values ranged from 7.03 to 92.84 whereas, the GCV values ranged from 0.07 to 60.77. The GY and BM, showed high values of PCV and GCV in each environment and under water-stressed respective well-watered water regime, which indicates a wide verity genotypic variation of these traits. Phenotypic expression of these traits would be good for the identification of genetic potential and are efficient to be further used for breeding crop improvement

- ➢ There was no clear trend in studied traits for heritability across both environments and water regimes, due to reduced effects by environmental stress. The Heritability estimates under WW ranged from -0.34 to 55, while under WS ranged from -0.29 to 43. However, overall most traits had low heritability in both water regimes. Therefore, one should be careful in selection for drought-tolerance using the studied traits.

6.3 THE IMPLICATION OF THE RESEARCH FINDINGS FOR SOYBEANS DROUGHT-TOLERANCE IN SOYBEAN IN PLANT BREEDING

Breeding for drought-tolerance is the major objective in plant breeding programmers for the improvement of grain yield and sustain production under environmental stress. Therefore, screening of drought-tolerance using agronomic traits in a well-watered regime in multiple environments leads allows the selection of superior genotypes with improved yields under a wide spectrum of environments.

- ➢ The phenotypic differences are important as they simultaneously reflect the influence of genes and environmental factors. Wide genotypic variation in genotypes allows for soybean improvement by selecting genotypes from different clusters to preserve genetic diversity critical for breeding.

- ➢ The present study illustrated that the screening of soybean genotypes for drought-tolerance using morphological and physiological traits is effective.

- ➢ The existence of genotypic and phenotypic differences among traits indicates substantial variation among soybean genotypes. This allows the selection of best-performing genotypes based on studied

traits under well-watered and water-stressed regimes. In addition, these findings suggested promising selection for genetic variability among genotypes which can be further evaluated for drought-tolerance.

➢ The correlation coefficients were used to identify the best criterion for selecting drought-tolerance genotypes. The observed association of grain yield was determined between SMOI and BM under a water-stressed and well-watered regime. This suggests that the selection of high-yielding soybean genotypes will incorporate with genotypes having high SMOI and BM and under water stress, low yield can induce the SMOI and BM. Therefore, selecting for SMOI and BM can improve soybean yields under both water regimes.

➢ Obtaining high yielding genotypes under both water regimes indicted the approach used in the study was effective since it was able to give positive results.

➢ The drought-tolerance indices having a significant correlation with yield under both under well-watered and water-stressed is suitable in selection breeding programs for drought-tolerance. The observed correlation between grain yield under both water regimes for MRP, GMP, MP, MRP, HM and REI indicates that the six indices were comparably effective for the selection and prediction of better grain yielding genotypes under well-watered and water-stressed.

➢ The estimates of heritability are important in the breeding programs for quantitative traits, thus providing knowledge on the extent to which character can be passed to the next generation. In research, for the evaluation of drought-tolerance, broad-sense heritability is used by breeders to predict the magnitudes of traits that may be contributed by the environmental effect or genetic effect. In this case, traits with high environmental effects are considered to be susceptible to water stress.

➢ The high genotypic coefficient was in accordance with broad-sense heritability was observed for grain yield under both water regimes. This allows direct selection using the phenotypic expression for the enhancement of traits. There was no clear trend in trait for heritability across both environments and water regimes, due to reduced effects by environmental stress. High heritability for PH was obtained, suggesting that is less likely to be affected by the environment.

6.4 RECOMMENDATIONS

Based on the findings of the study, the following suggestions were recommended for future purposes

➢ For clear trends and effective selection for drought tolerate in soybean lines, further evaluation of more yield-related traits across more field environments and over more seasons is essential to obtain reliable results.

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- The screening of drought-tolerance using morpho-physiological traits in soybean lines is highly recommended. Hence, the studied agronomic traits were effective in discriminating genotypes under both water regimes.
- The LL, STD, FLW, BM, GY and SMOI could be effectively used for selection in improving yields in soybeans under water-stressed conditions.
- The G10, G12, G22 and G29 were the best potential genotypes for improving drought-tolerance in soybean lines.
- The approach of using PCAs, Biplot and cluster plot analysis was very helpful in identifying high-yielding drought-tolerant genotypes, discriminating and grouping genotypes based on their water stress responses. Therefore, wise to use them for data analysis in plant breeding research projects.
- For future studies for clear and effective selection for drought tolerate in soybean lines, further evaluation of more yield related traits across more field environment   and over more seasons is essential to obtain reliable results.

6.5 APPENDIXES

Appendix 1: The mean values of yield and yield-related traits studied in 36 soybean genotypes under water-stressed and well-watered regimes in the greenhouse environment.

Genotypes	PH			STD			LW			LL		
	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	52.17	52.17	0.01	6.34	10.00	-57.85	9.67	6.00	37.95	4.83	6.50	-34.58
2	51.50	67.33	-30.74	6.50	8.67	-33.31	8.33	4.67	44.00	5.33	6.34	-18.86
3	61.50	57.17	7.05	7.34	8.00	-9.07	7.33	4.92	32.95	6.33	5.84	7.82
4	49.00	45.67	6.81	6.83	7.67	-12.23	9.17	4.50	50.93	5.33	5.34	-0.09
5	32.67	44.17	-35.19	6.00	9.67	-61.08	7.84	5.17	34.08	4.83	6.83	-41.41
6	53.33	45.84	14.05	6.17	7.67	-24.31	10.67	5.17	51.57	5.00	5.50	-10.00
7	43.00	48.67	-13.17	5.50	7.67	-39.45	7.33	4.00	45.43	4.34	5.17	-19.15
8	49.00	47.00	4.08	5.67	8.50	-50.04	11.67	4.50	61.42	4.50	5.50	-22.22
9	47.83	50.33	-5.23	6.17	8.50	-37.88	9.83	6.17	37.28	5.33	6.17	-15.67
10	58.83	54.84	6.79	6.50	9.33	-43.54	9.34	5.67	39.26	6.17	6.84	-10.78
11	55.17	41.00	25.68	6.84	7.84	-14.63	8.00	4.67	41.63	6.50	5.17	20.54
12	39.83	69.67	-74.91	5.17	10.67	-106.58	8.67	6.34	26.93	5.34	7.50	-40.58
13	57.00	42.17	26.02	6.50	9.84	-51.31	9.17	4.50	50.90	6.00	7.17	-19.42
14	44.17	47.50	-7.55	6.34	9.84	-55.25	10.00	4.83	51.70	5.00	6.50	-30.00
15	67.00	59.84	10.69	7.67	11.00	-43.42	9.83	6.00	38.96	6.67	8.00	-19.94
16	51.00	58.67	-15.04	7.50	12.50	-66.67	9.00	4.50	50.00	5.67	9.00	-58.87
17	52.67	49.83	5.39	6.84	8.67	-26.85	9.33	5.67	39.23	6.00	6.00	0.00
18	48.17	49.84	-3.47	5.00	7.67	-53.40	9.84	5.00	49.16	5.67	4.67	17.64

Genotypes	PH			STD			LW			LL		
	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
19	59.34	57.34	3.37	7.50	11.17	-48.93	8.34	6.00	28.01	6.17	7.50	-21.65
20	65.00	58.50	10.00	7.00	10.17	-45.29	9.00	5.50	38.89	6.00	6.17	-2.83
21	46.67	47.84	-2.51	6.00	7.84	-30.58	11.50	5.17	55.09	5.34	5.34	0.00
22	37.33	50.33	-34.82	5.17	10.17	-96.81	9.84	6.34	35.59	5.67	7.00	-23.57
23	48.33	59.50	-23.11	6.00	8.33	-38.83	10.00	5.00	50.00	4.84	5.67	-17.27
24	39.50	33.50	15.19	6.17	6.50	-5.43	8.84	4.17	52.80	4.67	4.17	10.72
25	46.84	54.00	-15.30	6.67	9.00	-35.03	9.17	6.00	34.53	5.00	6.17	-23.40
26	43.67	58.33	-33.57	6.17	9.34	-51.42	10.00	5.33	46.70	6.84	6.50	4.90
27	31.67	28.67	9.47	5.67	6.83	-20.56	10.17	4.50	55.73	4.67	4.17	10.72
28	46.50	36.50	21.51	6.50	7.67	-18.00	8.50	4.17	51.00	6.67	4.83	27.53
29	63.50	73.67	-16.02	7.67	11.33	-47.72	9.17	6.67	27.28	7.34	7.00	4.57
30	43.50	33.34	23.37	5.67	5.84	-3.00	9.17	5.00	45.44	5.17	4.50	12.88
31	53.83	56.83	-5.57	7.84	12.34	-57.43	7.34	6.00	18.20	6.00	8.17	-36.08
32	45.00	53.50	-18.89	5.84	9.33	-59.90	9.50	4.67	50.84	4.50	5.17	-14.78
33	37.17	31.50	15.24	5.34	7.17	-34.30	9.00	3.67	59.22	4.34	5.00	-15.34
34	50.84	54.50	-7.21	5.50	8.67	-57.55	8.50	4.84	43.12	5.00	5.84	-16.70
35	38.67	46.84	-21.13	5.67	8.17	-44.00	9.84	4.50	54.25	4.17	5.50	-31.89
36	33.00	41.50	-25.76	4.67	8.50	-82.21	8.84	4.67	47.20	4.67	5.34	-14.36

Genotype	STC			CC			FLW			SMOI		
	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	38.24	47.44	-24.06	57.50	69.00	-20.00	130.17	74.40	42.84	8.30	6.95	16.27
2	43.12	43.83	-1.66	57.00	53.50	6.14	101.70	89.05	12.44	7.80	7.55	3.21
3	48.15	47.47	1.42	58.00	62.00	-6.90	91.63	73.43	19.86	6.55	7.70	-17.56
4	44.84	56.07	-25.05	44.00	56.00	-27.27	85.52	63.49	25.76	8.65	7.55	12.72
5	53.45	48.69	8.91	50.00	54.50	-9.00	78.44	29.98	61.78	7.90	7.85	0.63
6	52.04	48.17	7.44	56.00	73.50	-31.25	135.90	105.12	22.65	8.50	7.40	12.94
7	46.05	48.53	-5.39	57.00	60.50	-6.14	128.15	69.49	45.78	7.85	7.50	4.46
8	45.30	44.47	1.84	58.50	72.50	-23.93	81.97	81.79	0.23	7.05	7.60	-7.80
9	53.65	47.62	11.25	52.50	56.50	-7.62	133.45	98.97	25.84	7.70	7.90	-2.60
10	46.94	32.82	30.08	55.50	68.50	-23.42	85.49	78.27	8.44	8.10	7.65	5.56
11	34.57	33.60	2.81	46.00	54.00	-17.39	69.38	136.58	-96.86	7.85	7.75	1.27
12	47.25	55.77	-18.02	61.00	58.50	4.10	110.42	80.92	26.72	7.55	7.35	2.65
13	45.12	51.74	-14.67	61.00	62.50	-2.46	104.80	111.03	-5.94	7.85	7.70	1.91
14	47.63	55.79	-17.12	53.00	56.50	-6.60	123.07	103.39	15.99	7.65	7.80	-1.96
15	47.80	57.03	-19.31	54.50	59.00	-8.26	154.15	133.77	13.22	7.80	8.90	-14.10
16	48.90	44.42	9.17	52.50	58.00	-10.48	89.64	94.58	-5.52	7.65	7.75	-1.31
17	47.42	38.02	19.81	74.00	59.00	20.27	148.29	93.19	37.16	7.70	7.85	-1.95
18	48.55	52.22	-7.55	55.00	49.00	10.91	125.32	78.42	37.43	8.10	7.20	11.11
19	49.22	47.23	4.03	58.00	57.00	1.72	131.50	115.85	11.90	7.60	8.60	-13.16
20	31.78	50.80	-59.85	56.50	56.50	0.00	125.25	115.82	7.53	7.40	7.15	3.38
21	51.00	57.14	-12.03	57.00	53.50	6.14	111.34	67.54	39.34	7.80	7.90	-1.28
22	54.67	51.29	6.18	54.00	53.00	1.85	92.13	99.12	-7.58	7.55	7.95	-5.30

Genotype	STC			CC			FLW			SMOI		
	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
23	48.72	36.75	24.56	44.00	52.50	-19.32	68.74	64.76	5.78	7.55	7.45	1.32
24	58.10	39.74	31.61	43.50	57.50	-32.18	101.87	79.28	22.17	6.80	7.40	-8.82
25	51.20	54.72	-6.87	53.00	71.00	-33.96	102.05	128.80	-26.21	6.85	7.30	-6.57
26	42.44	44.25	-4.28	49.50	50.00	-1.01	92.34	71.29	22.80	8.35	7.55	9.58
27	56.25	40.87	27.35	54.50	56.00	-2.75	113.20	184.52	-63.00	7.15	7.55	-5.59
28	55.04	44.59	18.99	59.00	56.00	5.08	90.85	81.02	10.83	8.25	7.00	15.15
29	30.29	43.24	-42.76	59.50	64.00	-7.56	61.72	88.22	-42.94	8.05	6.90	14.29
30	61.52	52.64	14.44	48.50	58.50	-20.62	114.29	121.02	-5.89	8.00	8.30	-3.75
31	55.97	45.94	17.93	47.00	49.00	-4.26	67.95	96.77	-42.41	7.95	7.90	0.63
32	40.38	62.08	-53.74	51.00	54.50	-6.86	112.90	81.93	27.44	8.05	7.60	5.59
33	58.44	56.68	3.00	60.50	57.00	5.79	215.64	125.72	41.70	6.95	8.35	-20.14
34	54.57	38.37	29.69	43.50	55.50	-27.59	110.43	86.67	21.52	8.00	7.60	5.00
35	39.78	42.08	-5.78	60.50	61.50	-1.65	209.47	109.48	47.73	7.60	7.75	-1.97
36	43.89	50.45	-14.96	59.00	51.50	12.71	153.32	75.53	50.74	7.85	8.35	-6.37

Genotype	BM			SW			GY		
	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	2.43	0.71	70.78	19.00	15.00	21.05	1.20	0.59	51.05
2	0.58	0.56	3.45	11.00	18.00	-63.64	1.26	0.62	51.00
3	0.55	0.62	-11.82	13.00	17.00	-30.77	0.95	1.05	-10.58
4	1.19	0.49	58.65	11.00	14.00	-27.27	0.51	0.64	-26.73
5	0.89	0.64	27.68	15.00	16.00	-6.67	0.55	0.56	-2.75
6	1.53	0.64	58.17	14.00	14.00	0.00	0.42	0.61	-45.78
7	0.26	0.84	-221.15	16.00	15.00	6.25	0.70	0.74	-5.76
8	0.83	1.31	-58.18	15.00	18.00	-20.00	0.40	0.67	-69.62
9	0.86	0.60	30.81	14.00	15.00	-7.14	1.43	0.95	33.92
10	0.97	0.90	6.74	14.00	17.00	-21.43	1.26	0.56	55.78
11	0.76	0.60	20.53	16.00	14.00	12.50	1.18	0.76	36.02
12	1.05	0.43	59.52	14.00	15.00	-7.14	1.26	0.37	70.92
13	1.04	0.73	29.47	13.00	15.00	-15.38	1.39	0.71	49.28
14	0.27	0.63	-133.33	9.00	18.00	-100.00	0.46	0.68	-47.83
15	0.81	1.27	-56.17	15.00	18.00	-20.00	0.65	0.74	-13.95
16	0.47	1.22	-158.51	13.00	21.00	-61.54	1.05	0.68	34.93
17	1.96	0.53	72.89	14.00	13.00	7.14	1.00	0.86	14.00
18	0.78	0.41	48.08	12.00	15.00	-25.00	1.15	0.45	60.87
19	0.20	1.37	-600.00	12.00	20.00	-66.67	1.32	0.99	25.00
20	0.47	0.50	-7.53	14.00	20.00	-42.86	1.06	0.28	73.46
21	0.55	0.86	-56.88	12.00	17.00	-41.67	1.11	0.87	22.07
22	0.83	0.22	73.49	19.00	16.00	15.79	0.23	0.40	-71.74
23	0.60	0.50	17.50	14.00	18.00	-28.57	0.50	0.47	6.06
24	0.57	0.49	14.91	10.00	15.00	-50.00	0.57	0.89	-56.64
25	0.63	0.35	45.24	13.00	24.00	-84.62	0.89	0.40	55.37
26	0.66	0.62	5.34	12.00	16.00	-33.33	1.48	0.59	60.47
27	0.82	0.36	55.83	13.00	13.00	0.00	0.50	0.88	-76.00
28	0.77	0.36	53.25	14.00	14.00	0.00	0.81	0.55	32.72
29	1.21	0.51	58.26	16.00	19.68	-22.97	1.80	0.68	62.12
30	0.88	0.85	3.41	12.00	16.00	-33.33	1.27	0.58	54.15
31	0.58	0.72	-24.35	16.00	22.00	-37.50	1.48	0.86	41.89
32	1.22	0.49	59.67	16.00	14.00	12.50	1.24	0.57	53.85
33	0.38	0.45	-17.11	12.00	14.00	-16.67	1.03	0.81	20.98
34	1.29	0.85	33.85	13.00	18.00	-38.46	0.60	0.68	-14.29
35	1.09	0.53	51.83	10.00	13.00	-30.00	0.83	0.46	45.18
36	1.48	0.45	69.49	10.00	14.00	-40.00	1.42	0.59	58.80

Appendix 2: The mean values of yield and yield-related traits studied in 36 soybean genotypes under water-stressed and well-watered regimes in the greenhouse environment.

Genotypes	PH			STD			LW			LL		
	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	91.88	73.63	19.86	6.43	6.13	4.67	7.23	5.08	29.76	10.98	8.38	23.69
2	92.63	69.38	25.10	6.40	5.88	8.20	7.68	6.88	10.42	10.53	9.13	13.30
3	91.50	78.20	14.54	5.03	5.38	-6.97	6.93	5.68	18.05	10.30	7.55	26.70
4	92.88	69.43	25.25	5.63	5.43	3.56	6.98	5.80	16.85	9.30	8.05	13.44
5	115.75	66.93	42.18	5.58	4.95	11.21	7.53	5.60	25.58	9.68	8.95	7.49
6	97.25	79.63	18.12	5.33	4.35	18.31	5.83	5.63	3.43	8.83	7.65	13.31
7	91.33	75.83	16.98	5.43	5.55	-2.15	6.05	6.45	-6.61	8.33	9.55	-14.60
8	103.75	86.05	17.06	5.73	5.13	10.48	7.35	7.25	1.36	12.00	11.38	5.21
9	105.25	82.35	21.76	4.53	4.95	-9.39	5.75	5.58	3.04	8.25	7.63	7.58
10	101.15	74.53	26.32	6.30	5.45	13.49	6.85	6.68	2.55	10.63	10.13	4.71
11	105.13	82.38	21.64	7.73	6.33	18.12	8.20	8.20	0.00	10.58	12.93	-22.22
12	100.00	77.18	22.83	6.53	6.35	2.68	7.25	6.15	15.17	10.05	8.88	11.69
13	98.50	76.93	21.90	6.70	5.68	15.30	6.90	5.13	25.72	9.35	9.00	3.74
14	93.75	70.60	24.69	5.88	5.75	2.13	6.98	7.95	-13.98	9.80	10.55	-7.65
15	100.25	74.28	25.91	5.63	5.93	-5.33	6.35	6.78	-6.69	9.00	9.55	-6.11
16	98.48	79.00	19.78	4.98	5.58	-12.06	7.78	7.05	9.32	9.55	10.23	-7.07
17	95.25	79.05	17.01	6.70	5.23	22.01	7.43	6.18	16.84	8.48	7.55	10.91
18	95.63	90.43	5.44	5.93	4.38	26.16	6.73	4.38	34.94	10.63	6.93	34.82
19	93.38	70.28	24.74	6.50	6.43	1.15	6.95	6.15	11.51	8.83	9.43	-6.80
20	111.13	83.45	24.90	6.55	6.73	-2.67	7.93	6.45	18.61	12.38	9.75	21.21

21	97.63	72.28	25.97	7.00	5.13	26.79	7.05	6.45	8.51	10.50	8.60	18.10
22	96.00	69.00	28.13	6.53	5.43	16.86	7.40	6.65	10.14	10.40	9.88	5.05
23	90.70	66.60	26.57	6.45	5.70	11.63	8.18	7.40	9.48	12.90	9.23	28.49
24	92.00	74.18	19.38	5.75	3.90	32.17	6.13	5.60	8.57	8.50	7.30	14.12
25	99.58	73.30	26.39	6.80	5.28	22.43	7.48	7.38	1.34	10.38	8.60	17.11
26	100.63	85.08	15.45	6.28	5.05	19.52	6.63	6.25	5.66	8.13	9.23	-13.54
27	108.00	70.85	34.40	5.10	4.78	6.37	4.40	8.20	-86.36	6.25	10.08	-61.20
27	108.00	70.85	34.40	5.10	4.78	6.37	4.40	8.20	-86.36	6.25	10.08	-61.20
28	103.75	94.63	8.80	6.93	5.10	26.35	9.23	7.78	15.72	12.93	8.25	36.17
29	108.13	68.00	37.11	7.35	5.45	25.85	7.80	7.10	8.97	11.30	11.00	2.65
30	101.63	83.85	17.49	5.05	4.25	15.84	5.85	5.63	3.85	7.03	8.25	-17.44
31	101.25	77.10	23.85	5.98	5.65	5.44	5.98	6.48	-8.37	10.03	9.53	4.99
32	90.38	68.55	24.15	6.35	5.40	14.96	5.55	6.23	-12.16	8.85	8.10	8.47
34	90.00	70.63	21.53	4.68	5.38	-14.97	4.38	5.88	-34.29	7.63	8.00	-4.92
35	93.75	79.75	14.93	4.15	5.15	-24.10	4.58	6.10	-33.33	6.50	7.90	-21.54
36	89.00	87.10	2.13	6.05	4.90	19.01	7.48	4.65	37.79	10.65	6.58	38.26

Appendixes continued

Genotypes	STC			CC			FLW			SMOI		
	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	241.33	199.73	17.24	41.15	40.50	1.58	58.00	31.50	45.69	8.30	7.20	13.25
2	211.85	172.50	18.57	49.43	40.08	18.92	44.00	44.00	0.00	8.20	4.95	39.63
3	263.33	146.33	44.43	71.58	31.75	55.64	45.50	77.00	-69.23	6.90	8.10	-17.39
4	205.28	172.75	15.84	37.33	35.35	5.29	28.50	33.00	-15.79	7.30	7.30	0.00
5	136.23	119.88	12.00	42.23	33.68	20.25	55.00	37.00	32.73	7.60	5.65	25.66
6	131.75	114.10	13.40	38.93	39.13	-0.51	41.00	41.50	-1.22	8.80	5.85	33.52
7	201.03	172.73	14.08	51.27	31.88	37.83	40.33	60.00	-48.76	6.80	5.80	14.71
8	99.28	226.88	-128.53	43.98	41.68	5.23	42.50	44.00	-3.53	7.50	7.80	-4.00
9	242.75	151.58	37.56	38.85	44.08	-13.45	49.50	42.50	14.14	6.25	7.15	-14.40
10	245.55	168.48	31.39	44.83	33.35	25.60	58.00	61.50	-6.03	4.60	5.55	-20.65
11	163.05	214.23	-31.39	55.65	38.50	30.82	37.00	64.50	-74.32	7.05	7.15	-1.42
12	185.48	146.53	21.00	69.43	40.73	41.34	41.00	47.00	-14.63	7.35	7.05	4.08
13	201.60	119.88	40.54	54.28	34.68	36.11	47.00	42.50	9.57	7.20	5.05	29.86
14	178.20	163.75	8.11	38.85	42.98	-10.62	37.50	42.50	-13.33	7.55	5.35	29.14
15	210.53	330.98	-57.21	57.53	47.10	18.12	36.00	63.00	-75.00	7.50	6.30	16.00
16	192.55	291.00	-51.13	42.73	43.75	-2.40	38.50	41.00	-6.49	7.30	6.90	5.48
17	272.80	142.20	47.87	41.50	36.80	11.33	38.50	52.50	-36.36	5.95	5.30	10.92
18	150.00	140.73	6.18	38.48	33.43	13.13	37.50	47.50	-26.67	6.35	6.80	-7.09
19	164.73	90.60	45.00	56.20	33.33	40.70	44.00	48.00	-9.09	7.80	6.20	20.51
20	212.88	237.85	-11.73	45.35	41.88	7.66	42.50	60.50	-42.35	7.00	6.60	5.71

21	110.60	199.60	-80.47	39.98	35.08	12.26	47.00	52.50	-11.70	6.60	6.30	4.55
22	146.15	194.18	-32.86	62.70	37.78	39.75	37.00	59.50	-60.81	6.30	6.00	4.76
23	175.98	231.03	-31.28	49.95	44.25	11.41	58.00	57.50	0.86	7.25	6.60	8.97
24	168.70	223.68	-32.59	30.68	33.45	-9.05	38.50	42.50	-10.39	8.00	7.45	6.88
25	147.55	194.68	-31.94	68.18	39.40	42.21	45.50	42.50	6.59	7.65	7.40	3.27
26	282.28	250.15	11.38	44.28	43.00	2.88	32.00	41.00	-28.13	7.30	7.10	2.74
27	246.25	156.30	36.53	44.15	41.80	5.32	47.00	35.50	24.47	6.10	6.45	-5.74
28	246.25	156.30	36.53	44.15	41.80	5.32	47.00	35.50	24.47	6.10	6.45	-5.74
29	113.48	170.48	-50.23	45.93	35.65	22.37	55.00	51.00	7.27	6.85	7.60	-10.95
30	145.63	157.80	-8.36	41.43	45.83	-10.62	59.50	70.00	-17.65	6.20	7.05	-13.71
31	143.58	244.18	-70.07	30.98	36.30	-17.19	56.50	35.50	37.17	7.25	6.50	10.34
32	176.95	116.00	34.44	40.75	43.00	-5.52	45.50	47.50	-4.40	6.75	5.40	20.00
33	266.23	100.05	62.42	39.00	40.00	-2.56	47.00	35.50	24.47	7.80	7.30	6.41
34	174.25	187.65	-7.69	39.18	37.88	3.32	58.00	45.50	21.55	8.50	8.25	2.94
35	168.95	88.18	47.81	38.03	38.85	-2.17	42.50	37.00	12.94	7.30	7.25	0.68
36	95.33	194.15	-103.67	68.18	37.38	45.18	44.00	47.00	-6.82	7.10	6.45	9.15

Genotype	BM			SW			GY		
	WW	WS	%Rd	WW	WS	%Rd	WW	WS	%Rd
1	2.34	3.38	-44.44	15.60	19.85	-27.24	1.19	0.76	36.13
2	1.53	0.35	77.05	17.75	7.45	58.03	0.63	0.08	87.30
3	1.58	0.81	48.57	18.85	5.97	68.36	0.30	0.50	-66.67
4	0.49	0.31	37.76	15.60	16.75	-7.37	0.10	0.25	-163.16
5	0.81	0.52	35.40	12.10	15.15	-25.21	0.37	0.30	17.81
6	1.27	1.18	6.72	18.40	12.85	30.16	0.58	0.30	48.28
7	1.76	1.45	17.77	14.03	13.00	7.36	0.38	0.14	62.83
8	0.97	0.67	31.44	15.75	15.70	0.32	1.10	0.52	52.73
9	0.64	0.28	55.91	13.90	14.55	-4.68	0.49	0.41	15.46
10	0.79	0.68	14.01	12.60	9.55	24.21	0.92	0.73	20.65
11	1.83	2.35	-28.49	16.85	14.60	13.35	0.87	0.83	4.60
12	0.36	0.26	27.78	17.80	17.35	2.53	0.21	0.42	-97.62
13	0.89	1.15	-29.94	17.10	11.95	30.12	0.71	0.36	49.65
14	1.36	0.66	51.29	18.30	11.75	35.79	0.68	0.12	82.22
15	1.49	1.13	23.91	17.45	17.60	-0.86	0.41	0.27	34.15
16	0.32	0.88	-173.44	19.05	16.80	11.81	0.12	0.70	-483.33
17	1.06	0.69	34.60	16.60	26.70	-60.84	0.36	0.14	62.50
18	0.46	0.36	22.83	18.65	17.30	7.24	0.53	0.34	36.79
19	1.54	0.70	54.40	17.30	14.00	19.08	0.65	0.35	46.92
20	0.70	0.63	10.71	16.65	13.90	16.52	0.46	0.32	29.67
21	1.11	0.32	71.04	15.10	11.10	26.49	0.45	0.42	5.62
22	0.40	0.18	55.70	14.50	8.21	43.38	0.10	0.34	-235.00
23	1.04	0.57	45.41	16.70	17.45	-4.49	0.60	0.34	43.33
24	0.23	1.05	-356.52	14.65	20.70	-41.30	0.92	0.33	64.48
25	0.18	0.59	-225.00	22.40	16.95	24.33	0.40	0.54	-33.75
26	0.29	1.14	-291.38	23.50	16.50	29.79	0.42	0.46	-9.52
27	1.11	0.77	30.63	16.70	17.05	-2.10	0.15	0.29	-93.33
28	1.11	0.77	30.63	16.70	17.05	-2.10	0.15	0.29	-93.33
29	1.03	0.69	33.17	20.05	14.65	26.93	0.41	0.44	-8.64
30	0.51	0.58	-13.73	26.50	13.75	48.11	0.45	0.34	25.56
31	0.29	0.24	15.79	17.20	13.10	23.84	0.51	0.15	70.59
32	0.47	0.30	37.23	19.20	14.10	26.56	0.88	0.12	86.93

33		0.51	47.67	15.75	17.30	-9.84	0.97	0.62	36.08
34	1.59	0.79	50.63	12.35	14.25	-15.38	0.93	1.00	-8.11
35	0.55	0.85	-55.05	12.65	13.50	-6.72	0.81	0.48	40.99
36	1.00	0.44	56.50	16.65	13.75	17.42	0.96	0.39	59.90