

**A COMPARATIVE STUDY ON WATER DEFICIT STRESS TOLERANCE BETWEEN THE  
COWPEA (*VIGNA UNGUICULATA* [L.] WALP) AND SOYBEAN (*GLYCINE MAX* [L.]  
MERRILL)**

BY

**MULALO LUTOMBO**

DISSERTATION

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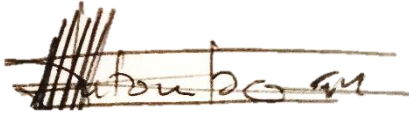
**UNIVERSITY OF LIMPOPO**

**SUPERVISOR: Dr PW Mokwala**

**2022**

## DECLARATION

I declare that **A comparative study on water deficit stress tolerance between the cowpea (*Vigna unguiculata* [L.] Walp) and soybean (*Glycine max* [L.] Merrill)** hereby submitted to the University of Limpopo, for the degree of Master of Science in Botany has not previously been submitted by me for a degree at this or any other university; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

A handwritten signature in brown ink, appearing to read 'Lutombo M', is written over a horizontal line. The signature is somewhat stylized and includes several vertical strokes on the left side.

29/06/2022

Lutombo M

## DEDICATION

I dedicate this dissertation to my son, Romuvhona and my fiancée, Enosencia.

## ACKNOWLEDGEMENTS

I wish to extend my special gratitude and word of appreciation to the following:

- First and far above all, I thank the Great and Almighty God for He has given me the opportunity to participate in this project and most importantly for giving me life to see it to completion.
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## ABSTRACT

The world has lately suffered prominent natural disasters, most with potential to extirpate all form of life. Drought comes in second to hurricanes in regards to causing severe economic impacts worldwide. Drought is a prolonged period of not enough rainfall or below average precipitation that results in shortage of water in the soil. Water shortage crisis and its effects can be easily observed in the agricultural sector. Each year, the agricultural sector and the food supply chains suffer a loss worth R100s trillions of the total average yield of agro-economic crop due to drought stress. Soybean production in sub-Saharan Africa is still low as is anywhere in Africa. The production could be much higher but local farmers suffer harsh environmental conditions such as pest, diseases and drought. Conventionally cowpea is considered the trunion of small farmers in Central and West African regions. This is because it is well adapted to areas with lower rainfall. The aim of this study was to investigate the similarities and dissimilarities between soybean and cowpea's response and tolerance to water deficit stress. Three genotypes were used for each plant, Red, Blonde and white (cowpea) and TGx 1937-f, PAN 1664R and LS 678 (soybean). Polyethylene glycerol 8000 was used to induce drought stress at five different levels of osmotic potential (of 0.00, -0.02, -0.27, -1.27, and -1.80 MPa) for evaluation of water deficit tolerance during germination. To assess both vegetative and reproductive growth plants were subjected to three drought stress levels; control- watered daily (no water stress), moderate (watered once in 5 days) and severe (watered once in seven days). Morphological parameters (number leaves, length of stem, root length, mass of the nodules, number of flowers, number of pods, seeds per pod, mean seed number per pod and mass of 100 seeds) were directly counted or measured on the plants. Samples were collected for physiological analyses including determination of proline content, determination of leaf relative water content, determination of malondialdehyde content and determination of chlorophyll content. Lastly, an experiment to assess the ability of cowpea and soybean to recover from. Plants were subjected to drought for 14 days followed by re-watering for two weeks and the samples were collected for physiological analyses mentioned above. The control set was watered daily. The results showed that the seed germination percentage and mean daily germination declined with the increase in water stress severity reaching lower than 10% germination at the lowest osmotic potential treatment (-1.80 MPa) for both cowpea and soybean. Germination results showed no significant differences between cowpea and soybean for all parameter. However, cowpea was significantly different from soybean with regard to

seedling growth parameters. Morphological results showed that soybean nodule weight significantly decreased as the stress severity increased. Cowpea nodule weight, number of stomata on both surfaces, and root length showed no significant difference between treatments and also between the genotypes. White genotype cowpea had a significantly lower root length and nodule weight and a slightly lower number of stomata but not significantly different from the other genotypes. Drought stress decreased the leaf relative water content of both cowpea and soybean under moderate and severe stress. However, cowpea performed distinctly superior to soybean. Cowpea with its lower nodule weight maintained no significant difference between all treatments. Water deficit stress affected leaf development. The highest number of leaves 1<sup>st</sup> count were 24.60 and 16.50 (control) and the lowest count was 19.33 and 14.50 (severe) for cowpea and soybean, respectively. The 1<sup>st</sup> count of the number of leaves of soybean genotypes showed a significant difference between genotypes for all water stress treatments with the exception of TGx 1937-f and PAN 1664R under severe stress, 20.16 and 20.83, respectively. With regards to water stress metabolites, the proline content and malondialdehyde content of both beans were significantly increased comparing control with stressed treatments. However, malondialdehyde content and proline content of soybean was significantly higher than cowpea for both control and stressed plants. The same trend was observed for stressed-rewatered experiment. The recovery response of cowpea was excellent and better than soybean which failed to recover. Considering morphological and physiological parameter during vegetative growth, the results suggested that cowpea response better to water stress than soybean. In conclusion, cowpea response and tolerate water deficit stress better than soybean. In future, it is necessary to genetically compare the two legume plants, to trace the genes that are responsible for such tolerance in cowpea.

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## ABBREVIATIONS AND SYMBOLS

ABA	Abscisic acid
AgriSA	Agri South Africa
ARF	Auxin response factors
CIA	Central intelligences agency
cm	Centimetre
cm <sup>2</sup>	Centimetre square
CRD	Completely randomised design
DAFF	Department of agriculture, forestry and fisheries of South Africa
DNA	Deoxyribonucleic acid
ER	Emergence rate
FAO	Food and agricultural organisation
G	Grams
GI	Germination index
GP	Germination percentage
IAA3	Indole-3-acetic acid
IITA	International Institute of Tropical Agriculture
JRC	Joint Research Centre
KJ	Kilo-joule
kPa	Kilopascals
L	Litre

LPWG	Legume Phylogeny Working Group
LSA	Leaf surface area
mM	Millimolar
MDG	Mean daily germination
Mg	Milligram
MPa	Pascal
MRL	Mean root length
MSL	Mean shoot length
NaOCl	Sodium hypochlorite
NCDC	United States National Climatic Data Center
OP	Osmotic pressure
PEG	Polyethylene glycol
RNA	Ribonucleic Acid
ROS	Relative oxygen species
RBP	Retinol binding protein
SVI	Seedling Vigour Index
TCA	Trichloroacetic acid
UCAR	Universal Corporation for Atmospheric Cesearch
WHO	World Health Organisation
°C	Degree Celsius
ha	hectare
kg	Kilogram

l	Litre
$\mu$	Micro
mg	Milligram
mm	Millimetre
$\text{m}^{-2}\text{s}^{-1}$	Per square metre per second
mM	Millimolar
%	Percent

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# **CHAPTER 1**

## **GENERAL INTRODUCTION**

## 1.1 THE STATE OF DROUGHT WORLDWIDE

The world has lately suffered prominent natural disasters, most with potential to extirpate all form of life. These catastrophic events and their severity have over the years tried-out both livelihood and economy. Drought, earthquake, floods, cyclones, hurricanes and volcanic eruptions are some examples of natural disasters. According to The United States National Climatic Data Center (NCDC, 2014), drought comes in second to hurricanes in regards to causing severe economic impacts worldwide. Drought is a prolonged period of not enough rainfall or below average precipitation that results in shortage of water in the soil (Hisdal and Tallaksen, 2000).

Chandio et al. (2016) reported that environmental problems such as drought, global warming and climate change are the major attributes to limited agricultural production. It is evident that the worst enemy of agricultural production is drought and prediction is that the worst of drought challenges maybe experienced in the near future (UCAR, 2020; Oo et al., 2019). On the other hand, the greatest challenge of the current century (21<sup>st</sup> century) and the paramount threat for the future is the disastrous increase in human population. According to Central Intelligence Agent (CIA) world factbook (2010), there has been massive increase in the human population where a fourfold increase was observed in the past hundred years with an estimated growth rate of 1.1% per year. Moreover, an increase from about 6.9 billion 10 years ago (2010), through 7.8 billion at present (2020) to 10.2 billion (predicted) by mid-21<sup>st</sup> – century (2050) is declared unavoidable (Kaneda and Bremner, 2014 and Knight, 2012; Worldmeter, 2020). Consequently, such drastic increase in mankind population highlights the challenge of food scarcity. According to World Health Organisation (WHO, 2009) the major cause of common illness in under-developed and developing countries is directly or indirectly linked to the diet. Thus, poor communities are stricken by food scarcity and suffer malnutrition which makes their immune system more susceptible to infections.

In recent times, the persistence of drought in different regions of the world threatens lives of millions of people. For example, Pakistan today experiences a worsened drought that started about 50 years ago (Farroqi et al., 2005). The government reported that shortage of food and water threatens close to three million people (FSIN, 2020). In China, the northern and southwest regions are under drought distress that caused a verge of starvation and austerity on farmers (Barriopedro et al., 2012). In Iran, close to 73% of the country is considered a desert and suffer outrageous drought (Amiraslani and Dragovich, 2011). Zimbabwe suffers

what is confirmed prolonged severe drought that started back in 2018 (Frischen et al., 2020). As a results more boreholes threatens to dry out as the ground water depletes (Frischen et al., 2020). Although drought is no longer considered a national disaster in South Africa, there are still areas that never had enough rain for more than seven years. Western Cape Province is repeatedly reported to be affected by severe drought since 2015 (JRC, 2018). Eastern Cape, especially the western half of the province suffers a worst drought which started 2015. Mahlalela et al. (2020) reported that livestock farmers in the region lost about half of the animals they kept before. According to the 350 Africa annual report (2014), seven out of ten Sahel and Southern Africa countries are affected by climate change. Thus, about 70% agricultural land is affected by drought. The more the agricultural land get barren the lesser the production of highly demanded crops such as cereals and food legumes.

## **1.2 IMPACT OF DROUGHT ON AGRICULTURAL PRODUCTION**

Water shortage crisis and its effects can be easily observed in the agricultural sector. Each year, the agricultural sector and the food supply chains suffer a loss worth R52 trillion of the total average yield of agro-economic crop due to drought stress (FAO, 2018). Water has an unrivalled importance in the normal physiological and biochemical functionality of a plant. It is reported that water makes up 90% of the biomass of most plants (Evert and Eichhorn, 2013). Inadequate water supply leads to water stress, thereby devastating transportation of metabolites and nutrients (Evert and Eichhorn, 2013). Drought and salinity are the fundamental cause of water deficit stress in plants (Lisar et al., 2012). Drought is experienced in various parts of the world every year, mostly in arid and semi-arid regions, affecting 70% of the agriculture in the Sahel and Southern Africa countries as reported in the 360 Africa annual report (2014). Since the ancient days, drought has been the considerable production restraining factor in the agricultural sector (Pena-Gallardo et al., 2019). Generally, its effects on plant growth and development are well documented, although the crucial biochemical and molecular response processes have not been completely elucidated. Water stress caused by drought negatively affects water potential and turgor of plant-cells. These lead to limited cell division and elongation, inhibiting growth and development.

Plants have different mechanisms through which they tolerate drought stress including morphological and physiological. When plants experience stress the accumulation of abscisic acid (ABA) and proline increases leading to senescence. Water deficit stress also affects opening and closure of the stomata leading to reduction in transpiration and gaseous

exchange as well as a decline in the rate of photosynthesis. Drought negatively affects plant photosynthetic metabolism and physiological processes by causing reduction in ribulose-1,5-bisphosphate (RuBP) regeneration and ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) activity (Rivas et al., 2016). These lead to plant shriveling, becoming flaccid, failing to properly flower and if drought is prolonged the plant may die (Fathi and Tori, 2016).

### **1.3 SOYBEAN PRODUCTION AND ITS POTENTIAL TO ELEVATE FOOD INSECURITY IN AFRICA**

Recent projections by UN estimates that the food demand in sub-Saharan Africa can increase by over 300% by 2050 (UN, 2020). These estimations are deduced from the reported increased population from 0.811 to 1.1 billion during the years 2000 to 2021 in the region (UN, 2020). On the other hand, both commercial and non-commercial farmers in the region experience poor production, failing to measure up to the demands. Worsening poverty and food insecurity mostly in the rural areas can be scrapped alternately by farming crops with good dietary qualities such as soybean (Jayne et al., 2018). The dry seed of soybean is composed of 19% oil, 36% protein, 35% carbohydrates (17% being dietary fibers), 5% minerals and essential vitamins (B-vitamins such as niacin, folacin and pyridoxing) (Hassan, 2013). Soybean production in sub-Saharan Africa is still low as is anywhere in Africa. However, sub-Saharan Africa has a potential to grow and maximise the production of soybean (Tefera, 2011).

This is evident since for years soybean has been one of the top three cultivated crops in South Africa (in terms of land planted and production) together with maize and sunflower. According to Department of Agriculture, Forestry and Fisheries (DAFF) the soybean production in South Africa tripled from about 500 000 in 2010 to over 1.5 million metric tons in the season 2017/2018 (DAFF, 2010; Bahta and Willemse, 2020). The production could have been much higher but local farmers suffer harsh environmental conditions such as pests, diseases and drought. Although modern technology practices have a potential to improve yield, there is an utmost need for genetically improved varieties that can withstand this constraint.

### **1.4 THE EFFECTS OF DROUGHT ON LEGUME PLANTS**

#### **1.4.1 Legumes and their economic importance**

Leguminosae (also called Fabaceae or legumes) is the third largest land flowering plant family with about 750 genera and up to 19 000 species (Ahmed and Hasan, 2014; LPWG,

2013; Timko et al., 2009). Species greatly show morphological diversity, some exhibit a form of herbs while others are trees (Menge et al., 2015). They also show variety in the way they interact with the environment and also their uses. Crops in the family Fabaceae are famously known for their fruits (legumes), their ability to fix atmospheric nitrogen and their tolerance to abiotic stress (Ahmed and Hasan, 2014). Botanically, legumes are formed from the unicarpelar ovary and are said to be dehiscent dry fruits (Pitchford, 1993). Crop legumes include peas (*Pisum sativum*), peanuts (*Arachis hypogaea*), alfalfa (*Medicago sativa*), cowpea (*Vigna unguiculata* [L.] Walp) and soybean (*Glycine max* [L.] Merrill) (Snapp et al, 2018). These plants have been the most important part of mankind as food and medication since before 6000 B.C (Ahmed and Hasan, 2014). They are the most important source of affordable crop oil and protein (Mangena, 2018).

Crop legumes are extensively commercialised, cultivated in over 180 million ha worldwide (Preissel et al., 2015). These makes them the second most cultivated family of plants, amounting to between 12 and 15% of earth's aerial land (Nedumaran et al., 2015). The literature reports that these plants are economically used to reduce the amount of nitrate (nitrogen fixation) and to improve the soil (Zahran, 1999; Graham and Vance, 2003; Rascia and La Rocca, 2008; Hirel et al., 2011). They are prominent for efficient use and tolerance of calcium (Ca) and aluminum (Al) (Arunakumara and Walpola, 2013; Graham and Vance, 2003). The historical time-lines and archaeological inference evident on the use of legumes for soil enhancement and crop rotation practice date back to the ancient civilisations of Greece, Egypt and Rome (Stoddard et al., 2009; Ahmed and Hasan, 2014). In fact, species such as Alfalfa (*Medicago sativa*) is conceptualised as one of the first plants to be cultivated and domesticated by man (Cook et al., 2005). Since then, these plants have been part of man's life as both food and medication for human and their livestock. Today they compose a total of over 27% of the basic crop production worldwide (Tassoni et al., 2020).

Legumes, predominated by soybean and peanuts contribute more than 35% of the world's processed vegetable oil. They are also an excellent source of high quality dietary proteins for human diet, poultry and pork production (Mangena, 2018). Moreover, legumes are: rich in plant sterols and fiber, low in fat, good supplement of animal proteins, rich in vitamins (such as vitamin K1), starch (carbohydrates), minerals (iron (Fe), phosphorous (P), calcium (Ca), etc.) amino acid. Furthermore, they are important to vegetarian and vegan diets as a source of protein (Kouris-Blazos and Belski, 2016). According to Polak et al. (2015) casual ingestion

of legumes alone or in combination with other dietary fruits and vegetables assist in the prevention of cardiovascular diseases, type 2 diabetes and some but not all cancers. The seed and foliage are also used in the production of medications, food colourants and colognes (Ahmed and Hasan, 2014).

#### 1.4.2 Cowpea versus soybean

Conventionally cowpea is considered the trunnion of small farmers in Central and West African regions. With an annual estimation of 12.5 million ha cultivation in the world and the worldwide production was 7.6 million tons and nearly 5.4 million tons (about 87%) produced in Africa back in 2019. Nigeria was the largest producer, contributed 61% and 85% of African production and world production respectively (IITA, 2018). Cowpea is the outmost cultivated legume in Africa (DAFF, 2014; Ngalamu et al., 2015). On the other hand, soybean is the paramount and most extensively cultivated legume in the world. An annual production of approximately 365 million tons was estimated for the season 2017/2018 (GSA, 2017).

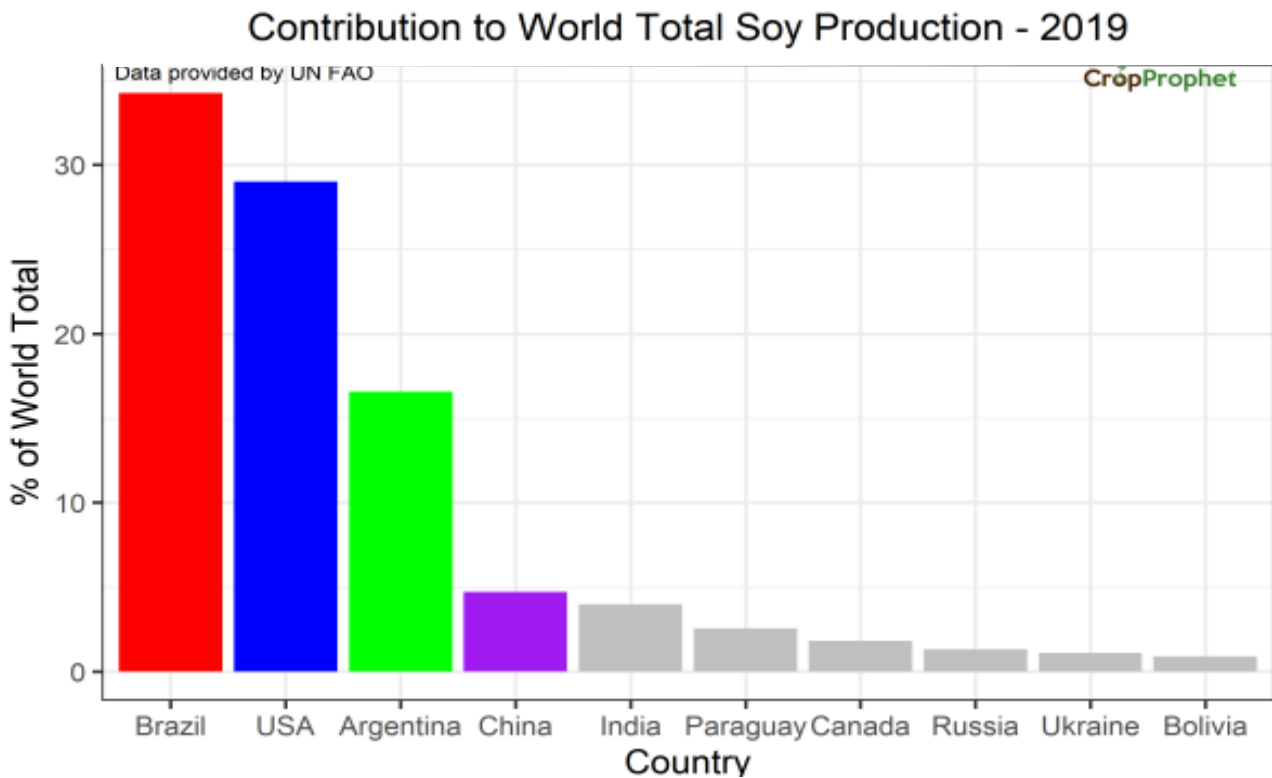
According to the USDA (Figure 1.1 below), Brazil was number one producer of soybean with a production of 108.0 million metric tons (32.42%) back in 2019. In the same season, none of the African countries was on the top ten of Soybean producers globally. This shows that, although soybean production increases worldwide, it is still difficult to maximise production in Africa. Soybean is by far nutritionally better than cowpea. In Table 1.1 below all nutritional values of soybean are higher than those of cowpea with the exception of saturated fatty acids and carbohydrates. Nevertheless, soybean remains superior and far better than cowpea. For example, soybean has more of mono- and poly-unsaturated fatty acids which are healthy, enhance good cholesterol and diminish bad cholesterol (Mahyara et al., 2016).

**Table 1.1:** Comparison in nutritional values between cowpea and soybean.

	<b>Per 100g</b>	<b>Cowpea</b>	<b>Soybean</b>
<b>General values</b>	Food energy (KJ)	1406	1866
	Dietary fibre (g)	10.6	9.3
	Saturated fatty acids (g)	0.33	2.88
	Monounsaturated fatty acids (g)	0.11	4.4

	Polyunsaturated fatty acids (g)	0.54	11.26
	Proteins (g)	32.52	36.49
<b>Minerals</b>	Potassium (mg)	1112	1797
	Copper (mg)	0.845	1.66
	Iron (mg)	8.27	15.7
	Magnesium (mg)	184	280
	Phosphorus (mg)	424	704
	Zinc (mg)	3.37	4.89
	<b>Sugars</b>	Carbohydrates (g)	<b>60.03</b>
Others (g)		6.9	7.33
<b>Vitamins</b>	Riboflavin (mg)	0.23	0.87
	Vitamin C (mg)	1.5	6
	Thiamin (mg)	0.85	0.87
	Pantothenic acid (mg)	1.5	0.79mg
	Vitamin B6 (mg)	0.36	0.38
<b>Amino acids</b>	Methionine (g)	0.34	0.55
	Tryptophan (g)	0.29	0.59
	Threonine (g)	0.9	1.77
	Lysine (g)	1.59	2.71
<b>Antioxidants</b>	Daidzein (mg)	0.10	62.9

**Note:** Values in red implies cowpea is better than soybean. **Source:** [www.foodstruct.com/](http://www.foodstruct.com/)



**Figure 1.1.** The top ten soybean producers in the world. **Source:** [www.cropprophet.com/top-ten](http://www.cropprophet.com/top-ten)

### 1.5 SCOPE OF THE RESEARCH

The study compared the responses of cowpea and soybean to water deficit stress induced by drought. Their similarities and dissimilarities were explored by evaluating parameters at three different levels.

1. Seed germination: Sterilised seeds of the soybean and cowpea were germinated in five different solutions (used to water the seeds) that had different osmotic potentials. Different osmotic potential levels were induced using polyethylene glycol (PEG-8000).
2. Morphology: The second study was on evaluating the morphological parameters. Both vegetative and reproductive structures produced during different levels of water deficit stress were evaluated. Fruit and nodules were harvested, dried, counted and weighed.
3. Physiology: In the third study, leaves were harvested and used for physiological analysis. Malondialdehyde, chlorophyll, proline and leaf relative water contents were determined.

## 1.6 AIM AND OBJECTIVES

### 1.6.1 Overall aim

The aim of the study was to compare between selected varieties of cowpea's (*Vigna unguiculata* [L.] Walp) and soybean's (*Glycine max* [L.] Merrill) response and tolerance to water deficit stress.

### 1.6.2 General objectives

The objectives of the study were to:

- (i) Determine the effects of water deficit stress on seed germination of selected cultivars of cowpea and soybean.
- (ii) Determine the level of water deficit stress that affect the growth and development of cowpea and soybean.
- (iii) Evaluate the effect of water deficit stress on phenotypic parameters as well as biochemical and physiological processes of the cowpea and soybean.
- (iv) Evaluate how seedlings and matured plants of cowpea and soybean recover from water deficit stress after rehydration.
- (v) Evaluate the effect of water deficit stress on the process of biological nitrogen fixation of the selected cultivars of cowpea and soybean

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## **CHAPTER 2**

# **EVALUATION OF WATER DEFICIT STRESS TOLERANCE OF SOYBEAN AND COWPEA DURING SEED GERMINATION**

## 2.1 INTRODUCTION

Cowpea and soybean are both most important grain legumes worldwide. Their seed are an important source of proteins and recommended nutrients for human diet and animal fodder. However, their seeds are by far the most important and most economical components (Grant-Downton and Dickinson, 2005; Alvarez-Venegas et al., 2020). Generally, seeds are an indispensable component of the world's life sustainability. Seeds' prominence lies on their ability to serve as a bridge between two generations of plants (Evert and Eichhorn, 2013). These facts about the potency of seeds reflect the extensity of research on seeds, their structures, uses and the germination process.

Under severe and extremely rising temperatures, getting a seed to germinate is as hard as growing a plant in such environmental conditions. Unfortunately, these challenges directly affect the production of commercial crops. Studies pointed out that significant crop loss has been suffered following drought or water stress that set during germination (Nambara et al., 2010; Weitbrecht et al., 2011; Vishal and Kumar, 2018). Carvelho et al. (2019) reported that the success of legume crops depends on the fast and uniform seed germination especially in regions with low water availability. The literature reports that water stress reduces the germination percentage and delays the inception of seed germination (Delouche, 2016).

Seed germination is defined as an emergence of a seedling from the seed. The process includes all events from imbibition (water intake), through activation stage to the radiating of embryonic axis (usually the radicle) from the seed coat (Delouche, 2016). Seeds are made of three main components, i.e. seed coat, dormant embryo (miniature plant) and endosperm (stored food). When seeds mature, they usually dry to only 10 to 20% water content (Evert and Eichhorn, 2013). Temperature, water content, pH of the medium used to germinate the seed have a direct effect on its germination (Chachalis and Reddy, 2000 and T aylorson, 1987; Rezvani and Zaefarian, 2017). Other environmental factors include oxygen (O) availability and light intensity. For example, most plant seeds germinate at temperatures between 18 and 33 °C. It is reported that lower temperatures interfere with imbibition process while higher temperatures reduce enzyme efficiency and sometimes denature them resulting in dead or rotten seeds (Rezvani and Zaefarian, 2017). Temperature and water availability also affect the rate and total percentage of seed germination (Morad, 2013).

Water has vital role in seed germination. Seeds are dry in nature and without water germination is rarely possible. Water helps prepare and initiate the process by softening the seed coat and promoting permeability. It dissolves and converts the insoluble stored food into

soluble absorbable form, and also provides dissolved oxygen (Weitbrecht et al., 2011). Thus, it initiates enzymes and bio-regulators necessary for biochemical and molecular mechanisms required for germination. Normally, seed germination occurs in three phases. Namely, 1<sup>st</sup> imbibition phase, 2<sup>nd</sup> latent phase and 3<sup>rd</sup> exponential growth phase. Although rapid absorption occurs during the 1<sup>st</sup> phase, water is still essential in all phases hence the continuous supply of adequate water is a necessity (Vishal and Kumar, 2018). Leubner-Metzger (2017) reported that shortage or limited water (drought) during seed germination, where seeds were initially imbibed promotes the second dormancy. In this study, the effect of water deficit stress during germination of soybean and cowpea seeds were evaluated. Different levels water deficit stress was induced using PEG8000.

### **2.1.1 Aim**

This study was aimed at evaluating and comparing the tolerance to water deficit stress during seed germination of soybean and cowpea.

### **2.1.2 Objectives**

Objectives of the study were to:

- i. Determine the effect of water deficit stress on germination parameters of cowpea and soybean seeds.
- ii. Evaluate the effect of water deficit stress on parameters of the germinated seedlings.

## 2.2 LITERATURE REVIEW

### 2.2.1 Germination process in legume plants

Legume seeds have a seed-coat (testa) that covers the two large cotyledons that surrounds the embryo which is made of radicle and plumule. The cotyledons also serve as an endosperm (Jennings and Foster, 2020). Seed germination is susceptible to other environmental conditions than the basic requirements (availability oxygen, adequate water and optimal temperature). Such factors include pH, light intensity and/ or nitrate (Rezvani and Zaefarian, 2017). More importantly, water is considered as the influential environmental factor that affects the most vulnerable and crucial stage of plant life cycle, i.e. germination (Finch-Savage and Leubner-Metzger, 2006). Excess water can also inhibit germination. Therefore, water deficit stress is the key environmental factor that has a notable effect on seed germination (Rezvani and Zaefarian, 2017).

The germination process differs and they also differ in their tolerance to water deficit, undergo germination differently. For example, the cotyledons of most monocotyledonous plants such as corn (*Zea mays*) and barley (*Hordeum vulgare*) are developed under-ground while dicotyledonous plants such as legumes have their cotyledons developed above-ground (Baskin and Baskin, 1998; Baraka, 2018; Copeland and McDonald, 2001). Germination process in legumes usually requires enough water to break the dormancy and softening the testa sufficiently (Baraka, 2018). In legumes, germination starts with a rapid uptake of water into the seed through the micropyle, a process called imbibition (Robert et al., 2008; Weitbrecht et al., 2011). Commonly, the uptake of water by seed is a triphasic process: i.e. Phase I, - rapid uptake leading to the swelling and enlarged size of the seed, membranes disturbance, initiation of physiological processes such as protein synthesis and some metabolic activities, mending of the existing mitochondria, promotion of enzyme activities (such as DNA ligase) and DNA repair (Preston et al., 2009; Tuan et al., 2019; Weitbrecht et al., 2019). Phase II, - the plateau phase, associated with low water uptake. In this phase, the existing DNA and mitochondria keep on mending as the new proteins and mitochondria are synthesised (Woodstock, 1988; Khatami and Ahmadiania, 2018). The protrusion of radicle characterises the end of phase II and commencement of phase III. Phase III – begins with the mobilisation and distribution of solubilised organic stored food in and from the stored endosperm (cotyledons) and end with the elongation of the visible seedling. The processes in phase III requires increased water uptake (Manz et al., 2005; Weitbrecht et al., 2011).

### 2.2.2 The effect of water shortage during seed germination

Research shows that the repercussions of water stress during seed germination is unavoidable. Failure of planted seed to germinate has been the greatest nightmare of commercial farmers and the principal effect of drought on global agricultural production. As a result of the due challenges, Canadians faced a rapid rise in prices of most vegetables such as lettuce (*Lactuca sativa*), increasing with 40% as reported by Lean (2015). In South Africa, the food prices increased by 15% to 20% back in 2016 (Vollgraaf et al., 2016). The country was reported to be the number one producer of white corn in Africa. However, in 2015 South Africa had imported the product from other countries due to drought. Prevention of seed germination and early development inhibition due to drought were the main cause of dropped production (Vollgraaf et al., 2016; Manderson et al., 2016).

In a study conducted by Batool et al. (2014), a significant decrease in seedling development and seed germination parameter for corn was observed as a result of limited water supply during germination. Germination index, seedling fresh and dry weight and seedling shoot length were all negatively affected by shortage of water. Jajarmi (2009) also obtained similar results when the response of various cultivars of wheat was studied under water stress. The study reported that water stress decreases imbibition process, and the highest reduction was recorded for the highest water stress level. The herbaceous legume *Trigonella suavissima* subjected to controlled drought conditions had the rate of germination decreased (Akhalkatsi and Losch, 2005). Tukuki (1990), Majd et al. (2011) and Rohamare et al. (2014), used polyethylene glycol (PEG) to induce water stress during germination of various species and observed that the rate and total germination percentage were reduced and seedling growth was inhibited.

These studies bare evidence that shortage of water during seed germination significantly affect germination and seedling development. This is because the stored nutrients in the seed cannot be dissolved or transported when there is not enough water (Montes-Recinas et al., 2012). It is reported that when seed is exposed to water that is not enough to cause germination, it promotes secondary dormancy thereby making it even harder for seeds to germinate (Bentsink and Koornneef, 2008). Seeds also require sufficient oxygen for germination and water helps with the supply of easily accessed dissolved oxygen. Oxygen initiates the respiration process and promote the utilisation of stored food, conversion and synthesis of proteins necessary for seedling growth and development. Moreover, without or

with limited water activation of enzymatic activities that catalyse metabolic and biochemical reactions responsible for germination may not be possible (Schütz et al., 2002)

### 2.2.2 The need for seed surface sterilization in germination studies

A study by Ahmad et al. (2012) reported that both exogenous and endogenous microbial contaminants such as fungi and bacteria are common on seeds and explants collected from the open field. The most censorious stage in the study of seed germination and plant regeneration (*in vitro*) is to remove contaminants and obtain contaminate-free seeds and plant materials. It is also necessary to sterilise all equipments and culture media used in *in vitro* studies. Autoclave is the most commonly used for sterilisation of equipment and media set at 121°C and pressure of 15 Kpa for 15 minutes (Trigiano and Gray, 2005). Unfortunately, failure to completely eliminate microbial contaminants often hinder seed germination and other *in vitro* processes (Sabele et al., 2015).

Surface sterilisation protocols are continuously reported to be effective enough for removal of exogenous contaminants and establishment of sterile seeds and explants for tissue culture and micro propagation studies *in vitro* (Okereke, et al., 2016). There are a number of surface sterilisation agents that have been used since the past century including sodium hypochlorite, calcium hypochlorite, mercuric chloride, chlorine gas and ethanol (Oo et al., 2018; Ahmad et al., 2012). Sodium hypochlorite, calcium hypochlorite and ethanol are the most commonly used decontaminating agents, especially for seeds of various species (Sabele et al., 2015). Although chlorine gas and hydrogen peroxide are less commonly used, they are reported to have been effective for sterilisation of the well-known *Arabidopsis* and soybean seeds (Ahmad et al., 2012).

Other reporters highly recommend the use of commercial bleach (hypochlorite, NaOCl) and polysorbate 20 (Bakhsh et al., 2016; Bonde et al., 1999; Fedzlinal and Daniel, 2021). Oftentimes when the seeds are collected from the open field or stored in improper conditions, these agents regrettably fail to completely remove contaminants. However, the highly toxic and most dangerous agent, mercuric chloride is used with prescribed safe handling procedure and requires special collection and disposal of the resulting hazardous waste.

#### 2.3.4 Seed viability

The moisture status of the seeds is usually high during the period of harvest and then starts to diminish with time, i.e., the longer the storage period the lower the moisture content. This concept “seed viability” is defined as the ability of the seed to germinate into an established plant given conducive environmental conditions (Balesevic-Tubic et al., 2010; Basu, 2020). Seed storage period is by far the most important cause of seed deterioration. Studies reported that seeds stored for a long period of time show a low total germination percentage and germination speed. Other causes encompasses storage conditions (mostly dry and cool environment), plant species and genotype (Mangena, 2019). To the greatest extents; seed deterioration (loss of viability) is the most neglected factor of seed germination (Shaban, 2013). However, this phenomenon is a significant and considerable impediment of germination, regeneration, seedling vigour, growth and development patterns as well as production of commercial field crops (Basu, 2010; Shaban, 2013).

#### 2.3.5 Polyethylene glycol as an agent of water deficit stress

Since the recent decades, researchers have been widely using the higher molecular weight polyethylene glycol (PEG) compounds such as PEG-6000 and PEG-8000 to simulate matric potential and achieve drought stress effects for germination and plant water relation studies (Violita and Azhari, 2021) as well as in animals (Naigamwalla et al., 2000). The PEG is an osmotic-regulating substance that is non-volatile, non-toxic, colourless, inert and exceptionally soluble in water and organic solvents. The inert nature of PEG makes it an excellent substance to encompass in the seed germination medium since it does not move into the cells, gradually affirming the no toxicity nature as past studies reported (Emmerich and Hardegee, 1990; Jadav et al., 2016). To date, the response of various agronomic crops to drought stress have been studied in *in-vitro* including soybean (Khaliliagdam et al., 2013), zucchini (*Cucurbita pepo* L.) (Atashi et al., 2015), watermelon (*Citrullus vulgaris* L.) (Bakhshandeh et al., 2017), and lemon balm (*Melissa officinalis* L.) (Atashi et al., 2014). The results of such studies show that lower osmotic potential (more negative) significantly lessened the germination percentage, germination speed and other parameters as the trend was noticed in all these studies.

Previously it was reported that there was a significant difference between different seedling traits of various drought tolerant wheat genotypes when different concentrations of PEG-6000

were used during germination (Kaufmann and Ross, 1970; Emmerich and Hardegree, 1990; Elkheir et al., 2016). It is evident that germination and early development of seedling are very sensitive to water deficit stress that leads to delayed onset, rate and uniformity of germination causing an overall poor plant performance (Abro et al., 2021). PEG is also used as a priming agent in osmopriming studies (Brancalion et al., 2008; Kareem et al., 2020). According to Jisha et al. (2010) PEG solution modulates metabolic processes required for germination by regulating seed water absorption rate. It also helps with initiating membrane repairing system when used as a priming agent (Kareem et al., 2020). This technique is frequently used in the agricultural sector in many developed countries (Elkheir et al., 2016).

Osmotic-regulating substances such as polyethylene glycol of 6000 and 8000 molecular weight (PEG6000, PEG8000), and sodium chloride (NaCl) have proved to be effective in generating drought conditions during the germination of seeds of most commercialised species including maize, wheat, rice and bean (Shahriari et al., 2014; Sayar et al., 2010; Mokhberdoran et al., 2009; Domínguez et al., 2014). PEG has been used with certain precautions (PEG must be of higher molecular weight to avoid diffusion through the cell wall), to simulate matric potential in germination studies (Kaufmann and Ross, 1970).

## 2.3 MATERIALS AND METHODS

### 2.3.1 Plant material

Soybean and cowpea mature seeds (three cultivars each) were obtained from the Department of Biodiversity, University of Limpopo (Turfloop campus). The seeds were planted in a nursery on the campus during the spring season of 2019 for production of new seeds. For the purpose of this study cowpea varieties were named according to the seed colours.



**Figure 2.1:** Seeds of soybean and cowpea that were used in the experiments, each plant with three varieties/cultivars. The first three are cowpea varieties followed by three soybean cultivars: A. Red, B. Blonde, C. White, D. TGx1937-1F, E. PAN 1664R and F. LS 678.

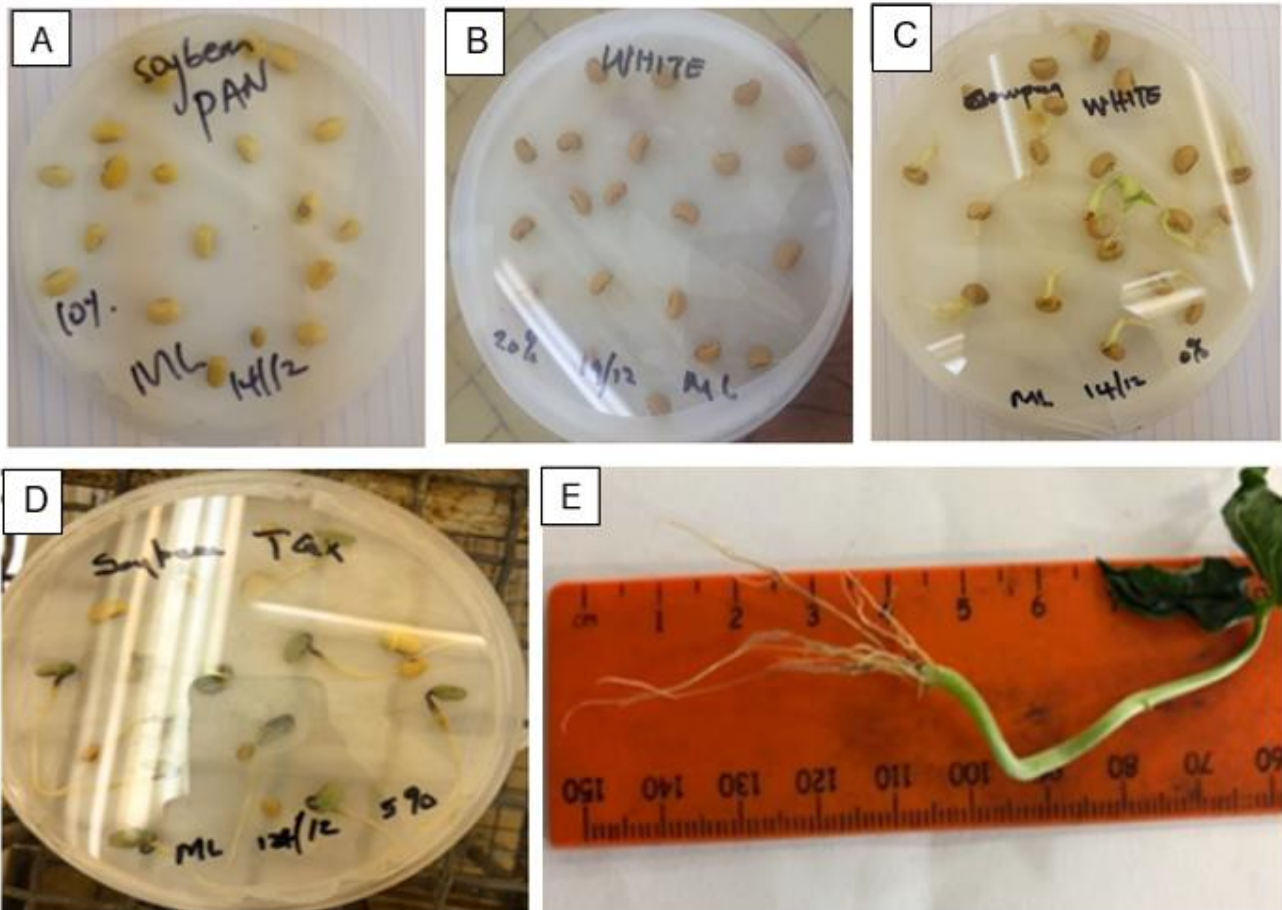
### 2.3.2 Seed surface sterilisation

A total of 20 mature dry seeds not older than 3 months per variety were randomly selected. Seeds were washed in clean running tap water and a drop of dish washing soap; carefully rinsed; and dried using a paper towel. Washed and dried seeds were placed in 6 x 1.5 cm petri dishes and placed inside a desiccator jar. A clean 250 ml beaker was placed inside the

desiccator jar and 10 ml of sodium hypochlorite (bleach) was added into the beaker; 3 ml hydro-chloric acid (HCL) was added into the beaker containing bleach and desiccator was immediately closed allowing the accumulation of chlorine gas to sterilise the seed for 16 hours. After 16 hours, the petri dishes with seeds were removed from the desiccator jar and placed in a laminar hood with the lid opened. Equipments used for the germination experiment including beakers, Whatman filter paper, test tubes etc. were autoclaved at 121 °C for 15 minutes.

### 2.3.3 Preparation of polyethylene glycol 8000 (PEG-8000) solutions

Polyethylene glycol 8000 (PEG-8000) solution of five different concentrations that are equivalent to the osmotic potential of 0.00, -0.02, -0.27, -1.27, and -1.80 MPa (thus, 0, 3.75, 7.5, 15.0, and 22.5% PEG-8000) were prepared according to Jain et al. (2013). An appropriate amount of PEG-8000 was weighed and completely dissolved in warm distilled water. Sterilise Whatman No.1 filter paper was lined in a 15 cm x 1.5 cm petri dishes. Volumes of 15ml of the PEG-8000 solutions were poured onto the lined filter paper in each petri dish. A total of 20 surface sterilised seeds without physical damage per cultivar were placed on a wet filter paper and the petri dishes were tightly closed with parafilm and transferred for incubation into a growth room in triplicates. The petri dishes were arranged in the growth room in a Completely Randomised Design (CRD). The temperature in the growth room was 24°C, light intensity of 150-200  $\mu\text{mol}^{-2}\text{s}^{-1}$  and 8:16 light period. Germination was recorded for 10 days in intervals of 24 hours. Seeds with radicle longer than 2 mm were considered germinated. The data collected was used to calculate the germination and seedling parameters following Table 2.1 below.



**Figure 2.2:** Seeds incubated for germination: A and B, soybean PAN 1664R and cowpea white varieties respectively on day 0. C, cowpea white cultivar on day 2. D, soybean TGx 1937- 1F cultivar on day 5. E, an example of how the shoot and root length were measured.

**Table 2.1.** Description of formulae used to study germination and seedling parameters of soybean and cowpea seed germinated under induced water stress using PEG8000.

Parameter	Symbol	Equation	Reference
Mean daily germination	MDG	$MDG = NG/t$	Czabator, 1962; Gairola et al., 2011
Emergence rate (%)	ER	$ER = (GT3/GT7) \times 100$	Islam et al., 2009
Seedling vigour index	SVI	$SVI = (SL \times Gp/100)$	Islam et al., 2009
Germination percentage (%)	GP	$GP = (NG/n) \times 100$	ISTA, 1993; Faijunnahar et al., 2017

<b>Germination index</b>	GI	$GI = \sum(GT/Tt)$	Islam et al., 2009
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**Note:** NG= total number of germinated seeds; n=the total number of tested seeds; GT3= number of seed germinated at day 3; GT7= number of seeds germinated at day 7; N= the number of seeds germinated; t= number of days; SL= seedling length (mm); GT= number of germinated seeds; Tt= period for the final and last germinated record.

#### 2.3.4 Data analysis

The obtained data for analysis within bean type was presented as mean values of all replicates for all treatments. For comparison between bean types, each value represent a mean of three cultivars in triplicates per bean type. Analysis of variance (ANOVA) was done using IBM SPSS statistic 27 to determine if there is significant difference between treatments, cultivars and/or between cowpea and soybean with regards to water deficit tolerance at 95% level of confidence. The student t-test was further used to compare and determine which parameters and treatments between the cultivars and/or cowpea and soybean account for water deficit tolerance and/or susceptibility. This analysis were conducted using student t-LSD (least significant difference) at 5% level of confidence. The statistical outcomes are recorded under appendix 1 and 2.

## 2.4 RESULTS AND DISCUSSION

### 2.4.1 Germination parameters

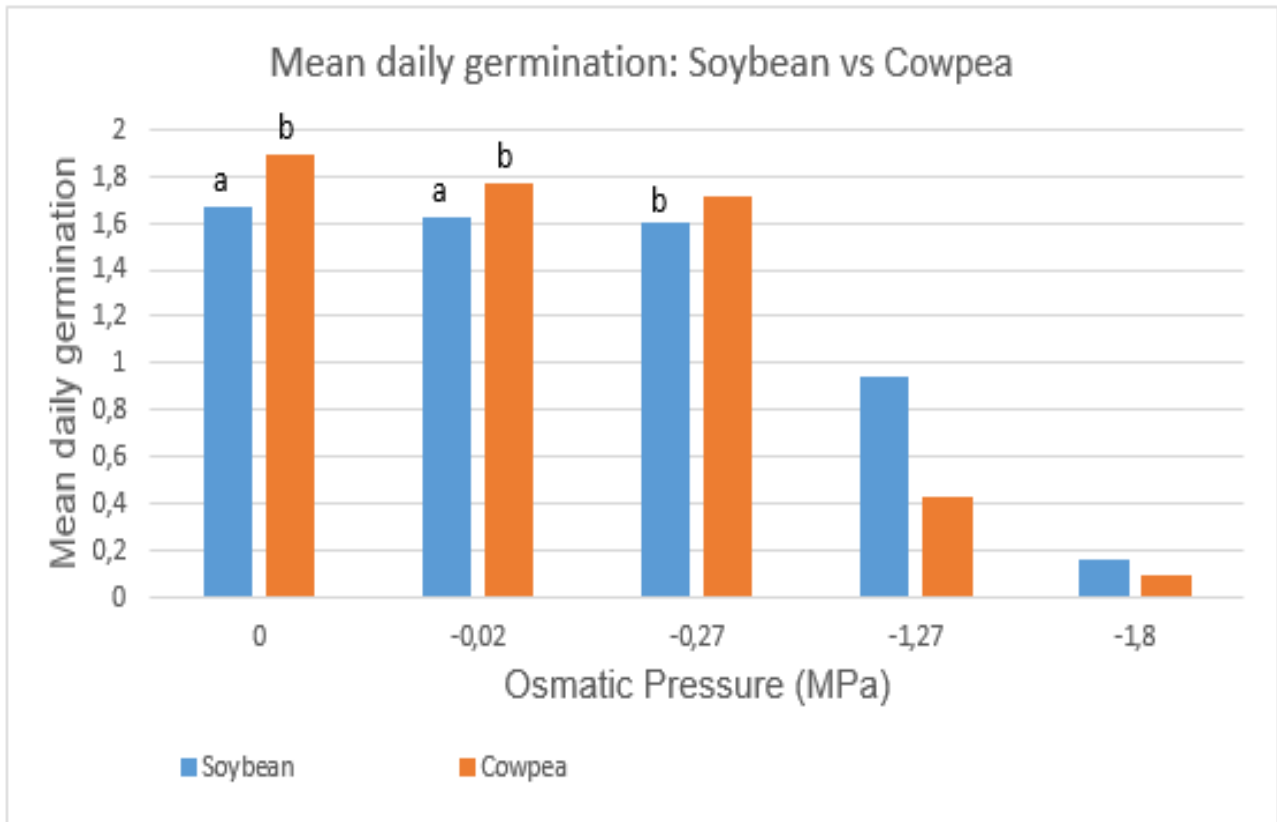
#### 2.4.1.1 Comparison between bean types

The drought tolerance level of cowpea and soybean during seed germination was assessed from daily counting of germinated seeds by determination of GP, MDG, ER and GI (Table 2.1). Germination percentage and MDG estimates the viability of the population of seeds. The seed viability can be affected by an inadequate supply of water during early phases of germination. When water supply suddenly decrease or ultimately stops, secondary dormancy is induced which leads to lower viability and therefore lower GP, MDG, ER and GI (Vishal and Kumar, 2018). In this study, water stress was induced using different concentrations of PEG-8000 which equals different osmotic potential (OP) levels i.e. 0, -0.02, -0.27, -1.27, -1.80MPa, the higher the concentration of PEG-8000 the lower the OP of the solution (Money, 1989).

Three genotypes from each plant type were tested. For all studied parameters no significant difference ( $P \geq 0.05$ ) between replicates were detected (Appendix 2). The seed MDG declined with the increase in water stress severity reaching lower than 5% germination at the lowest OP treatment (-1.80MPa) for both cowpea and soybean. The MDG of cowpea at the highest at 0 OP (1.89) and the lowest at -1.80 MPa (0.09). The MDG at the OP's in between were 1.77, 1.71 and 0.43 at -0.02 MPa, -0.27 MPa and -1.27 MPa as shown in Figure 2.3 below. There was no significance difference between MDG at 0 MPa, -0.02 MPa, and -0.27 MPa, however, the control OP compared to -1.27 MPa and -1.80 MPa showed a significant difference at  $P \geq 0.05$ . The difference between MDG at OP and MDG at -1.80 was found to be over 95% decreased (Figure 2.3). In average, cowpea had higher GP and MDG than soybean at 0, -0.02, and -0.27MPa although soybean was mostly higher for the two lowest osmotic potential, -1.20 and -1.80 MPa for almost all parameters as shown on the figure 2.3 and table 2.3.

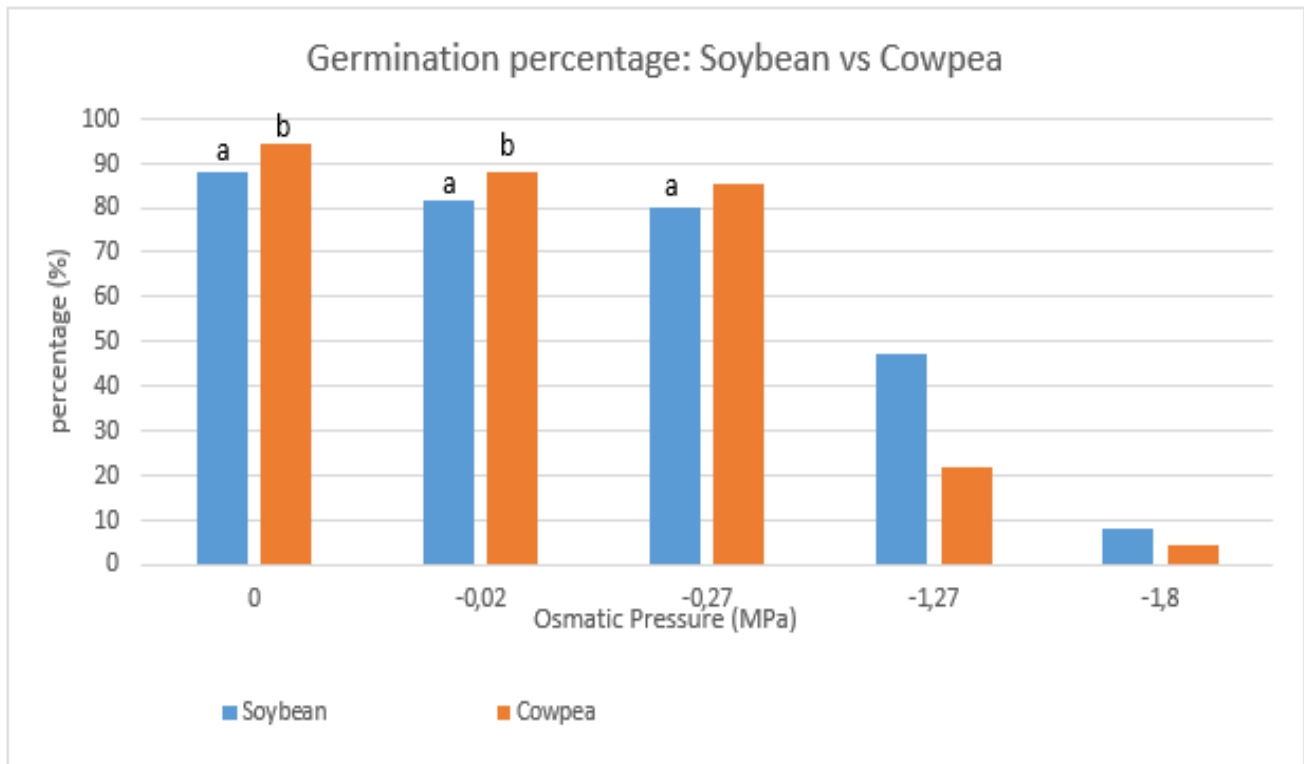
The results of this study agree with the findings by Murillo-Amador et al., 2000; AL-Taisa et al., 2010; Sayar et al., 2010; Jadav et al., 2016 and Toosie et al., 2014 who concluded that osmotic potential ranging from 0 to -1.5 MPa (induced by PEG-8000) reduced germination percentage of various commercial plant species especially when osmotic potential drops

below -0.3 MPa. The water availability at -0.3 MPa is limited below the required water for germination to occur (Baque et al., 2018). Murillo-Amador et al., 2002 and Sayer et al., 2010 also stressed that the effect was mainly due to osmotic potential than did ion toxicity.



**Figure 2.3.** Cowpea vs. soybean. Mean daily germination (MDG). The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

Although the GP of the lowest two OP levels -1.27 MPa (21.67 %) and -1.80MPa (4.45 %) were severely and considerably affected, there was no significant difference (Appendix 1 and 2) or changes in GP of cowpea at -0.02 MPa (88.22%) and -0.27MPa (85.55%) as compared to corresponding control, 0 MPa (94.44%) (Figure 2.4). The same trend was observed for soybean, however, both MDG and GP values were lower than corresponding cowpea value but not statistically different. These results suggests no germination difference between cowpea and soybean in terms of germination under water stress. They both can tolerate shortage of water during germination, however, their tolerance is limited to OP higher of equal to -0.27 (Figure 2.3 and 2.4).



**Figure 2.4.** Cowpea vs soybean. Germination percentage (GP). The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

These results agree with what Murillo-Amodor et al. (2002) who reported on Cuarenteno and Pacene cultivars of cowpea response to PEG-6000 induced stress. Several studies revealed that the GP of the various crop plants decreased at OP of between -0.4 and -0.8 MPa (Khaliliagdam et al., 2013; Atashi et al., 2014; Atashi et al., 2015; Bakhshandeh et al., 2017). This was reported to be due to the osmotic-regulating effects of higher molecular weight PEG solutions which limits the availability of water to the seed at the high osmoticum. Limited water during germination inhibits provision of dissolved oxygen, absorbable soluble food, the initiation of germination enzymes and bio-regulators necessary for biochemical and molecular mechanisms required for germination and also promote secondary dormancy (Weitbrecht et al., 2011; Leubner-Metzger, 2017; Vishal and Kumar, 2018). Soybean response at -0.02 which was 81.11% showed no considerable change compared to 87.78% at 0MPa (control) but -0.27 was significantly different to control with GP of 78.88 (Figure 2.4). The overall comparison of cowpea and soybean GP showed no significant difference between the two bean types at all OP levels (Appendix 2 and Figure 2.4).

**Table 2.2.** Results on the influence of PEG-8000 on emergence rate (ER) and germination index (GI) of cowpea and soybean genotypes.

Parameters	Osmotic potential (MPa)				
	0	-0.02	-0.27	-1.27	-1.80
<b>ER</b>					
Red	89.28 <sup>ab</sup>	28.80 <sup>cde</sup>	29.63 <sup>cfg</sup>	66.67 <sup>adf</sup>	33.33 <sup>beg</sup>
Blonde	85.74 <sup>a</sup>	98.14 <sup>b</sup>	75.88 <sup>c</sup>	36.11	50.00 <sup>abc</sup>
White	72.48 <sup>ab</sup>	31.62 <sup>cd</sup>	48.52 <sup>ef</sup>	66.67 <sup>ace</sup>	50.00 <sup>bdf</sup>
TGx 1937-1F	61.99 <sup>a</sup>	76.60 <sup>a</sup>	85.33 <sup>a</sup>	62.23 <sup>a</sup>	16.67 <sup>a</sup>
PAN 1664R	72.54 <sup>a</sup>	73.74 <sup>a</sup>	69.05 <sup>a</sup>	40.48 <sup>a</sup>	33.33 <sup>a</sup>
LS 678	72.02 <sup>a</sup>	74.73 <sup>a</sup>	60.35 <sup>a</sup>	26.98 <sup>a</sup>	41.67 <sup>a</sup>
<b>GI</b>					
Red	4.43	2.52 <sup>ab</sup>	1.98 <sup>ac</sup>	1.35 <sup>bcd</sup>	0.32 <sup>d</sup>
Blonde	3.02 <sup>ab</sup>	3.01 <sup>ac</sup>	3.12 <sup>bc</sup>	0.75	0.06
White	2.81 <sup>a</sup>	2.54 <sup>ab</sup>	1.81 <sup>b</sup>	0.27 <sup>c</sup>	0.31 <sup>c</sup>
TGx 1937-1F	2.39 <sup>a</sup>	2.45 <sup>a</sup>	2.60 <sup>a</sup>	2.70	0.40
PAN 1664R	2.23 <sup>a</sup>	1.86 <sup>a</sup>	2.18 <sup>a</sup>	0.99 <sup>a</sup>	0.21
LS 678	2.91 <sup>a</sup>	2.98 <sup>a</sup>	2.12 <sup>a</sup>	1.00	0.53

**Note:** The data is presented as mean value of all replicates. Values in the same row with the same alphabet are not significantly different at p-value  $\leq 0.05$ . n=9.

Apart from the GP; MDG, ER and GI (Table 2.1) are also considered the most informative parameters in the study of plants germination. ER and GI are the ratio of the seeds germinated at day 3: seeds germinated at day 7 and total germinated seeds: time taken for them to germinate, respectively. These assess the quality of the overall germination process of defined seed population and give more about seeds viability under adverse conditions (Mangena, 2020). Considering all the parameters, the difference between cowpea and soybean was not found to be significant (Appendix 1 and table 2.3). However, the stress treatments displayed a significant decrease on MDG and GI, compared to their respective controls, showing the same trend as GP (Figure 2.4 and Table 2.2). ER showed no significance difference at all OP levels, suggesting that water deficit stress impose no effect on ER of both cowpea and soybean (Table 2.2).

**Table 2.3.** Results on the influence of PEG-8000 induced stress on emergence rate and germination index of cowpea and soybean.

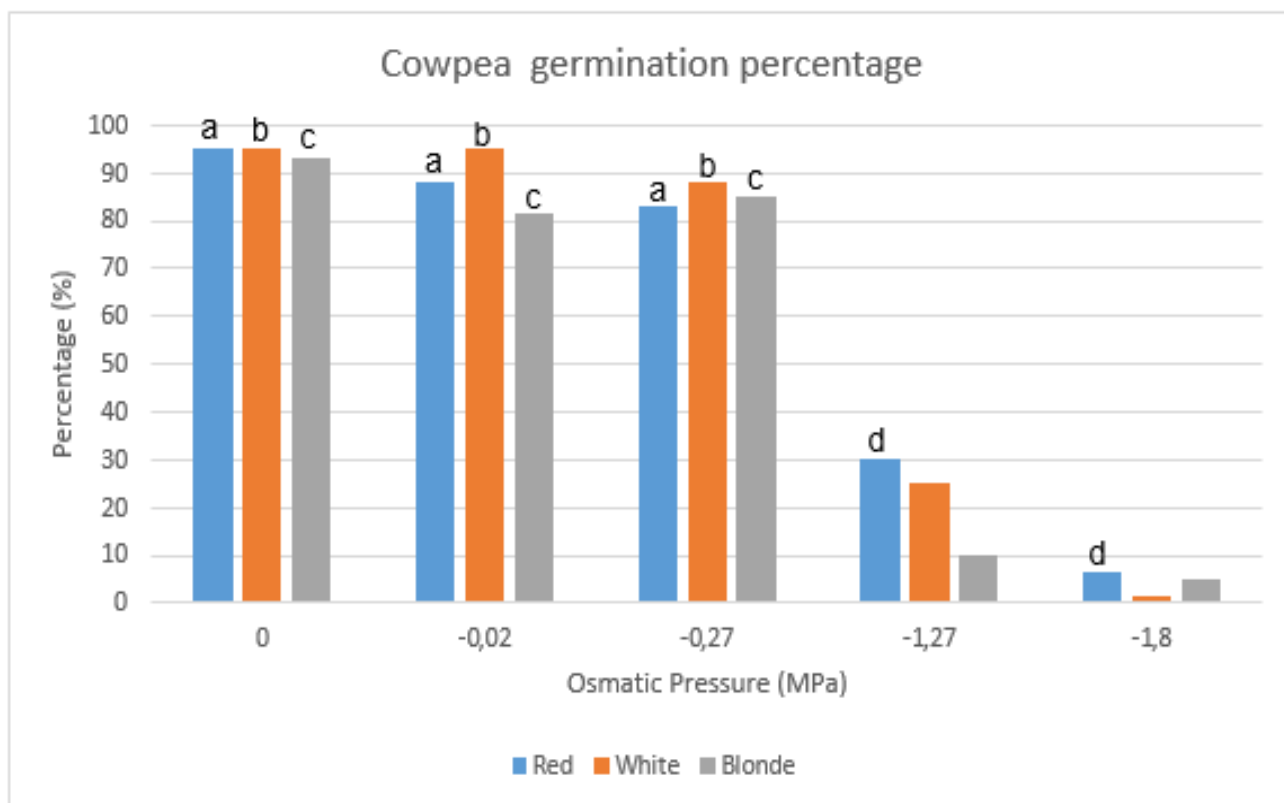
Parameters	Osmotic potential (MPa)				
	0	-0.02	-0.27	-1.27	-1.80
<b>ER Cowpea</b>	82.50 <sup>a</sup>	52.86 <sup>a</sup>	55.68 <sup>a</sup>	56.48 <sup>ab</sup>	44.44 <sup>b</sup>
<b>ER Soybean</b>	68.85 <sup>a</sup>	75.02 <sup>a</sup>	71.58 <sup>a</sup>	43.23 <sup>a</sup>	30.56
<b>GI Cowpea</b>	3.42 <sup>a</sup>	3.36 <sup>a</sup>	2.31 <sup>a</sup>	0.79 <sup>b</sup>	0.23 <sup>b</sup>
<b>GI Soybean</b>	2.51 <sup>a</sup>	2.43 <sup>a</sup>	2.30 <sup>a</sup>	1.58	0.38

**Note:** The data is presented as mean value of all replicates. Values in the same row with the same alphabet are not significantly different at p-value  $\leq 0.05$ . n=27

#### 2.4.1.2 Comparison within bean types

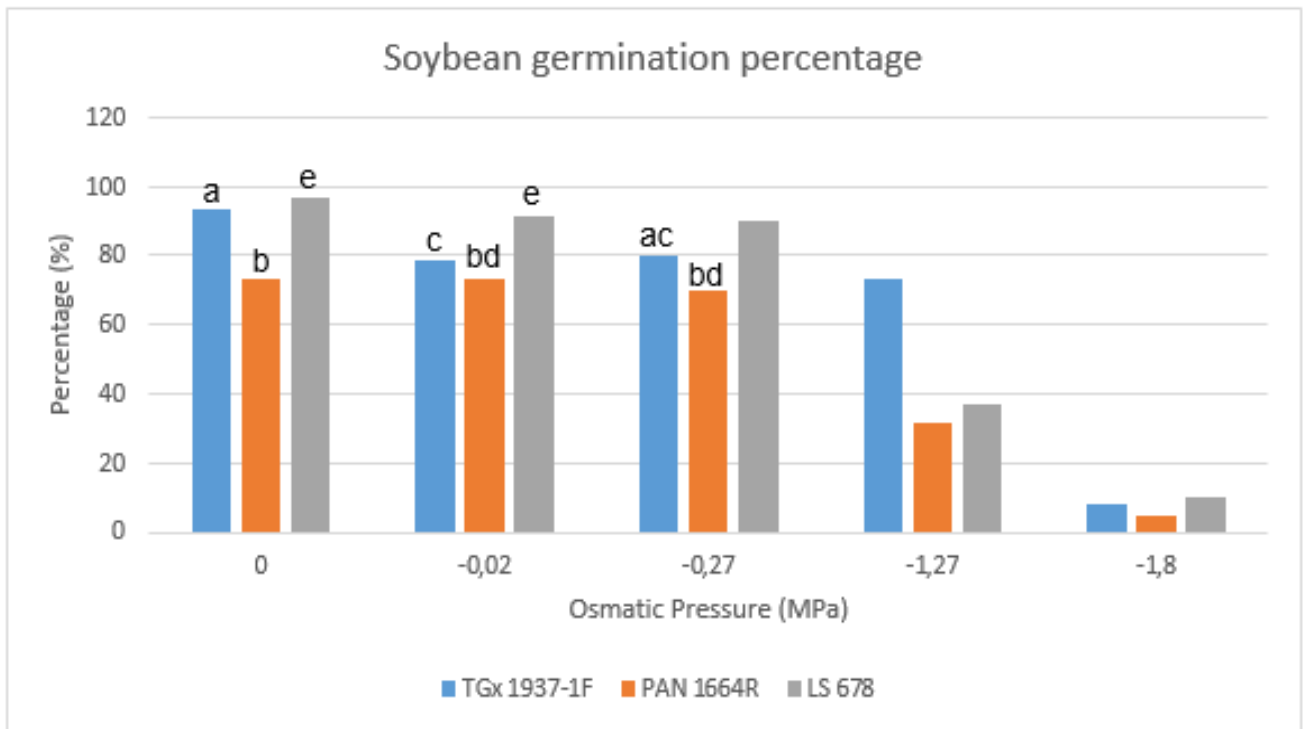
To trace the tolerance of each genotype per bean plant (cowpea and soybean), each genotype was evaluated and assessed for the above-mentioned parameters in triplicates. Cowpea genotypes showed no significance difference between each other and the decrease in GP with the increase in water stress severity was observed. The red and white genotypes had the highest and same GP of 95% at 0 MPa and Blonde recorded 93.33% for the same treatment. The GP between -0.02 MPa and -1.27 MPa ranged between 95% (White at -0.02 MPa) and 10% (Blonde at -1.27 MPa) as presented in Figure 2.5 below. All three genotypes recorded the lowest GP at -1.80 MPa; Red, White and Blonde recorded 6.67%, 1.67%, 5.00% respectively. The GP's at -1.27 MPa and -1.80 MPa were observed to be significantly lower compared to the corresponding controls.

This implies that the GP of cowpea is affected by OP lower or equal to -1.27 MPa. It was also observed that the GP of White genotype was significantly lower than the GP of Red and Blonde at -1.80 MPa, implying GP was more susceptible to the lowest (-1.80 MPa) OP than Red and Blonde. Blonde had lowest GP records for all treatments except at -1.80 MPa (Figure 2.5).

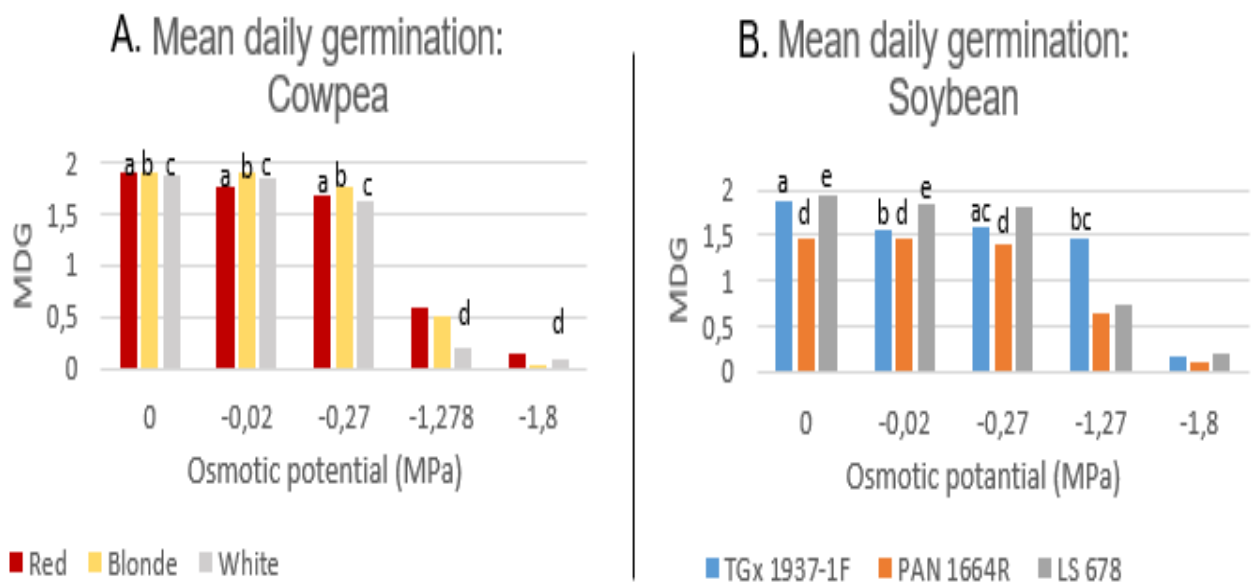


**Figure 2.5.** Germination percentage (GP) of cowpea genotypes germination percentage. The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

A similar trend was observed for soybean genotypes. Control recorded the GP which was significantly higher than the GP at -1.27 MPa and lower. LS 678 had higher GP (96.07%, 91.67%, 90.00%, 30.67% and 10% at 0 MPa, -0.02 MPa, -0.27 MPa, -1.27 MPa and -1.80 MPa, respectively) followed by TGx 1937- 1F (93.33%, 78.33%, 80.00%, 73.33% and 8.33% at 0 MPa, -0.02 MPa, -0.27 MPa, -1.27 MPa and -1.80 MPa, respectively), which had the highest GP at -1.27. PAN 1664R had the lowest GP at all OP levels as compared to TGx 1937 and LS 678 (73.33%, 73.33%, 70.00%, 31.67% and 5% at 0 MPa, -0.02 MPa, -0.27 MPa, -1.27 MPa and -1.80 MPa, respectively) as shown in Figure 2.6. No significant difference was observed between PAN 1664R and LS 678 at -0.02 and -0.27 as shown in Figure 2.6. Moreover, no significant difference was observed between soybean genotypes.



**Figure 2.6.** Germination percentage (GP). Soybean genotypes germination percentage. The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .



**Figure 2.7.** Mean daily germination (MDG). A. Cowpea genotypes mean daily germination, B. Soybean genotypes mean daily germination. The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

It was also observed that ER of all soybean genotypes at -0.02 MPa was higher than control ER. This was explained by various researchers who found that osmo-conditioning using PEG polymers alone or with other compounds at lower concentration improves germination and seedling parameters (including GP, ER, GI and SVI) of various plants including soybean. Baque et al. (2018) suggested that the germination improvement is due to the reserve mobilisation of food material, activation and synthesis of some enzymes, DNA and RNA synthesis that started during osmotic priming. In the case of the current study, the same mechanisms could have occurred as a result of PEG-8000 initiation of the above mentioned processes that started during the imbibition phase which resulted in germination being promoted. Lastly, the estimation of the time it takes for a certain germination percentage to occur (GI) was determined only for the last and final germination record according to Islam et al., 2009.

#### 2.4.2 Seedling growth and development

To assess the growth and development of seedlings, the root and shoot length were measured using a ruler as shown in Figure 2.2 above. Although soybean showed better comparative response to lower OP (-1.27 and -1.80 MPa) with regard to the germination parameters, both root and shoot length of cowpea are longer than soybean (Table 2.4). This imply cowpea tolerance to water stress is better than soybean and it can grow better in drier area because of the longer root system.

**Table 2.4.** Results on the influence of PEG-8000 induced stress on mean shoot length (MSL), mean root length (MRL), and seedling vigour index (SVI) of cowpea and soybean as measured on day 10.

Parameters	Osmotic potential (MPa)				
	0	-0.02	-0.27	-1.27	-1.80
<b>MSL (mm)</b>					
Cowpea	56.95	48.12	32.94	18.47	2.58
Soybean	27.07 <sup>a</sup>	33.20 <sup>a</sup>	22.42 <sup>a</sup>	6.17	0.56
<b>MRL (mm)</b>					
Cowpea	98.72 <sup>a</sup>	88.70 <sup>a</sup>	72.60 <sup>a</sup>	34.4 <sup>a</sup>	11.97
Soybean	80.64 <sup>a</sup>	75.36 <sup>a</sup>	72.08 <sup>a</sup>	25.79	9.72
<b>SVI</b>					
Cowpea	147.68 <sup>a</sup>	106.00 <sup>a</sup>	97.26 <sup>a</sup>	9.75	0.58
Soybean	98.53 <sup>a</sup>	90.13 <sup>a</sup>	77.81 <sup>a</sup>	17.91	0.81

**Note:** The data is presented as mean value of all replicates. Values in the same row with the same alphabet are not significantly different at p-value  $\leq 0.05$ . n=27.

The mean root length of seedlings of these two legume plants are highly affected by water deficit stress as outlined in Table 2.4. It is also clear that all the varieties of cowpea had their mean root length dropping with almost half from control (0 MPa) to the first treatment of PEG 8000 (-0.02 MPa). Red, White and Blonde had 105.57 mm, 90.97 mm and 108.63 mm root length at 0 MPa decreased to 56.17 mm, 56.4 mm and 57.23 mm at -0.02 MPa respectively. The mean root length of the cowpea seedlings showed a significant difference between different cultivars as they decrease from control to the treatment with the lowest osmotic pressure. Blonde had the longest root in the control (108.63 mm) and White the shortest for -1.80 MPa (13mm). Nathai and Singh (2013) and Onah and Donald (2009) reported that plants tolerate water stress by developing a very long, deep diving root system to access

deep water. This suggest cowpea is well adapted to drought condition from early stages of its vegetative growth.

## **2.5 CONCLUSION**

The data discussed in this chapter suggest that the water stress tolerance of the two bean plants (cowpea and soybean) during germination do not differ significantly. All germination parameters for soybean were lower than those of cowpea. However, no statistical difference was detected between the two plants. Considering the seedling growth parameters, all assessed parameters (Mean shoot length, mean root length and seedling vigour index) of cowpea were significantly higher than soybean. In conclusion, cowpea is well adapted to survive in conditions with limited water as compared to soybean.

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## **CHAPTER 3**

# **EVALUATION OF THE EFFECTS OF WATER DEFICIT STRESS ON PHENOTYPIC PARAMETERS OF SOYBEAN AND COWPEA PLANTS**

### 3.1 INTRODUCTION

Although the current generation disregards and consider plant phenotypic research of less meritorious (Petersen and Sobeberg, 1998), plant morphology still exhibit a crucial insight of plant responses to abiotic stress. Recently the modern plant biologist has circumscribed the importance of morphology by giving a greater affinity to molecular and biotechnology data than morphological data. Kaplan (2001) argued that the time for re-evaluating the role of plant morphology in the modern and technologised plant biology is overdue and also that the broader clarity on the relationship between morphology and physiological response is of greater significance.

Plant morphology is a valid scientific discipline that specifically deals with the study of both somatic and reproductive plant forms, patterns, colour and other physical features of plant organs (Mahmood et al., 2010). The concept refers to the general elements of the biological outermost appearance, arrangement aspects and their detailed structures. In contrast, anatomy and physiology refers to the cellular make-ups (internal structure) and functionality respectively (Evert and Eichhorn, 2013; Volkonen, 2008). However, the three terms are only separated conceptually but not practically, they are interrelated. The physical visible structure, an organ (for example, stem) is made of specific cells and is meant to carry out a particular function (Mahmood et al., 2010). This fact allows botanists to study one concept to understand the other. It is reported that when water uptake by plants is limited the physiological process called photosynthesis is slowed down, phenotypically expressed on the size of organs formed and the total accumulated plant biomass (Zlatko and Fernando, 2005; Usada, 2015 ).

Morphology has been useful in various fields of phyto-research including taxonomy, ecology and genetics for identification and relations of plants (Hassemer et al., 2020; Mestdagh et al., 2020). It was through morphological studies that Wilhelm Hofmeister discovered the eminent knowledge of alternation of generations in the plant life cycle (Hofmeister, 1851). Moreover, botanists continue to use morphological analysis to understand and trace the changes in plant's total structure or an individual organ due to unfavourable, harsh environmental conditions and other constrains (such as diseases) (Ponmurugan and Baby, 2007; Sourour et al., 2017; Kozminska et al., 2019; Hussain et al., 2019;). The literature outline that abiotic stress (such as salinity and water deficit stress) significantly affect the morphological

parameters of a plant, thereby inhibiting growth, development and cause a reduction in productivity (Zhang et al., 2013; Li et al., 2019; Kotagiri and Kolluru, 2017; El-Maaty et al., 2020). In this chapter, the effects of water deficit stress at three levels i.e. control, moderate and severe on somatic and reproductive morphological parameters of three soybean and cowpea varieties were evaluated by counting and analysing the parameters directly from the plants. Parameters such as fruit, and nodules were harvested, dried, counted and weighed.

### 3.1.1 Aim

This study was aimed at evaluating the effects of water deficit stress on phenotypic parameters of soybean and cowpea plants.

### 3.1.2 Objectives

The objectives of the study were to:

- i. Evaluate the effect of water deficit stress on both vegetative and reproductive morphological parameters of soybeans and cowpeas
- ii. Assess the similarities and dissimilarities between the responses of soybean and cowpea to three levels of water deficit stress.
- iii. Determine the level of water deficit stress that affect the growth and development of soybean and cowpea.

## 3.2 LITERATURE REVIEW

### 3.2.1 Water deficit stress

Water availability in the soil is an essential environmental factor that has a notable effect on agro-economic crops production worldwide. Water deficit stress on plants is an important abiotic stress caused by an inadequate or insufficient water availability in the soil, subsequently resulting in the plant failing to uptake enough water (de Oliveira et al., 2013). Lack of water in the soil is usually caused by prolonged periods of aberrant dry weather (drought). Periodically, water deficit stress occurs although there is adequate water in the soil. This happens when the concentration of salty elements or compounds (e.g. NaCl) in the soil is too high (salinity). Salinity affects the uptake of water and other nutrients by the plant roots causing water deficit stress (Evelin et al., 2009).

Water deficit stress is a devastating and restraining factor of plant growth and yield. It damages cells and disrupt the plant cellular, molecular, physiological and metabolic activities (Shukla et al., 2012; Sourour et al., 2017; El-Maaty, 2020). On average, commercial crop production is reduced by over 49% when exposed to dry conditions (Boyer, 1982; Shiferaw et al., 2014). Plants respond differently to dry environments, some are well adapted (e.g. *Helianthus annuus*) while others are very susceptible (e.g. *Zea mays*) (Hussain et al., 2018; ASR, 2019). The volume of water availability (degree of drought) and duration of dryness that induce water deficit stress differ from species to species. The time of plant developmental stage at which the dryness started is also crucial in determining whether the plant will be affected or not. Some species can only be affected if drought starts at early stage of development (McDowell et al., 2008). Species like *Zea mays* can survive mild to moderate stress but fail to withstand severe stress (Iglesias et al., 2018).

Plant leaves generally serve as a food factory of the entire plant, making them the most vital and integral part of the plant body (Evert and Eichhorn, 2013). Leaves are responsible for harvesting light, CO<sub>2</sub> intake, which are the two of three ingredients of photosynthesis (the process by which plants make their own food by converting light, carbon dioxide (CO<sub>2</sub>), and water to sugar) (Evert and Eichhorn, 2013). This is made possible by the presence of chloroplasts, the site of photosynthesis found in the leaves. Therefore, their surface area is directly linked to amount of food a plant can synthesise (Mullan and Pietragalla, 2011). The plant leaf area is also associated with another process which is called evapotranspiration

(Evert and Eichhorn, 2013). Reports suggest plants which grow in drier areas with high temperatures or suffer water stress have smaller leaves than plants growing in areas with adequate water supply and low sunlight availability (van der Vyver and Peters, 2017). Leaf area measurement helps commercial farmers and crop scientists to know how plants are adapting to the environment (Cowling and Field, 2002).

### 3.2.2 Morphology of soybean and cowpea

The success of plants vegetative and reproductive growth and development depends on the interaction of two major factors; genetic (internal, including tolerance resistance to abiotic stress, enzyme activity, rate and types of various biochemical reactions such as photosynthesis) and its environment (external, soil moisture, temperature). Normally, plants in the field, under natural conditions have their eminent environmental conditions naturally provided, although farmers manipulate them to maximum production of commercial crops (Nascimanto and Cecilia, 2008). The manipulation techniques include wise tillage management, use of specific fraternisers, weed and insect controls and others (van der Vyver and Peters, 2017). The environmental conditions have notable effect on the visible aspects of the plant growth- morphology. Farmers usually use the morphology to understand the physical response of plants under these straining conditions and decide which practise should be deployed to maximise production (Potocka and Szymanowski-Putka, 2018).

#### 3.2.2.1 Soybean

According to Azam et al. (2021) there are over 333 cultivars of soybean and are classified according to their morphological growth habit, day length and temperature requirements. The commercially cultivated soybeans are generally annual erect bush type although the wild and semi-wild varieties vary from spreading and vinery types (Goanka and Rosentrater, 2019; Poehlman, 1987). The stem growth habit can be determinate or indeterminate (Khan et al., 2018). The determinate type have their vegetative growth terminated and the terminal bud grows into an inflorescence. Flowering occurs throughout the plant, pod and seed develop and mature uniformly (Kato et al., 2015). The indeterminate type does not form a terminal inflorescence, the plant continues to grow taller while the lower parts produce flowers. Pods and seed at lower parts of the plant mature and ripe before those at the top (Kato et al., Khan et al., 2018).

Soybean suffers water stress when there is either a deficit in water availability or excess water in the soil. Excess water damages the root system and water shortage results in dehydration (Akhtar and Nazir, 2013; He, 2008). Day-length is also a vital factor of soybean growth and development (Nimje, 2019). Soybean is naturally a short day-length (less than 12hours) crop producing less dry matter. Cultivars growing in the summer seasons of the temperate region with long day-length (14-16 hours) turn to grow shorter with fewer nodes and flower earlier (Major et al., 2014; Nimje, 2019). Air and soil temperature affects both vegetative and reproductive growth of soybeans. The soil temperature of between 22 and 27°C is considered to be favourable for soybean root growth and air temperature of below 20 °C inhibit flowering or above 32 °C increases flower and pod abortion (Major et al., 2014).

### 3.2.2.2 Cowpea

Cowpea commercial crop cultivars show an extreme variation in growth habit, with many being vine, but other are modern with more upright, bush type cultivars (Timko, 2007). These cultivars are classified according to fruit or seed size, shape and colour (e.g. Black-eyed, Pink-eyed, Brown-eyed, Cream cultivars etc.) (DAFF, 2014). Cowpea is heat-loving and is well-adapted to hot areas, growing best during hot season (summer). It is a drought-tolerant crop with the optimal growth and development temperature around 20 to 30 °C. Cowpea is much less tolerant to cold soil but can tolerate higher rainfall areas. Most cowpea cultivars are photosensitive and they can flower within 30 days after sowing if the day-length is shorter (DAFF, 2014). The nodulation process that is due to the specific *Bradyrhizobium* spp. helps improve tolerance and adaptation to extreme hot regions (Jansen van Rensburg et al., 2007). Cowpea utilises soil moisture efficiently and can grow satisfactorily in areas with an annual rainfall of between 400 and 750 mm (DAFF, 2014).

**Table 3.1:** Soybean and cowpea morphological similarities and dissimilarities.

Trait	Soybean	Cowpea
Root	Soybean has a taproot system, consists of the primary root that may go deep into the soil depending on environmental conditions. They are usually 30-60	Cowpea has a long and strong taproot. The taproot can penetrate to the depth of 2.5 m in just 8 weeks after planting. The lateral roots profusely subtend from taproot and

	cm long and about 80-90% Of the dry weight occurring on the top 15 cm. Nodulation may start and evident visible nodules may be formed within 10 days after planting (BTE, 2021).	spread in the surface soil. Cowpea is able to form beneficial association with microorganism present in the rhizosphere, prompting formation of nodules (DAFF, 2014).
Leaves	Soybean has two main types; (i) primary ovate shaped with a petiole of 1-2 cm long and stipules at point of attachment and (ii) trifoliolate (very common) leaves that have a long petiole, the lamina is oval to elliptic-lanceolate shaped. They are alternately attached to the stem (Purcell et al., 2014)	The leaves of cowpea have been used as a diagnostic characteristic in the classification and differentiation of cultivars. This is because the size (ranging from 6 to 11 cm) and shape of cowpea leaves greatly differ and are generally alternate trifoliolate. Their colour is usually dark green, and linear-lanceolate to ovate in shape. The petiole length range from 5 to 25 cm long (Pottorff et al., 2012).
Stem	An old, mature and well developed stem may have 0-6 lateral branches. Stems can grow to as long as 20 cm to 100 cm depending on environmental conditions (Bennett et al., 2014; DAFF, 2010).	Cowpea stems usually have purple shades or marked with striae, they can be smooth or a bit hairy (SARI, 2012).
Inflorescence	The inflorescence is axillary raceme with a typical papilionaceous flowers of soybean are made of a tubular calyx with 5 sepals, a corolla (made of 1 standard petal, 2 lateral wing petals and 2 anterior keel petals). They have 10 stamens, 9 are fused and	The inflorescence is racemose attached to the stem with a very long peduncle (about 5-60 cm). Flowers are self-pollinating, with bright corolla (purple, pink, white, yellow and pale blue), conspicuous and alternately arranged in pairs on short pedicels (DAFF, 2014).

	the separated stamen is posterior (Purcell et al., 2014).	
Pod	Pods exhibit different colours depending on cultivars. They are about 2-7 cm long and 1cm width and containing the average of 2 to 3.5 seeds when matured. However, other cultivars can contain up to 5 seeds per pod. The pods are straight or slightly curved (rare) with short stalk and clustered together (Shurtleff and Aoyagi, 2015).	Usually the pods can be 7 to 30 cm long, containing 8 to 20 seeds in average. Pods are slightly curved, erect, crescent-shaped or coiled. Yellow, brown or purple in colour when ripe (Pottorff et al., 2012).
Seeds	Seed size may vary from 5-40 g per 100 seeds with most cultivars between 10 and 20 g. The seed colours and shape may differ from one variety to the next. Most seed are spherically shaped but some are flattened and elongated (Purcell et al., 2000).	Seeds vary significantly in size, shape and colour. The seeds are large (2 to 12 mm), kidney to more spherical shaped, and weigh 5 to 30 g per 100 seeds. Their colour and texture vary. They can have a smooth or rough coat and are sparkled, mottled or blotchy with colours including green, red, brown, blonde, black or various combinations (SARI, 2012)

### 3.2.2.3 Growth stages of soybean and cowpea

Growth stages of soybean and cowpea are differentiated as vegetative stages (V) and reproductive stages (R). The V and R growth stages are subdivided by the addition of numbers after either V or R to designate a specific V or R stage. For example, in V stages

there is V1, V2, V3 (numbers designates the number of nodes) through to Vn (where n represent the nth number of nodes or the last node count) as shown in Table 2.3 below, R stages are divided from R1 to R7 through to RH as shown in Table Below. The V-number designation excludes the first two stages VE (emergence stage) and VC (cotyledons stage).

<b>Table 3.2:</b> Description of vegetative and reproductive growth stages for soybean and cowpea according to Mark, 2014 and Martine et al., 1976	
Growth stage	Stage description
<b>Vegetative growth</b>	
VE- Emergence	Cotyledons produced at the first node
VC- Cotyledon	Unifoliate leaves unrolled sufficiently, with the leaf edges not touching. Cotyledons fall, leaving scars
V1- Node three	1 <sup>st</sup> trifoliate leaf fully expanded at node three, with leaf edge not touching
V2- Node Four	2 <sup>nd</sup> trifoliate leaf fully expanded at node four
V3- Node five	3 <sup>rd</sup> trifoliate leaf fully expanded at node five
V4- Node six	4 <sup>th</sup> trifoliate leaf fully expanded at node six + branching
Vn- Node n+2	n <sup>th</sup> trifoliate leaf fully expanded at node (n+2)
<b>Reproductive Growth</b>	
R1- Beginning bloom	One open flower
R2- Full bloom	50-100 % open flowers at one of the uppermost node
R3- Beginning pod	One pod expanded to maximum length
R4- Full pod	50 % of pods expanded to maximum length
R5- Beginning seed	One pod with fully developed seeds at one of the four uppermost nodes
R6- Full seed	50 % of pods with fully developed seeds at one of the four uppermost pod
R7- Physiological maturity	One pod reached mature colour
RH- Harvest maturity	80 % of pods reached mature colour

### 3.2.3 Plant organs under water stress.

#### 3.2.3.1 The root

Plant response to drought stress differs from one species to the other. It is evident that the roots are the first organ to detect water stress, both insufficient and excessive (Lisar et al., 2012). This is because roots are the primary organ in direct interaction with soil conditions and they play a vital role in response to water stress (Lisar et al., 2012; Sun et al., 2020). They detect and send chemical signals to the shoot (stems and leaves), leading to biochemical and molecular responses initiated and also the promotion of morphological changes that help protect the plant against water loss (Chaves, 2002). Some of such morphological changes happens on the roots. Jackson and Sperry (2010) reported that the function, structure (length and diameter) and movement of the root depends on soil water availability. In their experiment, it was evident that the production of cereal plants planted under drought conditions was increased by altering the root structure. Most plants tolerate water stress by developing a very long, deep diving root system to access deep water. This was also reported in the case of cowpea varieties (DAFF, 2014; Nathai and Singh, 2003), rice varieties and several other commercial crops (Onah and Donald, 2009).

#### 3.2.3.2 The stem

Drought stress negatively affects stem growth and development. It reduces stem elongation resulting in shorter stems, by affecting cell wall extensibility which is regulated by multiple enzymes including expansins (Lisar et al., 2012). The expansin activity and expression of genes encoding them can decrease due to severe stress. The inhibited activity of this hormone results in steady reduction in cell division and expansion leading to affected nutrient uptake and transportation, interference with phytohormones signalling which affects growth and development in general (Obidiegwu et al., 2015).

#### 3.2.3.3 The leaf

Leaves are one of the sites of photosynthesis. Water stress limits both leaf area and photosynthetic rate per unit area. The other mechanism of adaptation and drought tolerance is sclerophylly- the production of hard leaves which helps leaves not to suffer permanent damage due to wilting (Basu et al., 2016). Leaf shedding increases (number of leaves decrease) when plant suffer water stress. This is as a means to reduce transpiration, helping

with tolerance and dehydration avoidance as more water is retained. The rapid water loss due to transpiration can also be avoided by stomatal closure. Stomata are an essential leaf part that facilitate a critical role in the process of gaseous exchange such as in transpiration and photosynthesis. There are tens to hundreds of stomata on the upper and lower surfaces of the leaf with the lower surface having more (Lisar et al., 2012). Stoma is simply defined as a tiny opening made of specialised epidermal (subsidiary and guard) cells of the leaf (Osakabe et al., 2014). Light microscope have been used to view and study stomatal cells. Stomata function in controlling and monitoring water moisture balance, depending on environmental conditions and turgor pressure that is triggered by the osmotic flow of water in the guard cells (Urban et al., 2017). When the guard cells are turgid, the increase in the size of guard cells results in the opening of the stoma (Lisar et al., 2012). Under water stress the guard cells lose water, becomes flaccid and the stoma is kept closed completely, especially during water severe stress leading to the number of stomata decreasing (Reynolds and Tuberosa, 2008). These is another morphological change that plants deploy as a tool to tolerate water stress.

#### 3.2.3.4 Flowers, pods and seeds

Drought during reproductive growth substantially limits flowering, promotes flower abortion, poor pod filling and subsequently reduced fruit (seeds in case of beans) or production (Basu et al., 2016).

### 3.3 MATERIALS AND METHODS

#### 3.3.1 Experimental design

Two experiments were set up, one for vegetative growth assessment and the other for reproductive growth analysis. 15 seeds of three soybean and cowpea cultivars (with erect stem) were germinated in 15 cm x 1.5 cm petri dishes in triplicates. Soybean seeds were germinated November 2019 and cowpea were germinated November 2020. The germinated seeds were planted in specially designed tall pots that allows for root elongation – one seedling per pot (Figure 3.1A). There were 3 replicates for each cultivar per treatment making a total of 27 plants per bean type. The treatments were: Control – where plants were watered daily to field capacity; Moderate water deficit stress – where the plants were watered once a week (seven days); and severe water deficit stress – where the plants were watered once in ten days. The plants were arranged in a randomised block design and watered with Hoagland solution in the Glass house (100 ml each plant per week) with the natural environmental conditions. The stress was induced two weeks after plants were transplanted, growth stage-V3 (Figure 3.1C), five nodes and three fully expanded trifoliolate leaves (Table 3.2).

**Table 3.3:** Chemical composition of Hoagland nutrient solution used to provide plants with nutrients. The solution was prepared following Hoagland and Snyder, 1933.

Chemical nutrient formula	Concentration of stock solution (mM)	Grams of salt used (g)	Volume of stock solution per litre (L)
<b>Macronutrients</b>			
KNO <sub>3</sub>	1.00	101.10	6.0
Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	1.00	236.16	4.0
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub>	1.00	115.08	2.0
MgSO <sub>4</sub> .7H <sub>2</sub> O	1.00	246.48	1.0
<b>Micronutrients</b>			
KCL	25.00	1.864	2.00
H <sub>3</sub> BO <sub>3</sub>	12.50	0.773	2.00
MnSO <sub>4</sub> .H <sub>2</sub> O	1.00	0.169	2.00
ZnSO <sub>4</sub> .7H <sub>2</sub> O	1.00	0.288	2.00
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.25	0.062	2.00
H <sub>2</sub> MoO <sub>4</sub>	0.25	0.040	2.00

(85%MoO <sub>3</sub> )			
*Na.Fe.DTPA	52.7	30.00	0.3-1
*= Fe source, 10% Fe			

### 3.3.2 Assessment of morphological parameters

The following counts and measurements were carried out for each plant during or at the experiment termination;

#### 3.3.2.1 Vegetative growth

##### i. Number of leaves

The number of leaves were counted twice, firstly, three weeks after stress onset and secondly, three weeks after the first record.

##### ii. Length of the stem

The stem height was measured twice, firstly, three weeks after stress onset and secondly, at the termination of the vegetative experiment (at the beginning of R1).

##### iii. Root length and mass of the nodules

At the beginning of stage R1, the vegetative experiment was terminated. Plants were removed from the pots and the root length was measured. The nodules were also harvested, dried and weighed.

#### 3.3.2.2 Reproductive growth

##### i. Number of flowers

Open flowers were counted daily from R1 until the beginning of R3.

##### ii. Number of pods

Number of pods were counted from R3 until the beginning of R5.

##### iii. Seed per pod

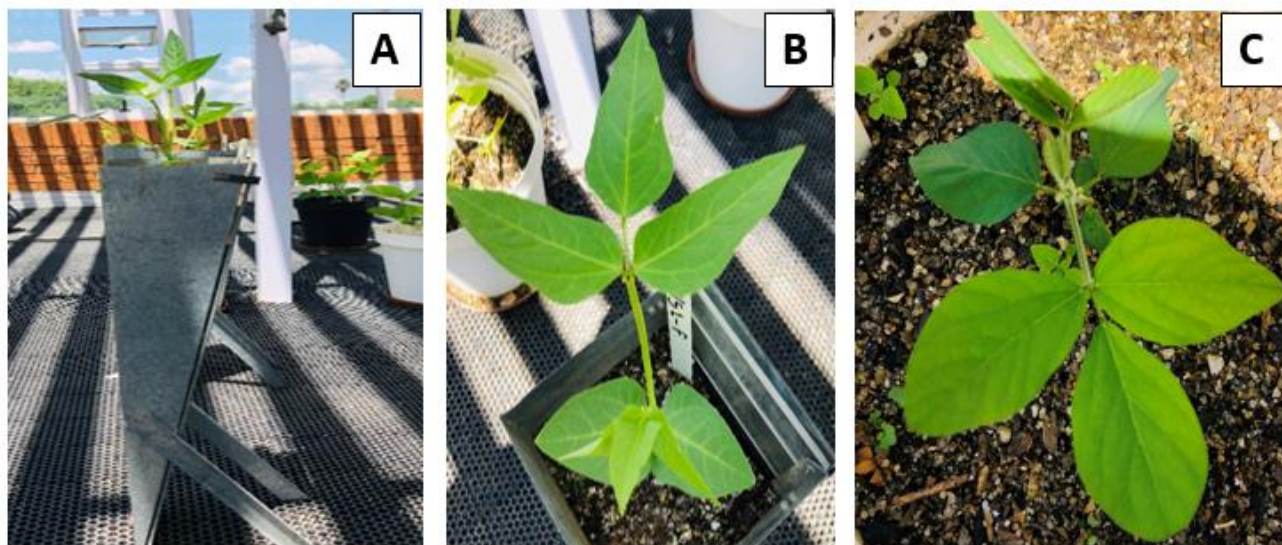
After all seeds were harvested at RH stage, the number of seeds per pod were counted.

##### iv. Mean seed number per pod

The mean seed number per pod was calculated by adding the total number of seeds per pod divided by the total number of pod for each plant.

#### v. Mass of 100 seeds

At the end of RH stage, all seeds were harvested, dried and the mass of 100 seeds was weighed.



**Figure 3.1:** Transplanted seedlings: A, The seedling of cowpea (Blonde) a week after transplanted into the specially designed tall pot. B, Cowpea (Red) seedling a day after transplanted. C, Soybean (PAN 1664- R) seedling a week after transplanted.

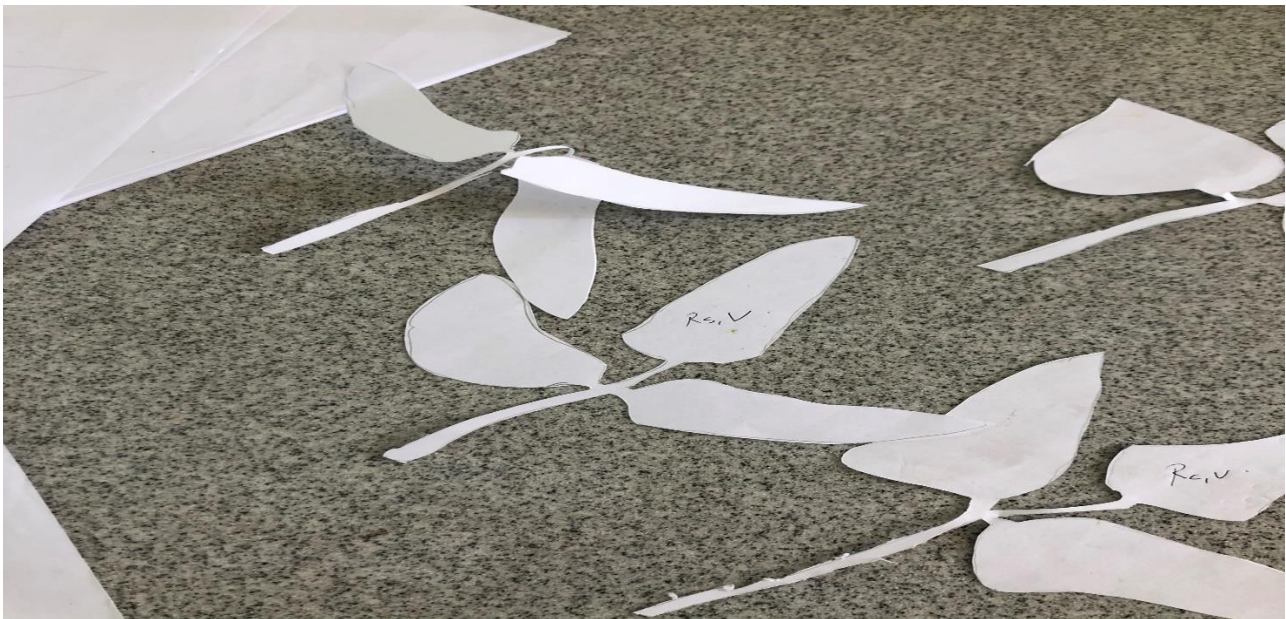
#### 3.3.2 Estimation of stomata per unit area

Three youngest fully expanded trifoliate leaf per plant were harvested. The “nail polish blotting method was used” to prepare the temporary stomatal slide following Elisabet et al. (2017) with modifications. Clear nail polish was applied on both abaxial and adaxial surfaces randomly making three small circular spots of about 7-10 mm diameter. The nail polish was allowed to completely dry, then the blotting thin film layer was peeled off using forceps. The blotting film was used to prepare a temporary light microscopy slide by placing it on microscopy slide and adding a drop of glycerin. The Leica DM500 Microscope from Leica microsystems was used to count the stomatal prints and the area of view for the 40X objective was calculated using a stage graticule. The area of view was 1.54 mm<sup>2</sup>.

### 3.3.3 Estimation of leaf surface area

The leaf surface area (LSA) of both cowpea and soybean was determined by harvesting the youngest fully expanded trifoliate leaf and traced on a blank print paper. The trace was cut out and weighed on an electronic balance in triplicates (Figure 3.2). The squares paper print of 5.0 cm x 5.0 cm was cut out and weighed. The formula below was used to estimate the surface area of each leaf:

$$\text{LSA} = 25.00 \text{ cm}^2 \times \text{mass of leaf trace} / \text{Mass of } 25.00 \text{ cm}^2 \text{ paper}$$



**Figure 3.2:** The blank print paper trace cuts of cowpea leaves.

### 3.3.4 Data analysis

The obtained data for analysis within bean type was presented as mean values of all replicates for all treatments. For comparison between bean types, each value represent a mean of three cultivars in triplicates per bean type. Analysis of variance (ANOVA) was done using IBM SPSS statistic 27 to determine if there is significant difference between treatments, cultivars and/or between cowpea and soybean with regards to water deficit tolerance at 95% level of confidence. The student t-test was further used to compare and determine which parameters and treatments between the cultivars and/or cowpea and soybean account for water deficit tolerance and/or susceptibility. These analysis were conducted using student t-

LSD (least significant difference) at 5% level of confidence. The statistical outcomes are recorded under appendix 3, 4 and 5.

## 3.4 RESULTS AND DISCUSSION

### 3.4.1 Vegetative parameters

#### 3.4.1.1 Comparison between bean types

The number of leaves of both bean plants were recorded twice. The first record was done three weeks after first day of stress (to assess early effects of water deficit) and second record was done three weeks after the first record (to assess late effects of drought stress). Based on statistical analysis, significant difference was detected between soybean and cowpea for the number of leaves (1<sup>st</sup> and 2<sup>nd</sup> count), shoot length (1<sup>st</sup> and final measurement), root length, number of stomata per unit area and LSA (Table 3.4). The number of leaves of cowpea were lower than the number of leaves that soybean recorded for all treatment on 1<sup>st</sup> and 2<sup>nd</sup> count. Cowpea recorded 16.50, 14.78 and 14.50 for control, moderate and severe on the first count respectively while soybean had 24.60, 20.44 and 19.33 on the first count respectively. Both beans had their control 2<sup>nd</sup> count increased compared to the 1<sup>st</sup> count, however, moderate (21.61) of soybean slightly but not significantly increased compared to moderate (20.44) 1<sup>st</sup> count, severe 2<sup>nd</sup> count decreased from 19.33 (1<sup>st</sup> count) to 15.67 (2<sup>nd</sup> count).

Moderate stress treatment of cowpea 2<sup>nd</sup> number of leaves count (16.11) significantly increased compared to the 1<sup>st</sup> count (14.78), however, no significant difference was found between severe 1<sup>st</sup> (14.50) and 2<sup>nd</sup> (14.89) count (Table 3.4). Soybean showed a strong significant difference in response to different stress treatment, both moderate and severe were significantly lower than control. However, no significant difference was spotted between moderate and severe which suggest that water stress only have mild to moderate effects on number of soybean leaves at early stage (Table 3.4). For final count, a significant decrease in number of leaves was observed with the increase in severity of the water stress. These results agree with the findings of Likoswe and Lawn (2008) who reported that soybean displayed a significant decrease in the number of leaves under stress conditions and attributed the effect to the rate of senescence that is rapid in soybean and slower in cowpea. Rivas et al. (2016) with similar finding argued that the less sensitivity of cowpea leaf to gaseous exchange promotes leaf retention and improves tolerance in general.

The shoot length of cowpea was longer than the shoot length of soybean for all treatment (control, moderate and severe) on both 1<sup>st</sup> and final measurements. Cowpea recorded 25.00

cm, 23.47 cm and 20.03 cm for control, moderate and severe treatment on 1<sup>st</sup> measurement respectively. On the second measure, it recorded 37.51 cm, 32.14 cm and 23.32 cm for control, moderate and severe treatments respectively. The soybean records were 24.75 cm, 19.68 cm and 15.78 cm (1<sup>st</sup> measurement) and 32.05 cm, 21.47 cm, 18.21 cm for control, moderate and severe treatments respectively. No significant difference was observed between the control treatments of soybean and cowpea shoot length (Table 3.4). However, soybean was significantly shorter than cowpea in the moderate and severe treatments (Appendix 5). These results implies that water stress cannot inhibit vegetate growth, especially stem elongation in cowpea while soybean suffers. The tolerance in cowpea is driven by the rapid recovery of photosynthesis reaction which allows plants to effectively use energy on growth and development (Rivas et al., 2016).

**Table 3.4.** The effects of induced water deficit stress on vegetative growth parameters of soybean and cowpea.

Parameters	Soybean			Cowpea		
	Control	Moderate	Severe	Control	Moderate	Severe
No. of leaves 1 <sup>st</sup> count	24.6±4.79 <sup>ab</sup>	20.44±3.47 <sup>a</sup>	19.33±2.38 <sup>b</sup>	16.5±4.54 <sup>c</sup>	14.78±3.84 <sup>cd</sup>	14.5±2.98 <sup>d</sup>
No. of leaves 2 <sup>nd</sup> count	32.11±4.21	21.61±2.62	15.67±2.30	22.83±4.99	16.11±3.85 <sup>c</sup>	14.89±3.23 <sup>c</sup>
Shoot height (cm) 1 <sup>st</sup>	24.75±2.90	19.68±2.64	15.78±2.68	25±3.23	23.47±2.10	20.03±1.61
Shoot height (cm) final	32.05±6.77	21.47±2.94	18.21±2.75	37.51±5.27 <sup>c</sup>	32.14±4.89 <sup>c</sup>	25.52±3.85
Root length (cm)	41.77±1.37	40.02±2.08	32.11±3.89	52.93±4.99	52.37±3.31	48.27±4.52

Nodule weight (g)	1.36±2.07	0.61±0.09	0.38±0.23	0.17±0.10	0.17±0.011	0.10±0.04
Leaf surface area (LSA) cm <sup>2</sup>	39.36±3.74	31.64±4.80	18.21±0.68	46.62±0.48 <sup>c</sup>	46.43±0.53 <sup>c</sup>	31.64±1.34
No. of stomata abaxial	9.14±2.58 <sup>a</sup>	9.51±2.31 <sup>a</sup>	7.54±2.23	11.70±2.27 <sup>c</sup>	11.63±2.08 <sup>c</sup>	10.63±1.88
No. of stomata adaxial	4.78±1.12 <sup>a</sup>	3.67±1.33 <sup>ab</sup>	2.81±1.42 <sup>b</sup>	6.15±1.20 <sup>c</sup>	5.30±1.23 <sup>cd</sup>	4.93±1.11 <sup>d</sup>
<b>Note:</b> Each value represent a mean± standard deviation of three cultivars in triplicates per bean type. Values in the same row with the same alphabet are not significantly different at P≤0.05. n=27						

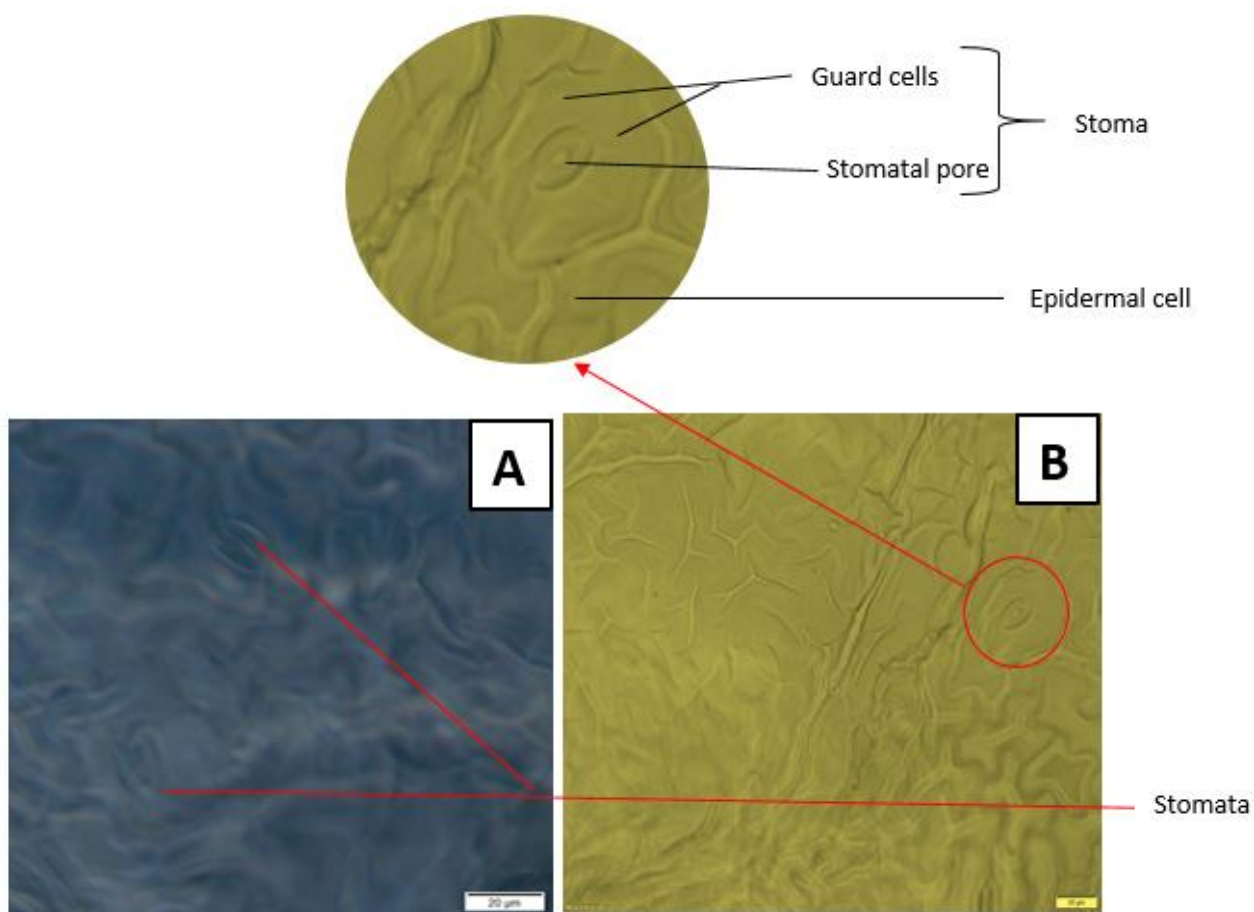
The healthy vegetative growth at early stages of cowpea under drought stress (especially under moderate stress treatment) is attributed to the root ability to vigorously grow although there is a limited water supply (Singh, 2003). The results on final measurements of shoot length pronouncedly showed that cowpea is taller than soybean with control recording 37.51 cm against 32.05 cm and severe recording the shortest height for both beans with 18.21 cm and 25.52 cm for soybean and cowpea respectively. Both cowpea and soybean stem significantly decreasing with an increase in severity of water stress (Appendix 5). These results agree with the study on growth and yield of soybean under drought that discovered that soybean shoot height, dry weight and dry matter distribution sharply decrease with the increases in stress conditions (Khan et al., 2016). Hussein et al. (2014) reported that withholding water at late stage of vegetative growth resulted in decreased plant height and fresh stem weight. The osmotic potential decrease due to water shortage and this phenomenon interrupts water intake which lead to reduction in stem elongation due to inhibited activities of expansins and its expression as stressed by Lisar et al. (2012) and Obidiegwu et al. (2015).

The root length was recorded once at the end of the vegetative growth experiment (beginning of R1), the results showed that the root length of cowpea was considerably longer than the root length of soybean. Soybean control treatment recorded 41.77 cm, moderate treatment (40.02) and severe treatment (32.11 cm) while cowpea had control (52.93 cm), moderate (52.37 cm) and severe treatment (48.27 cm). The root length of cowpea was significantly longer than soybean. Both beans' root length decreased with the increase in stress severity. However, unlike with soybean, cowpea shows no significant decrease comparing control treatment with moderate. The significant decrease was found between control and severe stress treatment. This results indicate that soybean is less tolerant of water stress than cowpea although cowpea is also vulnerable to severe stress (Table 3.4). A study on drought stress tolerance screening of cowpea cultivars collected worldwide reported that most cowpea cultivars produced a higher root dry weight (meaning had long root system) and it could be responsible for the higher water stress tolerance in cowpea (Carvalho et al., 2019).

DAFF (2014) and Nathai and Singh (2003) reported that cowpea tolerates drought stress by developing a very long, deep diving root system to access deep waters. Similar modifications are reported on selected rice varieties and lot other commercial crops (Onah and Donald, 2009; Avramava et al., 2016; Belachew and Stoddard, 2017; Burrige et al., 2017). The report by Hsiao and Xu (2014) stated that short-statured plants such as rice, wheat and beans use the "deep-root" technique to acquire more water and cope with stress. The root development of plants is directly influenced by auxin response factors (ARFs). These are responsible for activation of transcription factors and gene regulation. They function as both activators and repressors (Choi et al., 2018). Under drought stress, the overexpression of ARF 5, 7 and 8 influences the overproduction of auxin (specifically SHY2/ IAA3) which lead to longer root production (Mangano et al., 2017). Santos et al. (2020) reported that cowpea displays the same mechanisms and invest in root development when water stress is detected. In another study Gaofa et al. (2017) discussed the Vg50 cultivar of cowpea as revealing root growth enhancement under water stress, something that is not common with soybean.

The abaxial surface recorded relatively lower count of stomata compared to adaxial surface (Figure 3.3). The number of stomatal count showed a sharp decrease for both abaxial and adaxial surface of the leaves of soybean. No significant decrease was observed between control and stress treatments for both abaxial and adaxial surfaces of cowpea leaves (Table 3.4). The decrease in the number of stomata in both leaf surfaces is a morphological change

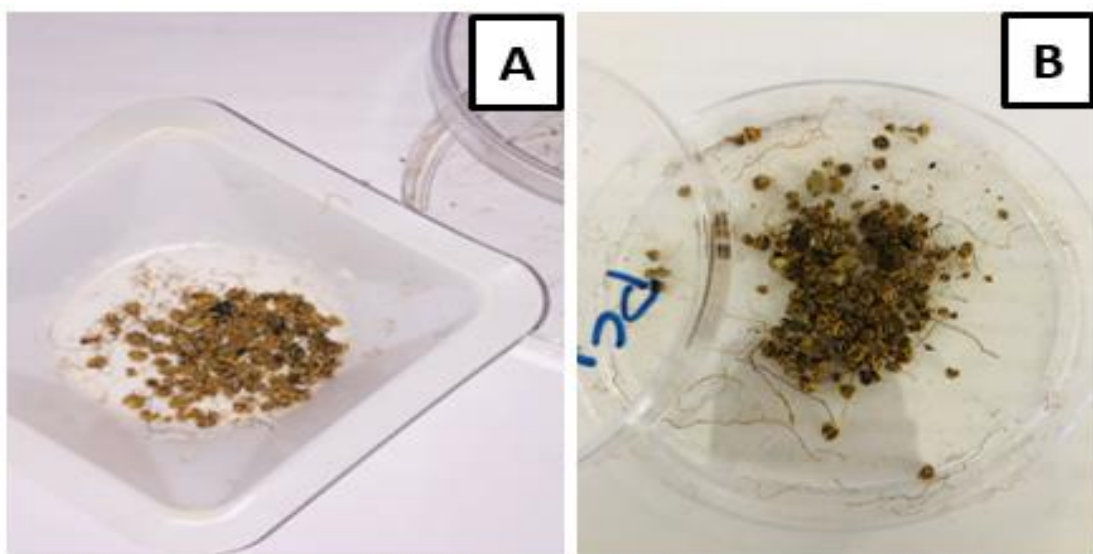
that plants deploy in response to water stress (Pirastesh-Anosheh et al., 2016). This is done to reduce the amount of water loss due to transpiration. However, the closing of stomata results in reduced CO<sub>2</sub> assimilation and the total net photosynthesis (Nemeskeri et al., 2015). Reynolds and Tuberosa, 2008 reported that the guard cells of the stomata lose water under drought conditions, becoming flaccid and kept closed most of the time, especially during water severe stress leading to the number of stomata decreasing.



**Figure 3.3:** Microscopic image: A, microscopic leaf tissues showing stomatal cells of cowpea (White) plant. B, Microscopic leaf tissues showing stomatal cells of soybean (LS 678) plant.

Leaf surface area (LSA) of cowpea was significantly bigger than LSA of soybean. There was no significant difference found between the LSA of cowpea at different treatment, control (46.62 cm<sup>2</sup>), moderate (46.43 cm<sup>2</sup>) and severe (31.64 cm<sup>2</sup>). The soybean LSA showed a significant decrease from control (39.36 cm<sup>2</sup>), moderate (31.64 cm<sup>2</sup>) and severe (18.21 cm<sup>2</sup>) as shown in Table 3.4. The leaf surface area (LSA) primarily estimates how much space is available for the essential processes such as photosynthesis, gaseous exchange and evapotranspiration have to occur since a leaf is their site. These processes are directly

proportional to LSA, for example, the increase in LSA increases the gross photosynthesis (Lisar et al., 2012). In this study the cowpea LSA was considerably higher than the LSA of soybean with cowpea reaching the largest LSA of 46.62 (control) and soybean recording the lowest 18.21 (severe). The LSA of soybean significantly decrease with an increase in stress severity. Cowpea showed no significant difference between control and moderate stress treatment but severe stress significantly differ from both control and moderate stress treatments. The nodule weight was the second vegetative parameters that soybean recorded a significantly higher figure than cowpea following number of leaves (Table 3.4). Soybean (1.36g) nodule weight was more than triple that of cowpea (0.17g) recorded for control (Figure 3.4). However, soybean nodule weight significantly decreased as the stress severity increased. Cowpea with its lower nodule weight maintained no significant difference between all treatments. The lower mass of cowpea nodules is not attributed to the water stress but the failure of cowpea to establish the symbiotic relationship with nitrogen fixing bacteria (*Bradyrhizobium sp*) or fungal microorganisms given the pot environmental conditions (Carvalho et al., 2019 ).



**Figure 3.4:** Nodules: A, Nodules of cowpea (Red) inside the weighing dish, B, Nodules of soybean (PAN 1664R) inside the petri-dish.

#### 3.4.1.2 Comparison within bean types

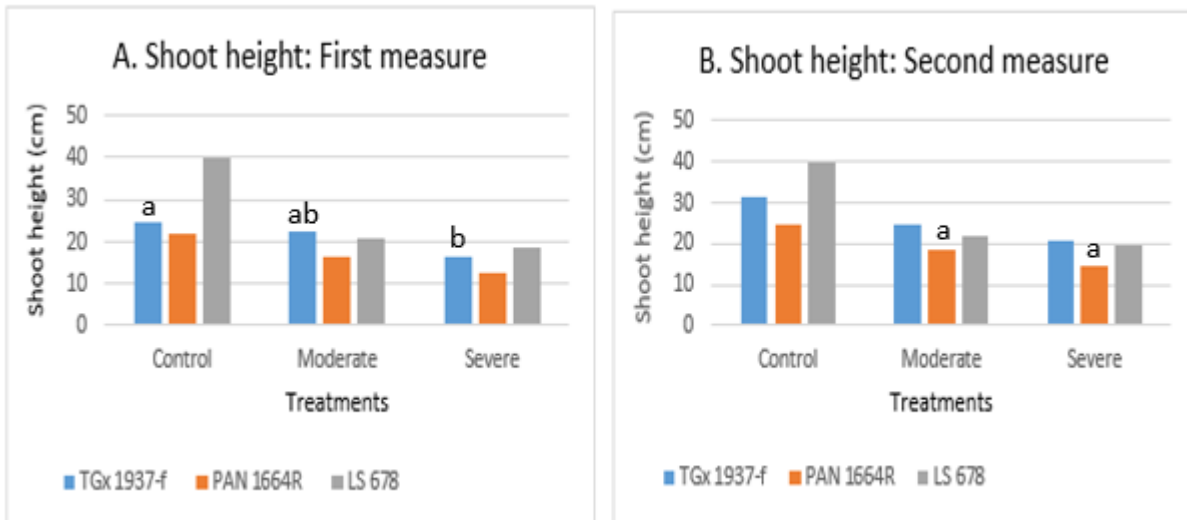
The 1<sup>st</sup> count of the number of leaves of soybean genotypes show a significant difference between genotypes for all water stress treatments (Figure 3.5A) with the exception of TGx 1937-f and PAN 1664R under severe stress, recording 20.16 and 20.83, respectively. TGx

1937-f and LS 678 control treatment was significantly higher than moderate and severe treatments but PAN 1664R showed no significant decrease in number of leaves from control to moderate and severe stress. This suggest water stress effects on leaves at early development stage of PAN 1664R were better tolerated than with TGx 1937-f and LS 678.



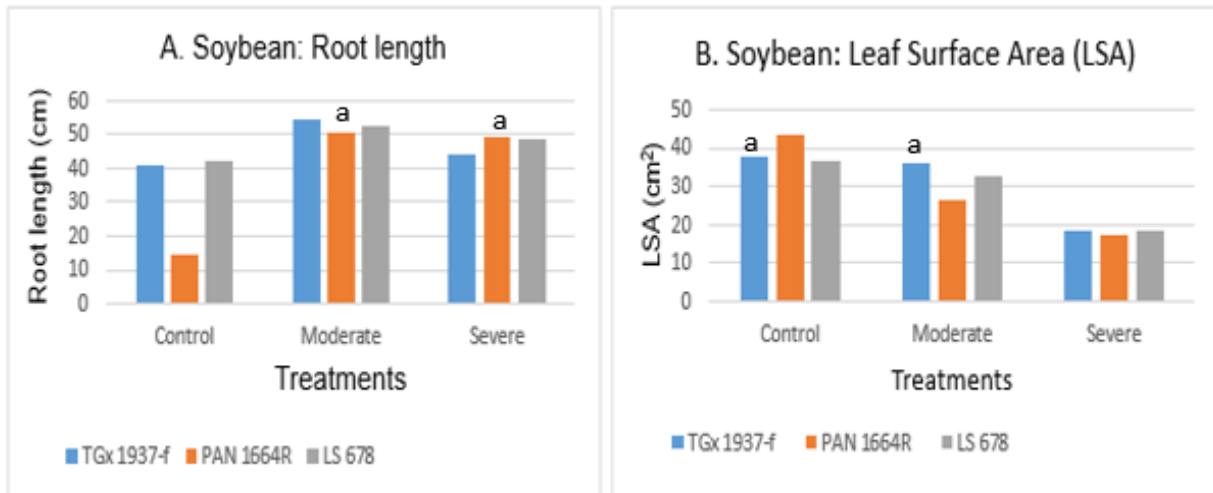
**Figure 3.5:** Number of leaves of soybean cultivars: A, First count of the number of leaves. B, Second count of the number of leaves. The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

The 2<sup>nd</sup> count results showed all genotypes had their number of leaves significantly decreasing with an increases in stress severity (Figure 3.5B). The same trend was observed for shoot height, root length, nodule weight, they all significantly decrease with the increase in stress severity.



**Figure 3.6:** Shoot height of soybean cultivars: A, First measure of the shoot height. B, Second measure of the shoot height. The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

The 1<sup>st</sup> and final measurements of the shoot height had the same response for all genotypes (Figure 3.6). PAN 1664R had the root length and nodule weight significantly longer/higher than LS 678 and TGx 1937-f. TGx recorded the lowest nodule weight for severe stress treatment. All genotypes decreased the number of stomata for both abaxial and adaxial surface with the increase in stress severity. There is a strong significant difference between treatments with control for LS 678 and TGx 1937-f recording the same highest number (4.89) and LS 678 recorded the lowest (15.55) for severe on adaxial surface as shown on table 3.5 below. LS 678 had the highest (11.00) for moderate, higher than 10.00 of control for abaxial surface count. Leaf surface area (LSA) of TGx 1937-f and LS 678 shows no significant difference to LSA of LS 678 for both control (37.89 and 36.56) and severe stress treatment (18.69 and 18.51) respectively. PAN 1664R had the largest LSA for control but moderate and severe were significantly lower compared to the other two genotypes.



**Figure 3.7:** The root length of soybean cultivars: A, Root length. B, Leaf Surface Area. The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

**Table 3.5:** Number of stomata on abaxial and adaxial surface of the both cowpea and soybean cultivars

Abaxial surface			
	Control	Moderate	Severe
TGx 1937-f	9.89±1.90	9.33±2.06 <sup>a</sup>	7.89±1.05 <sup>a</sup>
PAN 1664R	7.00±2.0	8.22±2.05	6.38±2.72
LS 678	10.00±2.65	11.00±2.12	8.22±2.44
Red	11.89±0.41 <sup>a</sup>	11.77±0.32 <sup>a</sup>	10.44±1.21 <sup>a</sup>
Blonde	12.67±0.36 <sup>a</sup>	13.00±0.98 <sup>a</sup>	11.78±1.48
White	10.56±1.10	10.11±1.66 <sup>a</sup>	9.67±1.40 <sup>a</sup>
Adaxial surface			
TGx 1937-f	4.89±0.93	4.00±1.22	2.44±1.42
PAN 1664R	4.56±1.24	4.33±1	3.44±0.73
LS 678	4.89±1.27	2.67±1.22	1.55±1.13
Red	6.89±0.42 <sup>a</sup>	6.00±1.33 <sup>ab</sup>	5.33±2.01 <sup>b</sup>
Blonde	6.22±1.12	5.33±1.30 <sup>a</sup>	5.07±1.01 <sup>a</sup>
White	5.33±1.22	4.55±2.09	4.44±0.84

**Note:** Each value represent a mean± standard deviation of three analysis in triplicates per cultivar. Values in the same row with the same alphabet are not significantly different at  $P \leq 0.05$ . n=9

Cowpea nodule weight, number of stomata on both surfaces and root length showed no significant difference between treatments and also between the genotypes with the exception of white which had a significantly lower root length and nodule weight and a slightly lower number of stomata but not significantly different from the other genotypes (Table 3.6). For LSA it was observed that genotypes show no significant difference, however, the LSA for control treatment was found to be significantly larger than LSA for severe stress treatment but not significantly different to moderate stress treatment for all genotypes (Table 3.6). This data suggest that LSA of cowpea can be affected only by a severe stress. Although no significance difference was observed, Blonde recorded the largest LSA for control and moderate stress treatments, 47.15 and 47.04, respectively. White had the smallest LSA record of 30.80 for severe treatment. The results on the 2<sup>nd</sup> leaf count and 1<sup>st</sup> and final shoot height measurement revealed a significant difference between treatments and between genotypes. However, a Red genotype showed no significant difference when control was compared with moderate stress. The 1<sup>st</sup> leaf count had no significant difference between treatments for both White and Blonde but Red control was significantly different to moderate stress treatment. White was significantly lower than Red and Blonde genotypes for all treatments.

**Table 3.6.** The effects of induced water deficit stress on vegetative growth parameters of three cowpea genotypes.

Growth and yield parameters	Control			Moderate			Severe		
	Red	Blonde	White	Red	Blonde	White	Red	Blonde	White
<b>Vegetative growth</b>									
No. of leaves 1 <sup>st</sup> count	20.00±1.14	18.00±4.05	11.50±2.07 <sup>b</sup>	17.67±0.82 <sup>a</sup>	15.67±4.55	11.00±0.89 <sup>b</sup>	17.50±1.76 <sup>a</sup>	14.33±2.25	11.67±1b.21
No. of leaves 2 <sup>nd</sup> count	27.17±0.98	25.00±1.41	16.33±1.75	19.33±1.21 <sup>a</sup>	17.00±3.36	12.00±1.41	18.17±1.70 <sup>a</sup>	15.33±2	11.17±0.75
Shoot height (cm) 1 <sup>st</sup>	25.75±1.08 <sup>a</sup>	26.41±4.84	24.75±1.62	24.18±1.20 <sup>a</sup>	24.75±1.62	21.50±1.94	20.57±1.52a	20.12±1.34	19.42±1.97
Shoot height (cm) final	39.12±0.69 <sup>a</sup>	42.02±4.95	31.40±0.86	38.52±0.78 <sup>a</sup>	29.97±0.84	27.95±2.10	29.97±3.87	26.85±1.36	21.77±2.73

Root length (cm)	54.90±2.59 <sup>a</sup>	50.40±2.58 <sup>b</sup>	63.70±4.27	54.08±5.30 <sup>a</sup>	50.20±0.94 <sup>b</sup>	52.45±3.32	44.06±7.55	49.33±1.68	48.45±5.48
Leaf surface area (LSA)	46.19	47.15	46.53	46.18	47.04	46.07	30.93	33.18	30.80

**Note:** Each value represent a mean± standard deviation of three analysis in triplicates per cultivar. Values in the same row with the same alphabet are not significantly different at P≤0.05. n=9

### 3.4.2 Reproductive parameters

#### 3.4.2.1 Comparison between bean types

Cowpea produced flowers that are significantly lower than soybean, soybean produced (77.22, 46.44 and 26.50) and cowpea produced (35.72, 27.22 and 17.94) for control, moderate and severe, respectively as shown in Table 3.7. Both cowpea and soybean showed a significant difference between control and the stress treatments (moderate and severe) (Table 3.7 and Appendix 5). The number of pods were slightly lower than the relative produced flowers as a result of aborted flower due to water stress (Figure 3.7). The pod numbers of soybean are significantly higher than cowpea (Table 3.7). Soybean pods numbers significantly decrease with the increase in stress severity.

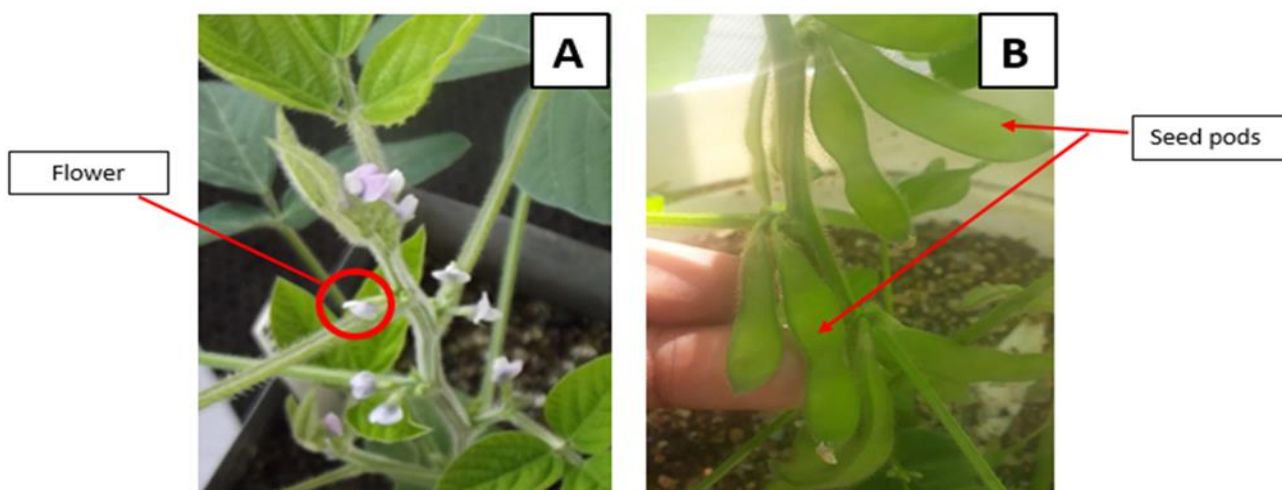
**Table 3.7.** The effects of induced water deficit stress on reproductive growth parameters of soybean and cowpea.

Parameters	Soybean			Cowpea		
	Control	Moderate	Severe	Control	Moderate	Severe
No. of flowers	77.22±17.8	46.44±7.55	26.5±6.86	35.72±7.94	27.22±2.76	17.94±2.78
No. of pods	43.17±4.58	38.33±7.32	25.72±7.81	18.72±1.00 <sup>a</sup>	18.38±1.50 <sup>a</sup>	15.0±3.04
Average pod length (cm)	4.17±0.31	3.40±0.36	2.7±0.36	20.4±1.90 <sup>a</sup>	19.93±1.81 <sup>ab</sup>	19.37±1.55 <sup>b</sup>
Mean number of seeds per pod	3.67±0.91	2.11±0.76	1.83±0.79	18.72±1.00 <sup>a</sup>	18.38±1.50 <sup>a</sup>	15±3.04
Weight of 100 seeds (g)	10.64±1.40	9.64±1.28 <sup>a</sup>	8.19±1.91 <sup>a</sup>	13.94±1.03 <sup>b</sup>	13.83±1.25 <sup>b</sup>	13.29±1.31 <sup>b</sup>

**Note:** Each value represent a mean± standard deviation of three cultivars in triplicates per bean type. Values in the same row with the same alphabet are not significantly different at  $P \leq 0.05$ . n=27

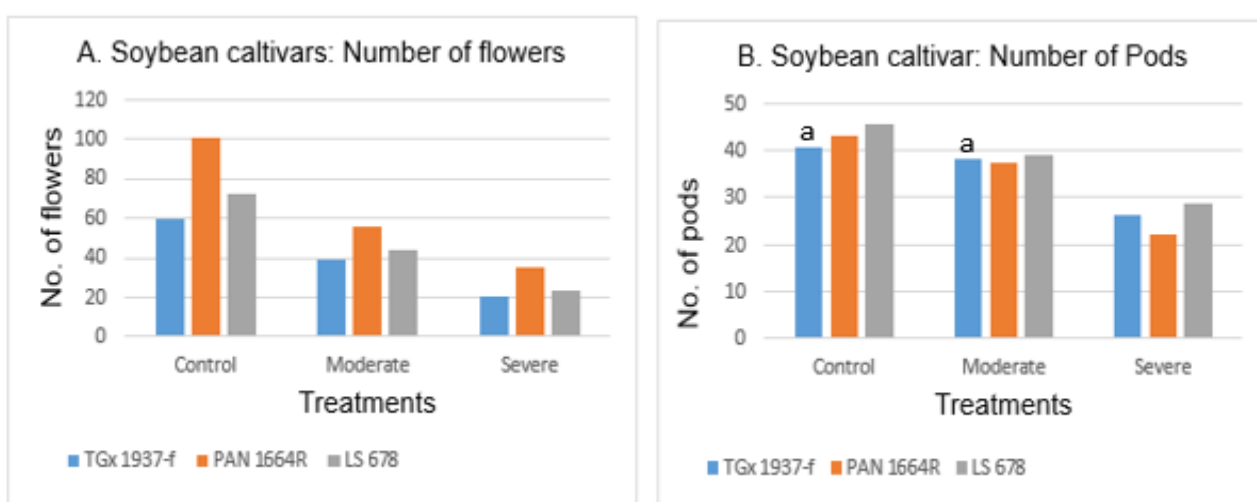
Cowpea had no significant difference between control and moderate but severe was significantly lower in pod numbers. The pods of cowpea are longer than the pods of soybean, therefore the difference is very significant. The length of the pod directly reflect to the number of seed per pod. For example, soybean average pod length for control was 4.17 cm containing the mean seeds of 2.67 per pod and cowpea recorded 20.4 cm containing mean of 18.72 seeds for the same treatment. The soybean average pods and mean seed per pod were significantly different across the all treatment, decreasing with the increase in severity of the water stress. Cowpea average pod length and mean seeds per pod were not significantly different with the exception of mean seeds per pod that significantly declined under severe stress.

### 3.4.2.2 Comparison within bean types



**Figure 3.8:** Flowers and seed pods: A, Soybean (TGX 1937-f) plant flowering and B, Soybean PAN 1664R filling the seed pods.

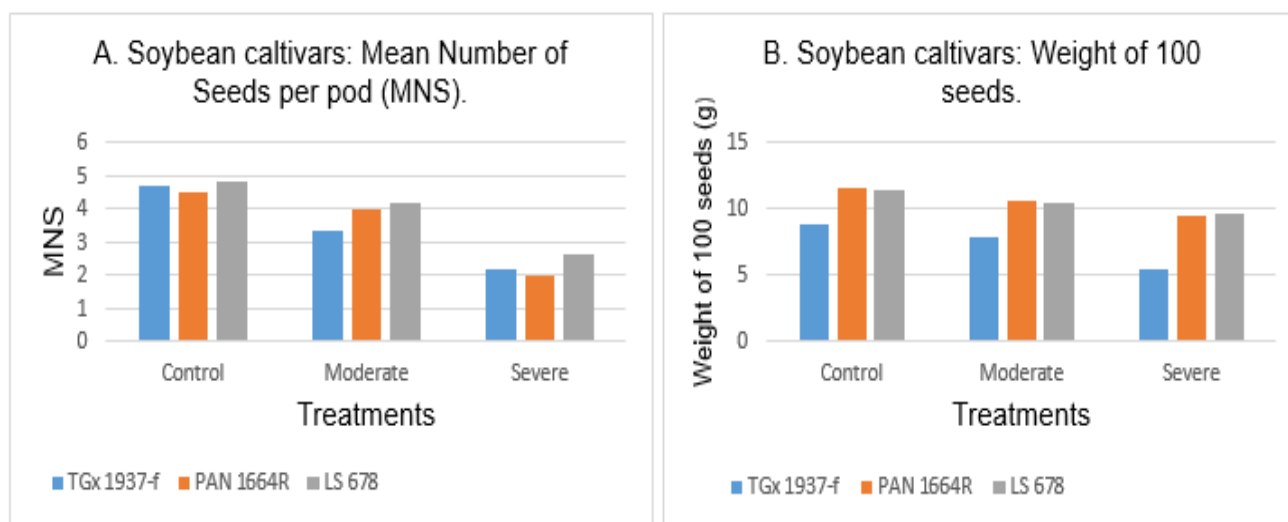
Soybean genotypes showed a great variation on the number of flowers produced, the control ranged from 59.00 (TGx 1937-f) to 100.33 (PAN 1664R) and severe between 20.67 (TGX 1937-f) and 23.83 (LS 1664R) (Figure 3.7). Genotypes were significantly different and the number of flowers decreased significantly with the increase in stress severity. The number of pods were slightly lower than the relatively produced flowers as a result of aborted flower due to water stress.



**Figure 3.9:** Soybean cultivars: A, Number of flowers B, Number of pods. The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

The decline was over 50%, e.g. over 100.33 flowers were produced by PAN 1664R under control but only 43.00 pods were formed. Just as the number of flowers were significantly

different, there was a decline in the number of pods formed with the increase in severity of water stress. The pods of soybean genotypes develop to the average pod length of between 4.5 cm (PAN 1664, control) and 2.4 cm (LS 678, severe) Figure 3.7. This data showed a significant difference between stress treatments but not between the genotypes.



**Figure 3.10:** Soybean cultivars: A, Mean number of seed per pod. B, Weight of 100 seeds. The bars with the same colour and same alphabet are not significantly different at  $P \leq 0.05$ .

Cowpea genotypes were found to produce flowers that are significantly different in terms of numbers and they significantly decrease with the increase in stress severity for all genotypes with the exception of White, where control was not significantly different from moderate stress. Red had about 40% decline from control to moderate stress treatment. Blonde produced the lowest number of flowers of 15.67 under severe stress compared to 20.17 and 18.00 of Red and White genotypes, respectively. Of all the total flowers produced only 41.26% in average developed in to pods. No significant difference was observed for both number of pots produced and the average length of the pots across all genotypes and all stress treatments. The pod length ranged between 22.3 (Red, control) and (18.10 white, severe). Mean seeds per pod of White was the highest at 19.68 and also the lowest recording 15.18. The three genotypes showed no significant decrease of the mean seeds per pod comparing control and moderate, however, severe was significantly lower than control.



**Figure 3.11:** A total of 100 seeds: A, Soybean (TGx 1937-f) seeds. B, Cowpea (blonde) seed and C, Soybean (PAN 1664R) seeds.

**Table 3.8:** The effects of induced water deficit stress on reproductive growth parameters of three cowpea genotypes.

Number of flowers			
	Control	Moderate	Severe
Red	59±1.79	39.17±1.17	20.67±1.63
Blonde	100.33±1	55.83±3.1	35±3.79
White	72.33±3.0	44.33±1.9	23.83±2.64
Number of pods			
Red	40.83±5.9	38.33±1.1	26.17±9.24
Blonde	43.00±4.3	37.50±6.4	22.17±8.33
White	45.67±1.6	39.17±1.3	28.83±5.08
Mean number of seeds per pod			
Red	4.67±1.03	3.33±1.0	2.17±0.9
Blonde	4.50±1.1 <sup>a</sup>	4.00±0.9 <sup>a</sup>	2.00±0.8
White	4.83±1.0 <sup>a</sup>	4.17±0.8 <sup>a</sup>	2.67±1.1
Weight of 100 seeds (g)			
Red	8.83±0.63 <sup>a</sup>	7.83±0.56 <sup>a</sup>	5.53±0.34
Blonde	11.58±0.6 <sup>a</sup>	10.66±0.49 <sup>ab</sup>	9.44±0.37 <sup>b</sup>
White	11.51±0.6 <sup>a</sup>	10.43±0.56 <sup>ab</sup>	9.59±0.40 <sup>b</sup>

**Note:** Each value represent a mean± standard deviation of three analysis in triplicates per cultivars. Values in the same row with the same alphabet are not significantly different at  $P \leq 0.05$ . n=27

### **3.5 CONCLUSION**

The finding of this study indicates that water deficit stress inhibits both vegetative and reproductive growth of soybean and cowpea. However, the impact on cowpea is not significant and its tolerance of moderate drought stress is excellent, especially on reproductive growth. Both moderate and severe stress devastated the vegetative and reproductive growth of soybean.

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## **CHAPTER 4**

# **EVALUATION OF THE EFFECTS OF WATER DEFICIT STRESS ON THE PHYSIOLOGICAL PARAMETERS OF SOYBEAN AND COWPEA**

## 4.1 INTRODUCTION

Harsh environmental conditions contribute to the growth and distribution of plants. During their lifetime, they are faced with several stress factors, both biotic and abiotic (Evert and Eichhorn, 2013). In the current times, the world is confined by a challenge of global climatic changes, thus water deficit stress is the most frequent, severe and important plant stress factor (Farooq et al., 2012; Rahduri and Hoseni, 2012; Fathi and Tori, 2016; Salehi and Bakhshayashan-Agdam, 2016). Drought directly affects plant development and production by hindering or arresting growth (Girmay and Gebreselassie, 2018), causing injuries or death. It also indirectly increase plant susceptibility to disease and pests (Despres-Loustau and Marcais, 2006).

Generally, plants struggle to survive water stress like any other living organisms. Moreover, drought is reported to have a significant impact on production of most common commercial crops (Nansamba et al., 2020; Mdungela at al., 2017). Although this fact is true, plants through evolutionary timelines have advancedly developed means to well adapt in harsh drought and other hostile conditions (Brodrribb and McAdam, 2017). Primarily, there are three strategies that plants use to persist water stress: desiccation tolerance, drought tolerance and drought avoidance (Brodrribb, 2017).

Plants such as mosses and ferns remarkably withstand almost total dehydration by censing metabolic activities triggered by low water content between the cells i.e. desiccation tolerance (Hoekstra et al., 2001). Plants such as most succulents exploit drought avoidance mechanism (Ogburn and Edwards, 2010). They avoid complete dehydration by keeping the stomata on the leaves closed during daytime to reduce evapotranspiration but open during night-time to collect carbon dioxide (CO<sub>2</sub>) that is used to complete photosynthesis during daylight (Crassulacean acid metabolism) (Evert and Eichhorn, 2013). Lastly; drought tolerant category: plants in this category thrive during drought periods by deploying a number of creative adaptations such as inhibition of new leaf formation, early leaf drop, cessation of shoot growth, early flowering or stomatal closure to retain water (Gupta et al., 2020). These plants closes their stomata to reduce transpiration but also lower gaseous exchange resulting in reduced CO<sub>2</sub> diffusion affecting regulation of carbon assimilation (Basu et al., 2016). Most species that are well adapted to drought are reported to promote the root growth with

morphological modifications (primary root elongate and branching diminished) as they arrest the growth of the shoot (Dinneny, 2019).

Although the literature on the general response of plants to water deficit stress is rich, the golden questions “how severe must a drought be to kill a plant and which plant will die and which will recover at that level of severity” remain partially answered for most species. In this chapter the effect of water deficit stress on physiological parameters of soybean and cowpea were compared. The chapter also investigated if soybean and cowpea plants can recover from the water deficit stress that had already set on.

#### 4.1.1 Aim

This study was aimed at evaluating and comparing the effects of water deficit stress on physiological parameters of soybean and cowpea plants.

#### 4.1.2 Objectives

Objectives of the study were to:

- I. Evaluate the difference between the physiological response of cowpea and soybean to water deficit stress.
- II. Evaluate the ability of soybean and cowpea to recover from water stress after rehydration.

## 4.2 LITERATURE REVIEW

Flaccidity of plant is an early and most commonly recognized symptom of water deficit stress (Dimkpa et al., 2019). Wilting can be critical as more water is lost and hydrostatic pressure inside plant cells decrease (Zhoy et al, 2020). When the related soil moisture level and relative humidity are low in surrounding locale with higher temperature experienced, drought is suffered (Farooq et al., 2012). Plant functionality fully depends on water, the limited water supply has a direct effect on growth and development processes (Akinci 1997; Lobell et al., 2013). It is obviously out of debate that land plants are exposed to drought at some stage of their life cycle. In response to this stress plants tend to develop, alter or activate adaptive mechanisms to mitigate the harmful impact of changing environmental conditions (Kooyers, 2015).

The response solemnly depend on period and extremity of water deprivation (Pugnaire et al., 1999). Water stress may extent from modest and of short period, to sharp severe and prolonged or steady period (Farooq et al., 2012). Different plant species and genotypes adapt differently, some adapt easily and others not so easily; losing the advantage to better compete or may die (Kooyers, 2015). Fundamentally, the effect of water stress at physiological level is observed as change in carbon and nitrogen metabolism caused by decreased photosynthesis (Aslam et al., 2015). It is reported that plant response to water occurs in three linked phases; short-term, mid-term and long-term (Bhattacharjee and Saha, 2014). Plants activate the short-term responses in just few seconds after detecting water deficit stress. The short-term responses of plants to water deficit stress are essentially associated with the stomatal regulation. This limit the time the stoma remains open to reduce the amount of water lost due to transpiration but maintain the CO<sub>2</sub> intake. However, the process of CO<sub>2</sub> intake under this circumstances is interfered with, leading to reduced CO<sub>2</sub> intake and directly affecting photosynthesis (Lisar et al., 2012). Following stomatal regulation, plants may modify the cell wall elasticity, fine-adjust the osmotic potential by absorbing solutes or may change their morphological features – the midterm response (Acclimation) (Bhattacharjee and Saha, 2014). Finally plants aims to limit growth and restrict resource usage by deploying different means; 1. Change in gene response, 2. Change in anatomical structures of specific organs (such as roots) or 3. Alterations of specific physiological mechanisms (Chen et al., 2013; Makhbul et al., 2011; Shao et al., 2008; Lisar et al., 2012).

Under in-vivo environment, these response can happen simultaneously or one at time depending on whether there are other interacting biotic or abiotic stresses involved. The worst of drought stress happens when cells have lost most of their protoplasmic water and very small amount remains, desiccation (Dinakar, 2012). Larcher (1987) reported that water stress is not only disruptive but it can be constructive, helping plant to improve their resistance mechanisms. This constructive effects of drought are responsible for evolutionary buildup of plant resistance adaptation, the tolerant and avoidance mechanisms. Water deficit stress evasion is achieved through a series of mechanisms at organ, system, tissue or cellular level that the whole plant undergo throughout its life span from germination to degeneration or death (Kooyers, 2015). For example plant organs undergo modification in respond to drought or plants can involve the process of osmotic adjustment which promote water uptake and turgor maintenance (Bray, 1997).

#### 4.2.1 Short-term response

Abiotic stress on plants is due to multiple factors. However, water stress, the most important and multidimensional in nature, affects plants at various levels. Basically water stress reduces water potential and turgor pressure of the plant cell (Jenks and Hasegawa, 2007). The reduction promotes the increase in solutes concentration within cellular organelles and matrices. The process of cell division and enlargement is hindered as a result, causing poor growth and limited production (Shao et al., 2009). On the onset and detection of water deficit stress by plants, the short-term response mechanisms are activated in just few seconds. This includes regulation reduction of water loss by transpiration, stomatal closure and enhanced CO<sub>2</sub> intake (Lisar et al., 2012).

##### 4.2.1.1 Stomatal closure

Transpiration refers to exhalation of gaseous water from the internal of the plant to the outside environment through the stomatal opening (Xingguo, 2002). Transpiration rate increases with increase in the total water vapor concentration within the leaf and decreases with the increase in the total resistance to water vapor transport across the boundary layers to the outside environment (Lisar et al., 2012). Stoma are minute openings made of the guard cells surrounded by special epidermal (subsidiary) cells on both adaxial and abaxial surface of the leaf or rarely on the stem (Damour et al., 2010). Stomatal closure allows for the movement

of the gases into and out of intercellular spaces of the cell. The essential and principal process that help with resistance build-up and restriction of transpiration rate in plants under drought stress, is stomatal closure (Buckley and Schymabski, 2014). In such unfavorable conditions the stomatal response is directly linked to the threshold water status, however, this response is more dependent on the soil moisture than it is to the leaf water content (Lisar et al., 2012). The whole mechanism is referred to as hydropassive and is triggered and monitored by the signaling hormone ABA (Lim et al., 2015).

The prominent ABA hormone accumulation increases in specialised areas such as xylem sap of the root and its concentration increase in various components and parts of the leaf of dehydrated plants (Frey et al., 2013). ABA also induce and regulate the expression of various genes that are responsible for or involved in stomatal closure and defense mechanism against water stress (Lim et al., 2015). The increase in ABA production is as a results of decreased PM-ATPase activities. This happens when pH of the cell wall increases due to water stress conditions leading to decline in PM-ATPase activates which then increase the production of ABA (Bauer et al., 2013). Since ABA cannot penetrate the plasma membrane due to low pressure of water steam in the leaf, it accumulates around guard cells promoting stomatal closure and thereby preventing further water loss due to transpiration (Schwartz et al., 2013). Morphologically it is observed that the number of stomata in both upper and lower surface of commercial crops significantly decreased in response to water stress (Lisar et al., 2012).

#### 4.2.1.2 CO<sub>2</sub> intake and photosynthesis

As discussed above plants sacrifice a lot of water in exchange for CO<sub>2</sub> during the opening of their stomatal pores. The absorbed CO<sub>2</sub> is converted into carbohydrates through the process called photosynthesis. Photosynthesis is explicitly susceptible to water deficit stress and its effects. It is reported that photosynthesis decreases with the decrease in relative water content and water potential especially in higher plants (Lowlar, 2002; Omae et al., 2007). This lower rates of photosynthesis caused by water stress are attributed to stomatal closure than it is a metabolic impairment (Flexas et al., 2006). Nevertheless, metabolic impairment is reported to also lower the rate of photosynthesis (Lisar et al., 2012). Metabolic impairment is explained as a more complex event than the stomatal regulation although the elementary importance of both is still unclear (Heidari, 2015).

Often a reduction in the rate of photosynthesis of plants is caused by water deficit stress. Water deficit stress decrease the leaf surface area decrease which lead to decreased photosynthetic rate per unit area (Heidari, 2015). The rate of leaf photosynthesis of both C<sub>3</sub> and C<sub>4</sub> plants is significantly reduced under drought conditions. Drought severely restrain photosynthesis and it is reported that some commercial crops have totally stopped photosynthesis under persistent and prolonged water stress (Mahammadkhani and Heidari, 2017). The photosynthesis of C<sub>3</sub> plants such as *Triticum sativum* is reported to be more sensitive to drought conditions than the photosynthesis of C<sub>4</sub> plants such as *Zea mays* L. The prevalence of C<sub>4</sub> plants in drought stricken areas is attributed to the lower number of stomata compared to C<sub>3</sub> plants (Maghsoudi et al., 2016). However, the primary photosynthesis processes such as Calvin cycle and electron transport chain of C<sub>3</sub> and C<sub>4</sub> are fundamentally similar, yet exhibiting significant difference and therefore respond differently to water deficit stress (Ye et al., 2013).

Although there are a number of co-factors associated with the decreased rate of photosynthesis due to water stress; low CO<sub>2</sub> intake, decreased assimilation rates in photosynthetic parts and reduction of photosynthetic pigments are the most prestigious (Omae et al., 2007). Of the remaining other co-factors, the impairment of photosynthetic apparatus such as the granum and thylakoids (chloroplast) due to the production of reactive oxygen species (ROS) like superoxide, is important (Lisar et al., 2012).

Sufficient literature is documented on decreased chlorophyll of photosynthetic parts under water stress and how it occurs (Flexas et al., 2006). Lisar et al. (2012) reported on four consecutive stages of chlorophyll synthesis. Water stress during these stages leads to inhibition of chlorophyll synthesis. These stages include: 1. the formation of 5-aminolevulinic acid (ALA), 2. condensation of ALA into prophytylloin which then form protochlorophyllide, 3. Light-dependent conversion of protochlorophyllide to chlorophyllide, 4. Synthesis of chlorophyll a and b. In most cases where agricultural plant were studied, it was demonstrated that chlorophyll is more sensitive to water stress than carotenoids (Lowlar, 2002; Maghsoudi et al., 2016). However, the increase in the content of xanthophyll pigments (has inhibitory role on production of ROS) with decrease of chlorophyll pigments have been reported for plants under drought stress (Latowski et al., 2011).

Ribulose-1, 5-biphosphate carboxylase (RuBisCo) is the key enzyme commonly known for its involvement in the critical steps of carbon fixation, its carboxylase role in the Calvin cycle and

oxygenase role in the adverse photorespiration process. RuBisCo's crucial role influences the biochemical response and physiology of the plants suffering water stress (Yadav et al., 2020). Under such conditions, a significant decline in the amount of Rubisco that leads to limited enzymatic activities of rubisco is reported in plants studied (Lowlar, 2002). This is evident that the reduction of carbon dioxide intake resulted from stomatal closure mechanism, affects rubisco activities. However, the photorespiration help maintain the carboxylase activities of rubisco (Lisar et al., 2012). In addition, the chloroplast may suffer oxidative damage causing a chloroplast volume reduction, rubisco and its functionality (Yadav et al., 2020). Rubisco activity is inhibited when the chloroplast is acidified due to water stress. Moreover, rubisco activity decline with lack of coupling factor ATPase, loss of RBP recognition site or damage in plastids that is releasing rubisco and structural damage of rubisco (Lowlar, 2002; Heidari, 2015). Water stress also affect activities of other photosynthetic enzymes including nicotinamide adenine dinucleotide phosphate (NADP) – dependent glyceraldehyde phosphate dehydrogenase, fructose-1, 6-bisphosphatase and phosphoribulose kinase (Ashraf and Harris, 2013). In the light reaction of photosynthesis, water stress obstruct the electron transport of both cyclic and non-cyclic type leading to lower transport rate (Kaur and Asthir, 2017). The reduced transport rate reduce the ATP and NADP<sup>+</sup> production as well as affecting the photophosphorylation process negatively. The reduction of synthesis process of ATP and inhibition of ATPase activities is responsible for decreased ATP levels in the chloroplast (Lowlar, 2002).

Fundamentally plant response mechanisms to water deficit stress results in notable changes such as photosynthetic metabolism, functionality and structural rearrangement (Xu et al., 2010). Photo-assimilation and the integrity of photosynthetic apparatus are influenced by several factors under drought conditions (Kaur and Asthir, 2017). Both PSI and PSII of the chloroplast are affected by water deficiency. Although the literature contradict, there is a significant higher number publications that reports that PSI is more sensitive and easily damaged by water deficit stress than PSII (Ye et al., 2013).

#### 4.2.2 Midterm response (Acclimation)

Plants have evolved and deploys multifarious response mechanisms for protection against various kinds of stress. At cellular level for instance, the water stress damage mitigation and alleviation mechanisms attempts and alter the metabolisms processes involved (Manns,

2002). This evolution allowed many plant species to adapt and survive dehydration to certain extent as reported (Osakabe et al., 2010). Hoekstra et al. (2001) reported on the plant tolerance of water stress at critical level and discussed two types of tolerance i.e. drought tolerance, defined as the tolerance of moderate dehydration and desiccation tolerance, defined as tolerance of further dehydration and also the ability to rehydrate. The accumulation of compatible solutes such as osmolytes leads to osmotic adjustment (Manns, 2002; Mahajan and Tateja, 2005). Compatible solutes are also involved in scavenging of the radical oxygen species and the protection of membrane structure as well as enzymes (Bhattacharjee and Saha, 2014).

#### 4.2.2.1 Cell wall elasticity and modulation of osmotic potential

The complex phenomenon of oxidative stress in plants is virtually comprised of all abiotic and biotic stress, with drought being the most prominent. The increase in production and accumulation of ROS resulted in oxidative stress in most commercial higher plants (Osakabe et al., 2014). Research also reported that the sense of this stress triggered the tightening and loosening or shrinkage of the cell wall. Plants avoid further water loss under water limited environment by altering the cellular volume through this shrinkage i.e. elastic adjustment of the cell wall (Lisar et al., 2012). Circumstantially, cell wall adjustment or elasticity is regarded one of the most important events that plants undertake to physiologically combat or adapt to water stress (Xu et al., 2010; Akinci and Losel, 2012). Cell wall elasticity is involved in the maintenance of cell turgor by causing a decline in cell turgor-loss volume as reported in several species (Bhattacharjee and Saha, 2014).

Basically, the matric potential of plants supporting medium such as soil or vermiculite decreases with the decrease in water availability to become more negative. Therefore, plants need to have a more negative water potential to be able to absorb water from such medium, this phenomenon is known as osmosis (movement of water from the region of lower concentration to the region of higher concentration) (Mahajan and Tateja, 2005). Osmotic adjustment which is simply the accumulation of the solutes or osmolytes by the cell help make its matric potential lower than that of the dry medium. Unlike the cell wall elasticity mechanisms, this technique of decreasing the water potential of the cell does not affect turgor or decrease the volume of the cell (Yadav et al., 2020).

From literature, it is evident that plants activate complex non-enzymatic and enzymatic (involving enzymes such as superoxide dismutase) antioxidant defense mechanisms to diminish toxicity due to ROS production (Verma et al., 2019). Under stress, especially salinity and water stress, osmotic adjustment plays a principal role to improve water status by manipulating osmolyte metabolisms (Hoekstra et al., 2001; Osakabe et al., 2014). Osmolytes are organic compounds with lower molecular weight in biological systems with the influence on properties of biological fluids such as amino acids, sugars etc. Literature suggests that the decline in ROS production in plants under water stress corresponds to the increase in osmolyte concentration and this enhances better tolerance to water stress (Hussain et al., 2019).

Glutamic acid or ornithine derivative and non-essential amino acid, proline (Pro) is a typical example of compatible osmolytes (Akinci and Losel, 2012). Although much about its biological function and role are poorly studied, Pro metabolism and its accumulation during osmotic stress due to environmental stress have been extensively studied and better understood (Maggio et al., 2002). Several living organisms including eubacteria, marine invertebrates and plants accumulate Pro in response to various extreme conditions such as salinity, drought, rising temperatures, infectious pathogens, nutrient deficiency and atmospheric pollution (Sardhi et al., 1995). In plants, Pro accumulation increases proportionally compared to other amino acids with the increase of severity of water stress in various plants (Osakabe et al., 2014). This is mainly due to the increased production and decreased degradation (Verma et al., 2019). Pro accumulation varies from one species to another and it can be up to 100 times greater compared to the control (Sardhi et al., 1995). Pro accumulation has been reported to play a vital role in plant stress tolerance and adaptation. The early study by Smirnoff and Cambes (1989) suggests that Pro is a ROS scavenger. It is believed to serve as a compatible osmolyte and assist as a means to store carbon and nitrogen (Akinci and Losel, 2012). Furthermore, pro accumulation is reported to be involved in stabilization of molecular structures such as proteins and other amino acids, help to maintain balanced cell redox status, buffers the pH of cytosol and also serve as a stress signal (Osakabe et al., 2014).

#### 4.2.3 Leaf relative water content (RWC)

Hundreds to thousands of genes underlie and facilitate the response mechanisms of plants to water stress. Understanding these underlying mechanisms is a treasure of knowledge for enhancing drought tolerance in crop plants. There are several methods that tested effective for screening the drought tolerance of commercial crops (Anithukumari et al., 2011; Spitters and Schopendonk, 1990). Anithukumari et al. (2011) reported that leaf relative water content (RWC) have been proven an important water stress indicator and have been used to study crops such as potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*) and barley (*Hordeum vulgare*) (Sade, 2015; Robinet et al., 2008). Several studies have focused on developing appropriate techniques for measuring RWC (Weatherley, 2001; Klar et al., 2017).

Leaf relative water content is the measure of how much (maximum amount) water can a tissue hold (Barrs, 1968; Boyer, 1968). Although leaf RWC interrelate with several leaf physiological parameters such as leaf turgor, photosynthesis, respiration and stomatal conductance, it is intimately linked to the growth and development of the whole plant (Kramer and Boyer, 1995; Yamasaki and Dillenburg, 1999). Leaf relative water content has been intensively studied and used to quantify water deficit stress in the leaf tissues (Robinet et al., 2008; Pirzad et al., 2011; Sade, 2015; Suresh et al., 2015). Solty-kaling et al. (2016) augured that RWC is probably the most accurate and pertinent method to assess the water balance and status of plant suffered water stress because it expresses the relative amount of water present on the plant tissues. This method has been used over decades (Borrs and Weatherlea, 1962; Polash et al., 2018), and it was then referred to as 'relative turgidity', it has gained experience and recognition over time in both the physiological and plant breeding research (Sade et al., 2015). Literature have reported that the normal RWC values range between 90% in fully turgid and to about 30-40% in severely desiccated and dying leaves, depending on species (Pirzad et al., 2011; Polash et al., 2018).

## 4.3 MATERIALS AND METHODS

### 4.3.1 Determination of leaf relative water content

The leaf relative water content (RWC) was determined following Mullan and Pietragalla, 2012 and Barrs, 1962. Clean and dry 15 ml sample tubes were weighed and their mass was recorded. Three youngest and fully expanded trifoliolate leaves were harvested from each plant and immediately placed into the pre-weighed sample tubes and the lid was tightly closed to avoid moisture loss/gain from the external environment. The tubes were immediately placed into a cold water bath (at about 10-15 °C) to arrest all physiological activities before taken to the laboratory. All the tubes containing the leaf samples were weighed and fresh weight was determined by subtracting the mass of empty sample tubes from the mass of sample tube containing leaf sample. The tubes were then filled with distilled water and kept at room temperature for 24 hours to allow the leaves to reach full turgor. After 24 hours the leaf samples were removed from the sample tubes and carefully blotted using a paper towel to remove excess distilled water and weighed to get the saturated weight. Finally the leaf samples were wrapped with labeled aluminum foil and dried in an oven at 95 °C for 24 hours. After 24 hours, the leaves were weighed again to determine dry weight. The dry weight was achieved by re-drying and re-weighing until a constant value was achieved. The following formula was used to determine the leaf relative water content:

$$\text{RWC} = \frac{Fm - Dm}{Sm - Dm} \quad (\text{Where: } Fm = \text{fresh mass, } Sm = \text{saturated mass and } Dm = \text{dry mass}).$$

### 4.3.2 Determination of proline content

Proline concentration was determined using the ninhydrin-acid method following Bates et al., 1973 and Kozminska et al., 2019. Approximately 0.5 g of plant material was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the solution was filtrated. Two (2) ml of filtrate was reacted with 2 ml ninhydrin acid and 2 ml of glacial acid in a test tube for 1 hour at 95 °C and the reaction was terminated in an ice bath. The reaction mixture was separated with 4ml toluene, mixed vigorously with a test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and absorbance read at 520 nm using toluene for a blank. The proline concentration was determined from a proline standard curve (Figure 4.1 below) and the proline content was calculated on a fresh weight basis as follows:

$$\text{Proline content } (\mu\text{mol/g}) = [(\mu\text{g proline/ml}) \times \text{ml toluene}] / 115.5 \mu\text{g}/\mu\text{mol}] / [(\text{g sample}) / 5].$$

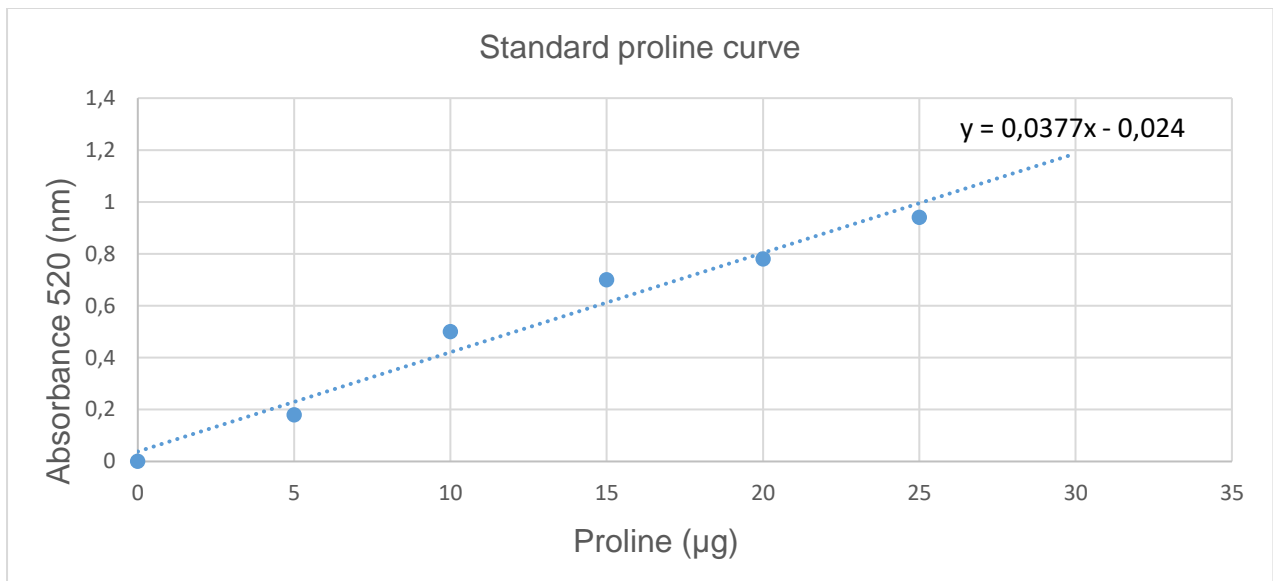


Figure 4.1: The standard curve between the absorbance of 520 nm and L-proline content.

#### 4.3.3 Determination of malondialdehyde content

Malondialdehyde (MDA) content is a widely used parameter as a measure of lipid peroxidation in plant tissue that increases under oxidative stress. The level of peroxidases was determined using the trichloroacetic acid (TCA) extraction method following Savicka and Skute (2010). Approximately 0.2 g leaf material was homogenized with 2 ml of 0.1% of trichloroacetic acid (TCA) and centrifuged at 14 000 rpm for 15 minutes. After centrifugation, 1ml of the supernatant was mixed with 2.5 ml of 0.5% TBA in 20% TCA and incubated in hot water at 95 °C for 30 minute. Thereafter, it was cooled immediately on ice to stop the reaction and centrifuged at 10 000 rpm for 30 minutes. Absorbance at 532 and 600 nm was determined, and malondialdehyde concentration was estimated by subtracting the non-specific absorbance at 600 nm from the absorbance of 532 nm, using the absorbance coefficient of extinction ( $155 \text{ nM}^{-1} \text{ Cm}^{-1}$ )

$$\text{MDA (mM)} = (A_{532} - A_{600}) / 155 \text{ nM}^{-1} \text{ Cm}^{-1}.$$

#### 4.3.4 Determination of chlorophyll content

The chlorophyll content was determined by soaking 0.20 g of leaf material in 100% acetone for 24 hours at room temperature in the dark according to Lichtenthaler and Wellburn (1983) and Zhai et al. (2020). After 24 hours, the samples were vortexed and centrifuged at 3500

rpm for 15 minutes. The absorbance of the collected supernatants were measured at 663 nm, 646 nm and 470 nm using the spectrophotometer. The analysis were done in triplicate for three cultivars per bean type. The following equations were used to calculate the concentrations of chlorophyll a, chlorophyll b and total carotenoids.

$$\text{Chlorophyll a } (\mu\text{g ml}^{-1}) = 12.21 A_{663} - 2.81 A_{646};$$

$$\text{Chlorophyll b } (\mu\text{g ml}^{-1}) = 20.13 A_{646} - 5.03 A_{663};$$

$$\text{Total carotenoids } (\mu\text{g ml}^{-1}) = (1000 A_{470} - 3.27 [\text{chl a}] - 104 [\text{chl b}] / 229).$$

#### 4.3.5 Evaluation of plant recovery after rehydration

The preliminary experiment was done to determine the time after the commencement of the water deficit treatments the plants start to suffer and/or showing dying signs. A follow-up experiment was performed wherein the water deficit treatments was followed by watering the plants to field capacity. Both cowpea and soybean were subjected to water stress by not watering for 14 days followed by three weeks of watering on daily bases. The analyses in 4.3.1 to 4.3.5 above were carried out to find out if the plants can recover from the water deficit stress that has already set-in. The analysis were done in triplicate for three cultivars per bean type.

#### 4.3.6 Data analysis

The obtained data for analysis within bean type was presented as mean values of all replicates for all treatments. For comparison between bean types, each value represent a mean of three cultivars in triplicates per bean type. Analysis of variance (ANOVA) was done using IBM SPSS statistic 27 to determine if there is significant difference between treatments, cultivars and/or between cowpea and soybean with regards to water deficit tolerance at 95% level of confidence. The student t-test was further used to compare and determine which parameters and treatments between the cultivars and/or cowpea and soybean account for water deficit tolerance and/or susceptibility. These analysis were conducted using student t-LSD (least significant difference) at 5% level of confidence. The statistical outcomes are recorded under appendix 6, 7 and 8.

## 4.4 RESULTS AND DISCUSSION

### 4.4.1 Physiological parameters

#### 4.4.1.1 Comparison between bean types

The assessment and analysis of relative water content (RWC) is an ultimate technique to measure plant hydrologic statuses. This is because it signifies the physiological consequences of the cellular water deficit (Shao et al., 2008). In this study, the RWC was decreasing with increasing drought stress levels (Table 4.1). The wide range of statistical difference was observed between cowpea and soybean under water stress conditions. This observations follows the same trend as the previous, the morphological observations made in chapter 3. The results revealed that the highest RWC (0.87) was found from cowpea control treatment and the lowest (0.34) was found from soybean severe stress treatment. Drought stress decreased the RWC of both cowpea and soybean under moderate and severe stress, however, cowpea performed distinctly superior to soybean. No considerable of significant difference was found between control (0.87) and stress treatment (0.86 and 0.74, moderate and severe respectively) for cowpea while soybean control (0.59) treatment was significantly different to severe (0.34) with the difference of 24%. Induced water stress was also reported to decrease RWC by Klar et al. (2017) and Polash et al. (2018) in young bean, Kabir et al. (2005) and Tahar et al. (2010) in matured dry beans species. The findings of this study also correlate with the study of Akhtar et al. (2015), where adverse effects of water stress on soybean were determined and found that RWC decreased with over 30% under stressed conditions compared to control.

The phenomenon of proline accumulation commonly occurs in plants under water stress (Osakabe et al., 2014). This amino acid has wide and diverse roles in plant, it acts as osmolyte for osmotic adjustment, stabilizes sub cellular structure, scavenging free radicals and maintain cell redox status under stressed conditions (Maggio et al., 2002). Its accumulation normally occurs in cytoplasm and it increases with the increase in water stress (Verma et al., 2019). The data presented in this study outlines that the proline content of soybean increased significantly (Appendix 7) from control (4.20  $\mu\text{mol/g}$ ) to the stress treatments, moderate (4.94  $\mu\text{mol/g}$ ) and severe (7.27  $\mu\text{mol/g}$ ) as shown in Table 4.1. The same trend of increased proline content with the increase in stress severity was observed for cowpea (Appendix 4.2). However, cowpea recorded lower proline content values compared

to soybean and no significant difference was observed between control and moderate stress but severe was significantly higher than control (Table 4.1). These results correlate with the findings of Zhang et al. (2014) who reported that the amount of proline found on the root of cotton cultivars was the highest at the most extensive water stress level. On the other study Wang et al. (2015) observed similar response, using different concentrations of NaCl to induce water stress, the proline content increased with increasing NaCl concentrations. They argued that the increased concentration of proline is an adaptive way of plants to counteract the effects of the water stress. However, the higher the concentration of proline the more grievous the effects of water stress.

Malondialdehyde (MDA) is also used as a drought stress indicator in plants (Pirzad et al., 2011). It is produced by membrane lipids in response to ROS. Several studies submitted that MDA content increased synchronously with the increase in water stress and that it allows to trace the degree of plasma membrane damage due to water stress (Akinici and Losel, 2012). When the MDA is lower, it suggest that the plasma membrane is less to no damage and that the plant tolerates water stress better. The results of the experiment of MDA extraction on this study showed that the highest MDA content (10.07 mM) was observed from soybean under severe stress treatment and the lowest MDA content (1.95 mM) was recorded from cowpea control treatment. It was also observed that soybean had a significantly higher MDA content than cowpea for all treatments as shown in Table 4.1 below. However, both bean plants had their MDA increasing with the increase in stress severity. As observed with proline content, no significant difference was found between control and moderate stress for cowpea but soybean showed a significant difference between the treatments. These results are in agreement with the findings of Hassan and Fuertes, 2015 who made similar observations on the response of cherry tomato to salt and water stress. The aldehyde residues are reported to be highly reactive and are involved in biochemical mechanisms aimed to improve tolerance to drought stress (Hassan and Fuertes, 2015).

The chlorophyll a, b and carotenoids content in cowpea leaves showed no remarkable difference between the control and stress treatments whereas the soybean photosynthetic pigments had lower concentrations than cowpea and showed a considerable decrease in all chlorophyll pigments. Soybean chlorophyll a, chlorophyll b and carotenoids contents declined by 22.6, 32.17 and 22.27 percent for severe and 11.21, 14.33 and 23.1 percent for moderate stress compared to the control treatment, respectively. Similar results were observed where

the runner bean (*Phaseolus coccineus*) was subjected to drought that lasted 10 days. The chlorophyll a, chlorophyll b and carotenoids contents declined by 18.52%, 21.58% and 19.47% compared with the well water treatment, respectively. Moreover, the same trend was observed on other parameters including proline content and malondialdehyde content (Latowski et al., 2011). It is reported that due to water stress the stomata are kept closed to avoid further water loss, however, this leads to the reduction of carbon dioxide intake and acidification of the chloroplast. The acidified chloroplast and limited carbon dioxide inhibits rubisco activities (Yadav et al., 2020).

**Table 4.1.** The effects of induced water deficit stress on physiological parameters of soybean and cowpea.

Parameters	Soybean			Cowpea		
	Control	Moderate	Severe	Control	Moderate	Severe
Leaf relative water content	0.59±0.02	0.55±0.03	0.34±0.10	0.87±0.02 <sup>a</sup>	0.86±0.05 <sup>a</sup>	0.74±0.05
Proline content (µmol/g)	4.20±0.65	4.95±0.83	7.27±1.30	1.59±0.61	2.18±0.88	3.13±0.29
Malondialdehyde content (mM)	3.92±0.52	7.08±1.04	10.07±0.99	1.95±1.16	2.72±1.08	3.53±1.70
Photosynthetic pigments (µgml <sup>-1</sup> )						
Chlorophyll a	18.48±0.51	10.00±0.40	7.83±0.64	20.16±0.75 <sup>a</sup>	19.48±1.21 <sup>a</sup>	18.50±1.80 <sup>a</sup>
Chlorophyll b	6.30±0.44	5.57±0.50	3.79±0.41	9.53±0.54 <sup>a</sup>	8.9±1.07 <sup>ab</sup>	7.49±0.59 <sup>b</sup>
Carotenoids	4.75±0.27	3.97±0.47	3.14±0.04	5.12±0.56 <sup>a</sup>	4.90±0.22 <sup>ab</sup>	4.13±0.96 <sup>b</sup>
<b>Note:</b> Each value represent a mean± standard deviation of three cultivars in triplicates per bean type. Values in the same row with the same alphabet are not significantly different at P≤0.05. n=27.						

#### 4.4.1.2 Comparison within bean types

The MDA and proline content of all soybean genotypes were increased with the increase in water stress severity (Table 4.2). TGx had the highest MDA and proline content for severe stress treatment and LS had the lowest records for the same treatment and analysis. A wide significant difference between the responses of the three genotypes was observed (Appendix

6). All genotypes (TGx, PAN and LS 678) MDA and proline content for severe stress treatment increased with over 50% compared with control treatment. The similar response was observed for cowpea genotypes. There was an increase in MDA and proline content with the increase in stress severity. However, no significant difference was observed between the treatments for all the genotypes. Red genotype was found not to be considerably different from Blonde genotype for MDR but significantly different from White. In view of proline content, cowpea genotypes showed no significance difference (Appendix 7).

**Table 4.2.** The effects of induced water deficit stress on physiological parameters of three soybean genotypes.

Parameters	Control			Moderate			Severe		
	TGx 1937- f	PAN 1664 R	LS 678	TGx 1937-f	PAN 1664R	LS 678	TGx 1937-f	PAN 1664R	LS 678
Leaf relative water content	0.61	0.61	0.57 <sup>b</sup>	0.58 <sup>c</sup>	0.52	0.56 <sup>b</sup>	0.56 <sup>c</sup>	0.64	0.44
Proline content (µmol/g)	4.65	4.62	4.95	5.47	5.57	3.80	8.16	8.16	5.48
Malondialdehyde content (mM)	4.31	4.25	3.21	8.26	6.83	6.14	11.41	9.71	9.08
Photosynthetic pigments (µgml <sup>-1</sup> )									
Chlorophyll a	17.99	18.4	19.01	9.64	9.94	10.4	7.15	8.42	7.42
Chlorophyll b	6.16	6.79	5.94	5.13	5.46	6.11	3.94	3.33	4.10
Carotenoids	4.60 <sup>a</sup>	4.59	5.07	4.61 <sup>a</sup>	4.42	3.49	3.11	3.12	3.19

**Note:** Each value represent a mean± standard deviation of three analysis in triplicates per cultivar. Values in the same row with the same alphabet are not significantly different at P≤0.05. n=9.

Considering RWC and photosynthetic pigments, the contrary results were observed. RWC and all photosynthetic pigment of both soybean and cowpea genotypes decreased with the increase in stress levels. There was no significant difference genotypes of both beans, with the exception of PAN (soybean) genotype that had RWC significantly lower than TGx and LS 678 for severe stress treatment. A considerable decline from control to moderate stress treatment of soybean genotypes, TGx, PAN and LS 678 decreasing with 12.14%, 18.4% and 9.8%, respectively. However, no significant decrease was observed for cowpea genotypes.

The highest recorded RWC for cowpea genotypes was 87.9% for Red, control treatment and the lowest for the same genotype was 71.9% for severe stress treatment (Table 4.1). The same trend as RWC was observed for photosynthetic pigments from both cowpea and soybean. Cowpea genotypes were not significantly different from each other and no considerable difference was found between different control and stress treatment. In the case of soybean, photosynthetic pigments decreased significantly with the increase in stress levels.

**Table 4.3.** The effects of induced water deficit stress on physiological parameters of three cowpea genotypes.

Parameters	Control			Moderate			Severe		
	Red	Blonde	White	Red	Blonde	White	Red	Blonde	White
Leaf relative water content	0.88 <sup>a</sup>	0.85 <sup>b</sup>	0.87 <sup>c</sup>	0.88 <sup>a</sup>	0.81 <sup>b</sup>	0.78	0.72	0.85 <sup>b</sup>	0.87 <sup>c</sup>
Proline content (µmol/g)	0.76	2.19	1.81	1.00	2.84	2.66	2.96	3.15	3.29
Malondialdehyde content (mM)	2.56	2.67	0.56	3.66	3.09	0.92	4.08	4.65	1.85
Photosynthetic pigments (µgml <sup>-1</sup> )									
Chlorophyll a	20.01 <sup>a</sup>	19.44 <sup>b</sup>	20.94	19.92 <sup>a</sup>	20.14 <sup>b</sup>	18.11	17.74	18.41	19.34
Chlorophyll b	9.43	9.04	10.11	10.13	8.41	8.16	8.14	7.31	7.01
Carotenoids	5.71 <sup>a</sup>	4.93 <sup>b</sup>	4.66 <sup>c</sup>	5.10 <sup>a</sup>	4.93 <sup>b</sup>	4.66 <sup>c</sup>	4.41	3.07	4.92 <sup>c</sup>

**Note:** Each value represent a mean± standard deviation of three cultivars in triplicates per bean type. Values in the same row with the same alphabet are not significantly different at P≤0.05. n=9.

#### 4.4.1.3 Recovery analysis on physiological parameters after rehydration.

The results obtained from the proline content, MDA and photosynthetic pigments to assess the recovery ability of the cowpea and soybean displayed the same trend as the results from 4.4.1.1 above. The proline content and MDA of both beans were significantly increased, comparing control with stressed-rewatered treatment (Table 4.4). However, MDA and proline content of soybean was significantly higher than cowpea for both control and stressed-

rewatered plants. Soybean proline and MDA content were increased from 3.80  $\mu\text{mol/g}$  and 3.91 mM (control) to 10.07  $\mu\text{mol/g}$  and 10.99 mM (stressed-rewatered) while cowpea increased from 1.56  $\mu\text{mol/g}$  and 1.00 mM (control) to 3.51  $\mu\text{mol/g}$  and 4.67 mM (stressed-rewatered), respectively. Over threefold increase was observed for both MDA and proline content for soybean. This results suggest that cowpea's abilities to recover from the effect of water stress is better than soybean. The results of the study done by Rivas et al. (2016) reports similar findings and the drought tolerance in cowpea species was explained as driven by less sensitivity of leaf gas exchange to water deficit and rapid recovery of photosynthesis after rehydration.

**Table 4.4.** The effects of induced severe water deficit stress on physiological parameters of soybean and cowpea after rewatering.

Parameters	Soybean		Cowpea	
	Control	Treatment	Control	Treatment
Proline content ( $\mu\text{mol/g}$ )	3.80 $\pm$ 0.60	10.07 $\pm$ 0.80	1.56 $\pm$ 0.65	3.51 $\pm$ 0.33
Malondialdehyde content (mM)	3.91 $\pm$ 0.85	10.99 $\pm$ 2.02	1.00 $\pm$ 0.49	4.67 $\pm$ 0.73
Photosynthetic pigments ( $\mu\text{gml}^{-1}$ )				
Chlorophyll a	18.41 $\pm$ 1.39	6.34 $\pm$ 0.31	19.67 $\pm$ 0.57	16.99 $\pm$ 1.92
Chlorophyll b	6.02 $\pm$ 0.09	3.18 $\pm$ 0.21	9.29 $\pm$ 0.79	6.40 $\pm$ 0.50
Carotenoids	3.93 $\pm$ 0.45	2.01 $\pm$ 0.23	5.40 $\pm$ 0.71	3.42 $\pm$ 0.63
<b>Note:</b> Each value represent a mean $\pm$ standard deviation of three cultivars in triplicates per bean type. Values in the same row with the same alphabet are not significantly different at $P\leq 0.05$ . n=27				

## **4.5 CONCLUSION**

The use of physiological data to assess the effect of water deficit stress of both soybean and cowpea correlates with what was observed morphologically (Chapter 3). The results indicates that cowpea is more tolerate and well adapted to environments with limited water supplies (drought) as compared to soybean. The observations made on RWC, MDR, proline content and photosynthetic pigments showed a considerable difference between the bean plants with cowpea showing a better response. Cowpea was also found to be able to recover from drought effects.

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## **CHAPTER 5**

# **GENERAL CONCLUSION, RECOMMENDATIONS AND FUTURE RESEARCH**

## **5.1 GENERAL CONCLUSION, RECOMMENDATIONS AND FUTURE RESEARCH**

### **5.1.1 General conclusion**

Soybean is by far most extensively used, both domestically and industrially as compared to cowpea. Its nutritional values are excellent and is believed to have a potential to alleviate the problem of malnutrition and hunger in poor countries. Soybean production is low and its supply is disproportional to the demands by the markets, however, cowpea supply is good, more especially in western Africa where it originated. The challenge of global warming and climate change intensify and further crumble the production of soybean.

The aim of this study was to investigate the similarities and dissimilarity between soybean and cowpea response to water deficit stress. This was aimed at providing the necessary information that could be used by biotechnologist to genetically improve soybean. The comparison could allow scientists to know what make cowpea survive in environment that soybean cannot. Based on data collected from this study, it was concluded that cowpea's response and tolerance to water deficit stress is better than soybean at all growth level and that drought significantly lower the production of soybean.

### **5.1.2 Recommendations**

From the results obtained in the current study, it is recommended that,

- i. The planting of seeds for the experiment should be done at the same season and the plant pots should be randomly kept in the same environmental conditions.
- ii. For germination study, increasing the days for observation from 10 days to 15-20 days is recommended.

### **5.1.2 Future research**

In future, it is necessary to genetically compare the two legume plants, to trace the genes that are responsible for such tolerance in cowpea.

## APPENDICES

A comparative study on water deficit stress tolerance between the cowpea (*Vigna unguiculata* [L.] Walp) and soybean (*Glycine max* [L.] Merrill)

**APPENDIX 1: ANOVA: Germination parameters**

## Cowpea Seedling Vigour

## Index

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	5819.04	4	1454.76	42.06	3.18E-06	3.48
Within Groups	345.84	10	34.58			
Total	6164.89	14				

## Soybean Seedling Vigour

## Index

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	2330.885	4	582.7213	8.765177	0.002632	3.47805
Within Groups	664.8141	10	66.48141			
Total	2995.699	14				

## Cowpea Germination Index

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	25.63302	4	6.408256	8.814492	0.002578	3.47805
Within Groups	7.270136	10	0.727014			
Total	32.90316	14				

## Soybean Germination

## Index

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	9.634228	4	2.408557	7.633312	0.004356	3.47805
Within Groups	3.155324	10	0.315532			
Total	12.78955	14				

## Cowpea Emergence Rate

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	2452.112	4	613.0281	1.252879	0.350156	3.47805
Within Groups	4892.954	10	489.2954			
Total	7345.067	14				

Soybean Emergence Rate

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	4688.92	4	1172.23	8.661603	0.002751	3.47805
Within Groups	1353.364	10	135.3364			
Total	6042.284	14				

Cowpea Germination  
percentage

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	23823.42	4	5955.856	4.30693	0.027824	3.47805
Within Groups	13828.54	10	1382.854			
Total	37651.96	14				

Soybean germination  
percentage

<i>Source of Variation</i>	<i>SS</i>	<i>Df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	46012.04	4	11503.01	428.2212	3.93E-11	3.47805
Within Groups	268.6231	10	26.86231			
Total	46280.66	14				

**APPENDIX 2:** The student t-test outputs for germination parameters

Cowpea Germination Index				
Treatment	Count	Mean	Variation	p-value
0	9	3.42	0.78	0.476
-0.02	9	3.56	2.04	
0	9	3.42	0.78	0.082
-0.27	9	2.31	0.50	
0	9	3.42	0.78	0.002
-1.27	9	0.79	0.29	
0	9	3.42	0.78	1.15E-06
-1.80	9	0.23	0.02	
-0.02	9	3.56	2.04	0.042
-0.27	9	2.31	0.50	
-0.02	9	3.56	2.04	0.001
-1.27	9	0.79	0.29	
-0.02	9	3.56	2.04	4.18E-04
-1.80	9	0.23	0.02	
-0.27	9	2.31	0.50	3.14E-11
-1.27	9	0.79	0.29	
-0.27	9	2.31	0.50	8.16E-09
-1.80	9	0.23	0.02	
-1.27	9	0.79	0.29	0.078
-1.80	9	0.23	0.02	
Soybean Germination Index				
0	9	2.51	0.13	0.422
-0.02	9	2.43	0.31	
0	9	2.51	0.13	0.226
-0.27	9	2.30	0.07	
0	9	2.51	0.13	0.106
-1.27	9	1.58	1.05	
0	9	2.51	0.13	0.001
-1.80	9	0.38	0.02	

-0.02	9	2.43	0.31	0.204
-0.27	9	2.30	0.07	
-0.02	9	2.43	0.31	0.001
-1.27	9	1.58	1.05	
-0.02	9	2.43	0.31	5.11E-14
-1.80	9	0.38	0.02	
-0.27	9	2.30	0.07	4.49E-06
-1.27	9	1.58	1.05	
-0.27	9	2.30	0.07	1.49E-11
-1.80	9	0.38	0.02	
-1.27	9	1.58	1.05	1.16E-08
-1.80	9	0.38	0.02	
Cowpea Emergence rate				
0	9	83.50	8.42	0.135
-0.02	9	52.86	4.59	
0	9	83.50	8.42	0.053
-0.27	9	55.68	3.79	
0	9	83.50	8.42	0.042
-1.27	9	56.48	11.21	
0	9	83.50	8.42	0.003
-1.80	9	44.44	2.59	
-0.02	9	52.86	4.59	0.028
-0.27	9	55.68	3.79	
-0.02	9	52.86	4.59	0.016
-1.27	9	56.48	11.21	
-0.02	9	52.86	4.59	0.006
-1.80	9	44.44	2.59	
-0.27	9	55.68	3.79	0.214
-1.27	9	56.48	11.21	
-0.27	9	55.68	3.79	0.002
-1.80	9	44.44	2.59	
-1.27	9	56.48	11.21	0.003

-1.80	9	44.44	2.59	
Soybean Emergence Rate				
0	9	68.85	0.13	0.422
-0.02	9	75.02	0.31	
0	9	68.85	0.13	0.226
-0.27	9	71.58	0.07	
0	9	68.85	0.13	0.106
-1.27	9	43.23	1.05	
0	9	68.85	0.13	0.001
-1.80	9	30.56	0.02	
-0.02	9	75.02	0.31	0.204
-0.27	9	17.58	0.07	
-0.02	9	75.02	0.31	0.001
-1.27	9	30.56	1.05	
-0.02	9	71.58	0.31	5.11E-14
-1.80	9	43.23	0.02	
-0.27	9	43.23	0.07	4.49E-06
-1.27	9	30.56	1.05	
-0.27	9	71.58	0.07	1.49E-11
-1.80	9	43.23	0.02	
-1.27	9	43.23	1.05	1.16E-08
-1.80	9	30.56	0.02	

**APPENDIX 3:** Soybean: The student t-test outputs of comparing treatments for morphological parameters

Number of leaves first count				
Treatment	Count	Mean	Variation	p-value
Control	9	16.50	20.62	0.114
Moderate	9	14.78	14.78	
Control	9	16.50	20.62	0.064
Moderate	9	14.50	8.85	
Moderate	9	14.78	14.78	0.404
Severe	9	14.50	8.85	
Number of leaves second count				
Control	9	32.11	17.15	8.43E-16
Moderate	9	21.61	6.84	
Control	9	32.11	17.15	1.89E-16
Moderate	9	15.67	5.29	
Moderate	9	21.61	6.84	1.11E-08
Severe	9	15.67	5.29	
Number of flowers				
Control	9	77.22	9.00	4.8E-08
Moderate	9	46.44	5.16	
Control	9	77.22	9.00	2.65E-13
Moderate	9	26.50	7.09	
Moderate	9	46.44	9.00	5.55E-10
Severe	9	26.50	7.09	
Height of the plant first record				
Control	9	24.76	8.41	1.99E-06
Moderate	9	19.68	6.98	
Control	9	24.76	8.41	1.46E-11
Moderate	9	15.78	7.17	
Moderate	9	19.68	6.98	5.14E-05
Severe	9	15.78	1.05	
Height of the plant final record				

Control	9	30.05	5.79	3.34E-07
Moderate	9	21.47	8.64	
Control	9	30.05	5.79	1.12E-09
Moderate	9	18.21	7.57	
Moderate	9	21.47	8.64	0.001
Severe	9	18.21	7.57	
Root length final record				
Control	9	52.24	12.22	0.002
Moderate	9	47.28	2.09	
Control	9	52.24	12.22	1.24E-06
Moderate	9	41.77	1.88	
Moderate	9	47.28	2.09	1.84E-11
Severe	9	41.77	1.88	
Number of stomata (lower surface)				
Control	9	9.14	6.67	0.290
Moderate	9	9.51	5.33	
Control	9	9.14	6.67	0.009
Moderate	9	7.54	4.98	
Moderate	9	9.51	5.33	0.001
Severe	9	7.54	4.98	
Number of stomata (upper surface)				
Control	9	4.78	1.26	0.007
Moderate	9	3.67	1.77	
Control	9	4.78	1.26	0.001
Moderate	9	2.81	2.00	
Moderate	9	3.67	1.77	0.126
Severe	9	2.81	2.00	
Leaf surface area				
Control	9	39.56	14.06	0.047
Moderate	9	31.64	3.04	
Control	9	39.56	14.06	0.001
Moderate	9	18.21	0.46	

Moderate	9	39.56	14.06	0.004
Severe	9	18.21	0.46	
Mean seed per pot				
Control	9	4.17	0.09	0.024
Moderate	9	3.40	0.13	
Control	9	4.17	0.09	0.003
Moderate	9	2.76	0.03	
Moderate	9	3.40	0.13	0.038
Severe	9	2.70	0.13	
Weight of 100 seeds				
Control	9	10.64	2.08	0.022
moderate	9	9.64	1.99	
Control	9	10.64	2.08	7.53E-05
Moderate	9	8.19	3.87	
Moderate	9	9.64	2.08	0.008
Severe	9	8.19	3.87	
Number of pods				
Control	9	43.17	2.97	0.012
moderate	9	38.33	3.65	
Control	9	43.17	0.97	7.8E-10
Moderate	9	25.72	0.34	
Moderate	9	38.33	3.65	8.69E-06
Severe	9	25.72	0.34	

**APPENDIX 4:** Cowpea: The student t-test outputs of comparing treatments for morphological parameters

Number of leaves first count				
Treatment	Count	Mean	Variation	p-value
Control	9	24.66	22.94	0.002
Moderate	9	20.44	12.02	
Control	9	24.66	22.94	8.29E-05
Severe	9	19.33	5.65	
Moderate	9	20.44	12.03	0.135
Severe	9	19.33	5.65	
Number of flowers				
Control	9	35.72	63.04	6.98E-05
Moderate	9	27.22	7.59	
Control	9	35.72	63.04	8.79E-11
Moderate	9	17.94	7.70	
Moderate	9	27.22	7.59	4.95E-12
Severe	9	17.94	7.70	
Height of the plant first record				
Control	9	25.00	10.44	0.052
Moderate	9	23.48	4.23	
Control	9	25.00	10.11	6.96E-07
Moderate	9	20.20	2.58	
Moderate	9	23.48	4.43	1.50E-06
Severe	9	21.00	2.58	
Height of the plant final record				
Control	9	37.11	28.82	0.001
Moderate	9	32.14	23.96	
Control	9	37.11	28.82	0.001
Moderate	9	25.53	14.83	
Moderate	9	32.14	23.96	3.67E-05
Severe	9	25.53	14.53	
Root length final record				

Control	9	56.33	1.61	0.012
Moderate	9	52.24	12.22	
Control	9	56.33	1.61	4.09E-05
Moderate	9	47.28	2.09	
Moderate	9	52.28	12.22	0.002
Severe	9	47.28	2.09	
Number of stomata (lower surface)				
Control	9	11.70	5.14	0.45
Moderate	9	11.63	4.34	
Control	9	11.70	5.14	0.032
Severe	9	10.63	3.55	
Moderate	9	11.63	4.32	0.035
Severe	9	10.63	3.55	
Number of stomata (upper surface)				
Control	9	6.15	1.44	0.007
Moderate	9	5.30	1.52	
Control	9	6.15	1.44	0.001
Severe	9	4.93	1.23	
Moderate	9	5.30	1.53	0.126
Severe	9	4.93	1.23	
Leaf surface area				
Control	9	46.62	0.23	0.333
Moderate	9	46.43	0.29	
Control	9	46.62	0.23	2.65E-05
Severe	9	31.64	1.79	
Moderate	9	46.43	0.29	2.93E-05
Severe	9	31.64	1.79	
Mean seed per pot				
Control	9	20.40	3.61	0.387
Moderate	9	19.93	3.29	
Control	9	20.40	3.61	0.352
Severe	9	19.36	2.41	

Moderate	9	19.93	3.29	0.351
Severe	9	19.36	2.41	
Weight of 100 seeds				
Control	9	13.94	1.12	0.391
Moderate	9	13.83	1.65	
Control	9	13.94	1.25	0.115
Severe	9	13.29	1.84	
Moderate	9	13.83	1.65	0.124
Severe	9	13.29	1.84	
Number of pods				
Control	9	18.72	2.57	0.262
moderate	9	18.38	2.25	
Control	9	18.72	2.57	2.94E-05
Severe	9	15	2.29	
Moderate	9	18.38	2.57	8.3E-05
Severe	9	43.23	2.29	

**APPENDIX 5:** The student t-test outputs of morphological parameters. Cowpea versus soybean

Number of leaves first count						
Treatment	Plant	Count	Mean	SDV	Variation	p-value
Control	Soybean	27	24.66	4.79	22.94	6.20E-08
	Cowpea	27	16.50	4.54	15.99	
Moderate	Soybean	27	20.44	3.47	12.03	2.47E-08
	Cowpea	27	14.78	3.84	14.78	
Severe	Soybean	27	19.33	2.38	5.65	2.17E-08
	Cowpea	27	14.50	2.97	8.85	
Number of leaves second count						
Control	Soybean	27	32.11	4.21	17.75	4.03E-07
	Cowpea	27	22.83	4.99	24.97	
Moderate	Soybean	27	21.61	2.62	6.84	8.21E-06
	Cowpea	27	16.11	3.84	14.81	
Severe	Soybean	27	15.67	2.30	5.29	0.210
	Cowpea	27	14.89	3.23	10.46	
Number of flowers						
Control	Soybean	27	77.22	17.86	19.04	7.89E-11
	Cowpea	27	35.72	7.94	13.04	
Moderate	Soybean	27	46.44	7.55	56.97	3.98E-12
	Cowpea	27	27.22	2.76	7.59	
Severe	Soybean	27	26.50	6.86	47.09	1.14E-05
	Cowpea	27	17.94	2.78	7.70	
Height of the plant first record						
Control	Soybean	27	24.76	2.90	8.41	0.406
	Cowpea	27	25	3.23	10.44	
Moderate	Soybean	27	19.68	2.64	6.98	1.7E-05
	Cowpea	27	23.48	2.10	4.43	
Severe	Soybean	27	15.78	2.67	7.17	8.42E-07
	Cowpea	27	20.03	1.61	2.58	
Height of the plant final record						

Control	Soybean	27	32.06	6.77	45.79	0.006
	Cowpea	27	37.51	5.37	28.82	
Moderate	Soybean	27	21.42	2.94	8.64	1.51E-06
	Cowpea	27	32.14	4.89	23.90	
Severe	Soybean	27	18.21	2.75	7.58	1.62E-07
	Cowpea	27	25.53	3.85	14.83	
Final Root length						
Control	Soybean	27	41.77	1.37	1.88	8.17E-13
	Cowpea	27	54.93	4.99	24.91	
Moderate	Soybean	27	40.02	2.08	4.33	1.98E-15
	Cowpea	27	52.37	3.31	10.95	
Severe	Soybean	27	32.11	3.38	15.14	1.44E-13
	Cowpea	27	48.23	4.52	20.42	
Number of stomata (lower surface)						
Control	Soybean	27	8.96	2.55	6.50	5.7E-05
	Cowpea	27	11.70	2.27	5.14	
Moderate	Soybean	27	9.52	2.31	5.34	0.001
	Cowpea	27	11.63	2.08	4.98	
Severe	Soybean	27	7.54	2.23	4.98	7.17E-07
	Cowpea	27	10.63	1.88	3.55	
Number of stomata (upper surface)						
Control	Soybean	27	4.78	1.12	1.26	3.33E-05
	Cowpea	27	6.15	1.14	1.44	
Moderate	Soybean	27	3.67	1.33	1.77	1.09E-05
	Cowpea	27	5.29	1.23	1.52	

Severe	Soybean	27	2.81	1.17	2.00	6.5E-08
	Cowpea	27	4.93	1.11	1.23	
Leaf surface area						
Control	Soybean	27	39.36	1.42	14.06	0.001
	Cowpea	27	46.62	2.14	0.23	
Moderate	Soybean	27	31.64	0.87	23.04	3.3E-05
	Cowpea	27	46.43	1.23	0.29	
Severe	Soybean	27	18.21	1.11	0.46	0.022
	Cowpea	27	31.64	1.95	1.79	
Average pod length						
Control	Soybean	27	4.17	0.31	0.09	6.38E-05
	Cowpea	27	20.40	1.90	3.61	
Moderate	Soybean	27	3.40	0.36	0.13	5.09E-05
	Cowpea	27	19.93	1.81	3.29	
Severe	Soybean	27	2.70	0.36	0.13	2.74E-05
	Cowpea	27	18.10	1.55	2.41	

**APPENDIX 6:** Soybean: The student t-test outputs, comparing treatments for physiological parameters.

Proline content				
Treatment	Count	Mean	Variation	p-value
Control	9	4.20	0.48	0.030
Moderate	9	4.94	0.77	
Control	9	4.20	0.48	9.74R-06
Severe	9	7.27	1.90	
Moderate	9	4.94	0.77	0.001
Severe	9	7.27	1.90	
Leaf relative water content (RWC)				
Control	9	0.59	1.90	1.11E-05
Moderate	9	0.55	5.12	
Control	9	0.59	2.41	1.09E-02
Severe	9	0.34	1.15	
Moderate	9	0.55	5.03	6.5E-08
Severe	9	0.34	9.11	
Chlorophyll a				
Control	9	18.48	21.94	3.33E-05
Moderate	9	10.00	3.12	
Control	9	18.48	11.40	1.09E-05
Severe	9	7.83	15.15	
Moderate	9	10.00	5.03	6.5E-08
Severe	9	7.83	2.11	
Chlorophyll b				
Control	9	6.30	1.44	1.22E-04
Moderate	9	5.57	20.12	
Control	9	6.30	3.40	0.003
Severe	9	3.79	15.15	
Moderate	9	5.57	5.03	6.5E-08
Severe	9	3.79	9.11	
Carotenoids				

Control	9	4.75	1.94	1.33E-05
Moderate	9	3.97	2.12	
Control	9	4.75	2.40	2.09E-05
Severe	9	3.14	5.15	
Moderate	9	3.97	7.03	0.002
Severe	9	3.14	1.11	
Malondialdehyde content				
Control	9	35.72	63.04	6.98E-05
Moderate	9	27.22	7.59	
Control	9	35.72	63.04	8.79E-11
Severe	9	17.94	7.70	
Moderate	9	27.22	7.59	4.95E-12
Severe	9	17.94	7.70	
Malondialdehyde content: Recovery				
Control	9	3.91	0.81	0.001
Moderate	9	8.63	10.89	
Control	9	3.91	0.81	4.79E-08
Severe	9	10.98	4.60	
Moderate	9	8.63	0.89	0.046
Severe	9	10.98	4.60	

**APPENDIX 7:** Cowpea: The student t-test outputs, comparing treatments for physiological parameters.

Proline content				
Treatment	Count	Mean	Variation	p-value
Control	9	1.59	0.42	0.069
Moderate	9	2.18	0.87	
Control	9	1.59	0.42	3.76E-06
Severe	9	3.13	0.09	
Moderate	9	2.18	0.87	0.005
Severe	9	3.13	1.90	
Leaf relative water content (RWC)				
Control	9	0.59	21.94	1.21E-07
Moderate	9	0.55	22.12	
Control	9	0.59	12.40	2.09E-02
Severe	9	0.34	15.15	
Moderate	9	0.55	5.03	8.5E-02
Severe	9	0.34	9.11	
Chlorophyll a				
Control	9	18.48	21.94	3.33E-05
Moderate	9	10.00	22.12	
Control	9	18.48	12.40	1.09E-05
Severe	9	7.83	15.15	
Moderate	9	10.00	5.03	6.5E-08
Severe	9	7.83	9.11	
Chlorophyll b				
Control	9	6.30	21.94	1.22E-04
Moderate	9	5.57	22.12	
Control	9	6.30	12.40	0.003
Severe	9	3.79	15.15	
Moderate	9	5.57	5.03	6.5E-08
Severe	9	3.79	9.11	
Carotenoids				

Malondialdehyde content				
Control	9	1.94	1.50	0.093
Moderate	9	2.72	1.31	
Control	9	1.94	1.50	0.022
Severe	9	3.53	3.25	
Moderate	9	2.72	1.31	0.137
Severe	9	3.53	3.25	
Proline content: Recovery				
Control	3	1.56	0.43	0.196
Moderate	3	2.20	0.90	
Control	3	1.56	0.43	0.005
Severe	3	3.51	0.11	
Moderate	3	1.56	0.90	0.043
Severe	3	3.51	0.11	
Malondialdehyde content: Recovery				
Control	9	1.00	0.27	0.001
Moderate	9	2.72	1.32	
Control	9	4.67	0.27	1.28E-09
Moderate	9	2.67	0.59	
Moderate	9	2.72	1.32	0.001
Severe	9	4.67	0.59	

**APPENDIX 8:** The student t-test outputs of physiological parameters. Cowpea versus soybean.

Proline content					
Treatment	Plant	Count	Mean	Variation	p-value
Control	Soybean	27	4.20	0.48	1.84E-07
Moderate	Cowpea	27	1.58	0.02	
Control	Soybean	27	4.94	0.77	3.80E-06
Severe	Cowpea	27	2.18	0.87	
Moderate	Soybean	27	7.27	1.90	7.97E-08
Severe	Cowpea	27	3.13	0.09	
Leaf relative water content (RWC)					
Control	9	0.59	21.94	1.11E-05	
Moderate	9	0.55	22.12		
Control	9	0.59	12.40	1.09E-02	
Severe	9	0.34	15.15		
Moderate	9	0.55	5.03	6.5E-08	
Severe	9	0.34	9.11		
Chlorophyll a					
Control	9	18.48	2.14	3.33E-05	
Moderate	9	10.00	12.12		
Control	9	18.48	12.40	1.09E-05	
Severe	9	7.83	14.15		
Moderate	9	10.00	5.03	6.5E-08	
Severe	9	7.83	0.11		
Chlorophyll b					
Control	9	6.30	9.94	1.22E-04	
Moderate	9	5.57	5.12		
Control	9	6.30	12.40	0.003	
Severe	9	3.79	3.15		
Moderate	9	5.57	4.03	6.4E-08	
Severe	9	3.79	3.11		
Carotenoids					

Malondialdehyde content					
Control	Soybean	27	3.92	0.31	0.001
Moderate	Cowpea	27	1.95	1.51	
Control	Soybean	27	7.08	1.22	1.99E-07
Severe	Cowpea	27	2.72	1.31	
Moderate	Soybean	27	10.07	1.10	3.24E-08
Severe	Cowpea	27	3.53	3.26	
Proline content: Recovery					
Control	Soybean	27	3.80	0.36	0.006
Moderate	Cowpea	27	1.56	0.43	
Control	Soybean	27	6.23	0.13	0.001
Severe	Cowpea	27	1.56	0.43	
Moderate	Soybean	27	10.07	0.64	9.66E-05
Severe	Cowpea	27	3.51	0.11	
Malondialdehyde content: Recovery					
Control	Soybean	27	3.91	0.81	1.51E-07
Moderate	Cowpea	27	1.00	0.27	
Control	Soybean	27	8.64	10.89	5.56E-05
Moderate	Cowpea	27	2.72	1.32	
Moderate	Soybean	27	10.98	4.60	1.68E-07
Severe	Cowpea	27	4.67	0.59	

**APPENDIX 9:** Results on the effects of induced water deficit stress on vegetative and reproductive growth parameters of three soybean genotypes.

Growth and yield parameters	Control			Moderate			Severe		
	TGx 1937-F	PAN 1664R	LS 678	TGx 1937-F	PAN 1664R	LS 678	TGx 1937-F	PAN 1664R	LS 678
Vegetative growth									
No. of leaves 1 <sup>st</sup> count	29.67±1.21	18.83±1.72	25.50±1.38	24.00±3.28	17.33±1.26	20.00±1.26	20.16±2.04	20.83±1.17	17.00±1.90
No. of leaves 2 <sup>nd</sup> count	36.67±1.63	27.5±1.87	32.17±1.94	24.00±2.83	20.50±1.76	20.33±1.37	17.33±1.63	16.67±1.21	13.00±0.89
Shoot height (cm) 1 <sup>st</sup>	24.38±1.23	21.95±1.81	27.93±1.45	22.21±0.89	16.27±0.39	20.57±0.44	16.13±0.91	12.71±1.35	18.50±1.20
Shoot height (cm) final	31.67±3.25	24.62±0.47	39.88±2.18	24.37±1.39	18.27±1.44	21.78±1.70	20.37±1.27	14.70±1.03	19.55±0.73
Root length (cm)	41.17±1.23	41.72±0.94	42.43±1.69	54.08±4.53	50.20±0.94	52.45±3.32	44.06±7.55	49.33±1.68	48.45±5.48
Leaf surface area (LSA)	37.89	43.62	36.56	35.81	26.40	32.72	18.68	17.43	18.51
No. of stomata abaxial	9.89±1.90	7.00±2	10.00±2.65	9.33±2.06	8.22±2.05	11.00±2.12	7.89±1.05	6.38±2.72	8.22±2.44
No. of stomata adaxial	4.89±0.93	4.56±1.24	4.89±1.27	4.00±1.22	4.33±1	2.67±1.22	2.44±1.42	3.44±0.73	1.55±1.13
Reproductive growth									
No. of flowers	59±1.79	100.33±1.63	72.33±3.27	39.17±1.17	55.83±3.76	44.33±1.86	20.67±1.63	35±3.79	23.83±2.64
No. of pods	40.83±5.98	43.00±4.34	45.67±1.63	38.33±4.23	37.50±6.41	39.17±11.03	26.17±9.24	22.17±8.33	28.83±5.08
Mean number of seeds per pod	4.67±1.03	4.50±1.05	4.83±0.98	3.33±1.03	4.00±0.89	4.17±0.75	2.17±0.98	2.00±0.89	2.67±1.03
Weight of 100seeds (g)	8.83±0.63	11.58±0.58	11.51±0.65	7.83±0.56	10.66±0.49	10.43±0.56	5.53±0.34	9.44±0.37	9.59±0.40
<b>Note:</b> Data is presented as the mean± standard deviation values of all the replicates and repetitions.									

**APPENDIX 10:** The effects of induced water deficit stress on vegetative and reproductive growth parameters of three cowpea genotypes.

Growth and yield parameters	Control			Moderate			Severe		
	Red	Blonde	White	Red	Blonde	White	Red	Blonde	White
Vegetative growth									
No. of leaves 1 <sup>st</sup> count	20.00±1.14	18.00±4.05	11.50±2.07	17.67±0.82	15.67±4.55	11.00±0.89	17.50±1.76	14.33±2.25	11.67±1.21
No. of leaves 2 <sup>nd</sup> count	27.17±0.98	25.00±1.41	16.33±1.75	19.33±1.21	17.00±3.36	12.00±1.41	18.17±1.70	15.33±2	11.17±0.75
Shoot height (cm) 1 <sup>st</sup>	25.75±1.08	26.41±4.84	24.75±1.62	24.18±1.20	24.75±1.62	21.50±1.94	20.57±1.52	20.12±1.34	19.42±1.97
Shoot height (cm) final	39.12±0.69	42.02±4.95	31.40±0.86	38.52±0.78	29.97±0.84	27.95±2.10	29.97±3.87	26.85±1.36	21.77±2.73
Root length (cm)	54.90±2.59	50.40±2.58	63.70±4.27	54.08±5.30	50.20±0.94	52.45±3.32	44.06±7.55	49.33±1.68	48.45±5.48
Leaf surface area (LSA)	46.19	47.15	46.53	46.18	47.04	46.07	30.93	33.18	30.80
No. of stomata abaxial	11.89±2.03	12.67±2.92	10.56±1.24	11.77±1.92	13.00±1.87	10.11±1.45	10.44±1.51	11.78±2.05	9.67±1.58
No. of stomata adaxial	6.89±0.78	6.22±1.48	5.33±0.70	6.00±1.12	5.33±1	4.55±1.24	5.33±1.42	5.071±0.71	4.44±1.01
Reproductive growth									
No. of flowers	59±1.79	100.33±1.63	72.33±3.27	39.17±1.17	55.83±3.76	44.33±1.86	20.67±1.63	35±3.79	23.83±2.64
No. of pods	40.83±5.98	43.00±4.34	45.67±1.63	38.33±4.23	37.50±6.41	39.17±11.03	26.17±9.24	22.17±8.33	28.83±5.08
Mean number of seeds per pod	4.67±1.03	4.50±1.05	4.83±0.98	3.33±1.03	4.00±0.89A	4.17±0.75	2.17±0.98	2.00±0.89	2.67±1.03
Weight of 100seeds (g)	8.83±0.63	11.58±0.58	11.51±0.65	7.83±0.56	10.66±0.49	10.43±0.56	5.53±0.34	9.44±0.37	9.59±0.40
<b>Note:</b> Values are presented as mean± standard deviation of all replicates.									

**APPENDIX 11:** The effects of induced water deficit stress on physiological parameters of soybean and cowpea genotypes.

Parameters	Control			Moderate			Severe		
<b>Soybean</b>									
	TGx 1937-F	PAN 1664R	LS 678	TGx 1937-F	PAN 1664R	LS 678	TGx 1937-F	PAN 1664R	LS 678
Leaf relative water content	0.61	0.57	0.60	0.58	0.56	0.52	0.56	0.44	0.64
Proline content ( $\mu\text{mol/g}$ )	4.65 $\pm$ 0.36	4.62 $\pm$ 0.22	3.33 $\pm$ 0.22	5.47 $\pm$ 0.14	5.57 $\pm$ 0.09	3.80 $\pm$ 0.31	8.16 $\pm$ 0.49	8.16 $\pm$ 0.19	5.48 $\pm$ 0.38
Malondialdehyde content (mM)	4.31 $\pm$ 0.10	4.25 $\pm$ 0.10	3.21 $\pm$ 0.02	8.26 $\pm$ 0.96	6.83 $\pm$ 0.12	6.14 $\pm$ 0.66	11.4 $\pm$ 0.11	9.70 $\pm$ 0.20	9.08 $\pm$ 0.10
<b>Cowpea</b>									
	Red	Blonde	White	Red	Blonde	White	Red	Blonde	White
Leaf relative water content	0.88	0.85	0.87	0.88	0.81	0.87	0.72	0.85	0.78
Proline content ( $\mu\text{mol/g}$ )	0.76 $\pm$ 0.01	2.19 $\pm$ 0.07	1.81 $\pm$ 0.18	1.02 $\pm$ 0.42	2.84 $\pm$ 0.54	2.66 $\pm$ 0.07	2.96 $\pm$ 0.38	3.15 $\pm$ 0.35	3.29 $\pm$ 0.16
Malondialdehyde content (mM)	2.56 $\pm$ 1.17	2.67 $\pm$ 0.79	0.59 $\pm$ 0.08	3.66 $\pm$ 0.08	3.09 $\pm$ 1.08	0.92 $\pm$ 0.12	4.08 $\pm$ 0.09	4.65 $\pm$ 1.98	1.85 $\pm$ 1.61
<b>Photosynthetic pigments (<math>\mu\text{g ml}^{-1}</math>) soybean</b>									
	TGx 1937-F	PAN 1664R	LS 678	TGx 1937-F	PAN 1664R	LS 678	TGx 1937-F	PAN 1664R	LS 678
Chlorophyll a	20.00 $\pm$ 1.14	18.00 $\pm$ 4.05	11.50 $\pm$ 2.07	17.67 $\pm$ 0.82	15.67 $\pm$ 4.55	11.00 $\pm$ 0.89	17.50 $\pm$ 1.76	14.33 $\pm$ 2.25	11.67 $\pm$ 1.21
Chlorophyll b	27.17 $\pm$ 0.98	25.00 $\pm$ 1.41	16.33 $\pm$ 1.75	19.33 $\pm$ 1.21	17.00 $\pm$ 3.36	12.00 $\pm$ 1.41	18.17 $\pm$ 1.70	15.33 $\pm$ 2	11.17 $\pm$ 0.75
Carotenoids	25.75 $\pm$ 1.08	26.41 $\pm$ 4.84	24.75 $\pm$ 1.62	24.18 $\pm$ 1.20	24.75 $\pm$ 1.62	21.50 $\pm$ 1.94	20.57 $\pm$ 1.52	20.12 $\pm$ 1.34	19.42 $\pm$ 1.97
<b>Photosynthetic pigments (<math>\mu\text{g ml}^{-1}</math>) cowpea</b>									
	Red	Blonde	White	Red	Blonde	White	Red	Blonde	White
Chlorophyll a	54.90 $\pm$ 2.59	50.40 $\pm$ 2.58	63.70 $\pm$ 4.27	54.08 $\pm$ 5.30	50.20 $\pm$ 0.94	52.45 $\pm$ 3.32	44.06 $\pm$ 7.55	49.33 $\pm$ 1.68	48.45 $\pm$ 5.48
Chlorophyll b	46.19	47.15	46.53	46.18	47.04	46.07	30.93	33.18	30.80
Carotenoids	11.89 $\pm$ 2.03	12.67 $\pm$ 2.92	10.56 $\pm$ 1.24	11.77 $\pm$ 1.92	13.00 $\pm$ 1.87	10.11 $\pm$ 1.45	10.44 $\pm$ 1.51	11.78 $\pm$ 2.05	9.67 $\pm$ 1.58
<b>Note:</b> Values are presented as mean $\pm$ standard deviation of all replicates									

