UNIVERSITY FOR DEVELOPMENT STUDIES

EVALUATION OF ELITE SOYBEAN (*Glycine max* (L) Merril) GENOTYPES FOR NITROGEN NUTRITION, WATER USE EFFICIENCY AND GRAIN YIELD IN THE GUINEA SAVANNA AGRO ECOLOGICAL ZONE OF NORTHERN GHANA

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DECLARATION

I, Matey Duut hereby declared that this is the result of my work and that no part of it has been presented for another degree in the University or elsewhere. Work by others which served as source of information has been duly acknowledged by references to the authors.

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DEDICATION

I dedicate this thesis to my beloved wife (Happy Laari) and children Salima Yennumi Duut and Saleem Yennukua Matey for being the inspiring force behind my success.

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ABSTRACT

Nitrogen is very essential for every crop production due to its function in plant growth and development. It is an essential component of DNA and proteins which are the building blocks of life. Notwithstanding its vast importance, nitrogen is worldwide considered as one of the most limiting factors of production. The need to meet the huge nitrogen requirement has necessitated the use of synthetic fertilizer which continued application affects soil health, environment and agricultural sustainability. Identifying breeding lines and developing new legume crop varieties that have the ability to fix enough atmospheric nitrogen and integrating these varieties into the farming systems is a best alternative to reduce the harmful effects that are occasioned by the continued application of the synthetic fertilizers. A field experiment was conducted at the CSIR-Savanna Agricultural research Institute research fields using 20 elite lines under rain fed condition. The objective of the study was to select soybean (*Glycine max* (L.) Merril) elite lines for improved nitrogen fixation, water use efficiency and grain yield. A randomized complete block design was used with three replications. Data collected include; the amount of nitrogen fixed, nitrogen derived from the atmosphere, and grain yield. The nitrogen (N) difference technique was used. The genotypes showed statistically significant variability for Amount of N-fixed, percent nitrogen derived from the atmosphere and grain yield. The mean symbiotic N contribution of the genotypes ranged from 53.6 Kg/ha – 370.5 Kg/ha. Also, the grain yield of the genotypes were observed to be significantly different among the genotypes. Genotypes, SAR-SL2/SPG-18-4, SAR-SLI1/USL-18-2, SAR-SL2/USL-18-1, Favour, and FT Cristaline showed superior performance for N-fixed and grain yield. There was high heritability observed amongst the selected traits and high phenotypic coefficient of variation and genotypic coefficient of variation which is required in breeding program for crop improvement. The research suggested that the genotypes should be re-evaluated in multi-locations and if they perform same they can be considered for release as varieties.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

The cultivated soybean (*Glycine max* (L) Merril) is a world round legume crop that has been considered globally to be among the top-traded commodities, with a variety of uses. It grows well under many ecologies especially in the tropical, subtropical, and temperate environments (Saryoko *et al*., 2017). The cultivated soybean species [*G. max* (L) Merril] which belong to the family Fabaceae and with the order Fabales according to (Sedivy *et al*., 2017), is taught to have originated from East Asia and was domesticated about the 9th century from the wild species of soybean (*G. soja* sieb and Zucc). Although soybean is widely adapted to many soil types and climatic conditions, its response to photoperiod is a key function in its production and adaptation (Bu *et al*., 2021). Brazil, the United States of America, and Argentina, respectively, are currently the leading producers of soybean with over 80% of the world's soybean production (Dohlman *et al*., 2022). Soybean was first recorded in Sub-Saharan Africa (SSA) by the Chinese traders around the 19th century (Khojely *et al*., 2018). Even though soybean is not a staple crop like maize and other crops in Sub-Saharan Africa (SSA), its variety of uses for food, feed for livestock and sources of raw materials for the processing industries and other importance has given it the potential to become a cash crop (Khojely *et al*., 2018). Soybean is currently cultivated throughout Sub-Saharan Africa (SSA) with the largest production in South Africa, Nigeria, Zambia, and Uganda (Khojely *et al*., 2018). The seed of soybean is rich in dietary protein, oil, and energy. It contributes about 59% of the world's total oilseed production and 70% contribution to world protein consumption (Soystats, 2018). The seed contains about 14-24% of oil and 35-52% protein of the dry seed weight (Vollmann, 2016). Its oil is more desirable nutritionally due to its high linoleic acids content, low saturated fatty acid content,

and high amount of vitamins E which is a fat-soluble antioxidant that prevents cell damage which may lead to chronic diseases such as cancer (Marg & Mehta, 2019).

It contains all the eight essential amino acids and all the essential minerals (Marg & Mehta, 2019). According to studies, the optimum rate of nitrogen supplied to cereal crops after soybeans is lower than after non-leguminous crops (Gomes *et al*., 2013). Soybean meal is one of the most important ingredients in livestock and aquaculture feeds (Hartman *et al*., 2011). Soybean farming in Ghana is critical for overcoming hunger and supplementing the expensive source of animal protein. As a result, the cultivation of this crop is essential in the country in order to provide sufficient and high-quality protein to the country's stable cereal-based meal. The use of seed oil to make products like biodiesel to supplement fossil-based diesel is increasing and therefore making its application in the industrial and pharmaceutical sector very broad. In the United States, its abundant cultivation has made it the second most important crop in crop revenue total contribution to the country (Soystats, 2018).

In Sub-Saharan Africa (SSA), the diverse benefits of soybean and its potential to be used as a cash crop has made it receive significant attention (Khojely *et al*., 2018). The crop was first brought to Ghana in 1910, and subsistent farmers in the Northern Region started its cultivation (Plahar, 2006). By far, soybean is relatively a new crop in Ghana and cultivated largely under rain-fed conditions by smallholder farmers. However, it has gained significant attention, especially in Northern Region due to its important role in the rural economy of many farm households (Adjei-Nsiah *et al*., 2018). Currently, Ghana's domestic soybean grain demand exceeds the supply and therefore requires significant soybean grain importation to fill the gap. This has led to an exponential increase in production since 2012 (MoFA, 2016). Demand for soybean grain for poultry feed by the poultry farmers, agro-processing industry, and human consumption of its products accounts for the increase in production (Adjei-Nsiah *et al*., 2018).

The high demand for soybean requires the cultivation of high-yielding improved varieties. The agencies mainly mandated to undertake the soybean breeding program in Ghana are CSIR-Savanna Agricultural Research Institute (CSIR-SARI) and the Crops Research Institute of Ghana (CRI). Their mandate areas are the northern and southern regions, respectively.

The improved varieties available to farmers as released by CSIR-SARI in the Northern Region include Salintuya I, Salintuya II, Quarshier, Jenguma, Afayak, Favour, Songda, and Suong Pungun. Low soil fertility is a major cause of low agricultural output in West Africa's Guinea Savanna (Kombiok *et al*., 2012). Farmers in this region often resort to the application of inorganic fertilizers to increase their yields, which is costly and can pollute air and water. Soybean is useful in fixing atmospheric nitrogen into the soil with its root nodules by forming a symbiotic association with the soil bacteria, rhizobia which can help to enrich the poor soils in the Guinea Savanna agro-ecology which are constraint by nutrients such as nitrogen and phosphorous (Ahiabor, 2011). Because of its role in plant growth and development, nitrogen is particularly important in soybean production, and the fixed form of nitrogen, ammonia (NH_3), is required as an essential component of DNA and proteins (Nelson *et al*, 2008). It is also needed for the biosynthesis of chlorophyll which is a main component of photosynthesis (Bano & Sheikh, 2016). At the global level, biological N_2 fixation (BNF) is a significant source of soil input. Inorganic fertilizer accounts for around a quarter (25%) of the earth's fixed nitrogen, while BNF contributes for about 60% of the earth's fixed nitrogen (Bano & Sheikh, 2016). This research aimed to assess symbiotic N_2 fixation and N contribution of different soybean genotypes using the N difference technique.

1.2 Problem Statement and Justification

Agricultural lands in the Guinea savannah agro-ecology of Ghana are constrained by poor soil fertility, especially low nitrogen (N) and phosphorous (P), and drought which often affects crop production (Ahiabor, 2011). Nitrogen is required for building proteins and DNA which are the

Centre of life (Nelson *et al*, 2008). Farmers often resort to the application of chemical fertilizers to increase their yields, which is costly and can pollute the air and water bodies (Eickhout *et al*., 2006). All over the world, there is an ecosystem perturbation that is caused by the excessive application of chemical or inorganic fertilizer (Guignard *et al*., 2017). There is a need for alternative means of ensuring the availability of nitrogen and employing biological nitrogen fixation seems a logical option. Although nitrogen is abundant in the atmosphere in the form of N_2 gas, nitrogen is limiting in most agricultural lands globally due to its inert form (Unkovich *et al*., 2008). Therefore, the ability of legumes to form an effective symbiotic association with Rhizobium and Bradyrhizobium to convert the atmospheric N_2 into ammonia enables them to fix a high quantity of symbiotic nitrogen (Oldroyd *et al*., 2011; Unkovich *et al*., 2008; Vitousek *et al*., 2013). This can help to enrich the poor soils in the Guinea Savannah agro-ecology which are constrained by nutrients such as nitrogen and phosphorous (Ahiabor, 2011). It can also help achieve sustainable agricultural development without any harm to the ecosystem.

1.3 General Objective

The study's main objective was to select soybean genotypes for improved nitrogen fixation, water efficiency, and grain yield in Northern Ghana's Guinea Savanna Agro-Ecological Zone.

1.3.1 Specific Objectives

i. To determine nitrogen-fixing efficiency of elite soybean lines
ii. To determine N contribution of selected soybean lines to the soil
iii. To determine water use efficiency of elite soybean lines
iv. To determine grain yield of elite soybean lines

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy

Soybean taxonomy has been revised several times since 1962. A review by Singh (2019) stated that Soybean belongs to the genus *G.* Wild, family Fabaceae, subfamily Papilionoideae, order fables, and tribe Phaseoleae and subtribe Glycininae. Singh (2019) split the genus *G.* into *G.* and *Soja* subgenera. The cultivated soybean (*G. max* (L) Merril) and the undomesticated soybean (*G. Soja* Sieb & Zucc.) belong to the Soja (Moench) F.J. Herm subgenus (Singh, 2019). He suggested that the cultivated and undomesticated soybeans both have 20 chromosomes (2n=40). However, the cultivated species has a narrow gene pool as compared with the undomesticated species (Kim *et al*., 2011). *G.* soja is a genetically diverse species that has been demonstrated to be more genetically varied than *G. max* (Kofsky *et al*., 2018). *G. max* and *G. soj*a are both cross-compatible and have typical meiotic chromosomal pairing (Chen & Nelson, 2004). According to Carpenter *et al*. (1986) as cited by Singh, (2019) *G. soja* exhibits various unfavorable genetic features, including lodging, shattering, and small seeds size, which are all intimately connected to desired qualities. The *G*. subgenus contains 26 species unique to Australia and the neighboring islands, all of which are wild and perennial (Singh, 2019). He also reported that the perennial *G*. species inherit features like biotic and abiotic stress resistance that aren't found in cultivated or wild soybeans and could be introgressed into *G. max* if a method for producing fruitful plants by intersubgeneric hybridization is established. Three species (*G. argyrea, G. canescens, and G. tomentella*) have successfully hybridized with cultivated soybean thus far.

2.2 Origin and Distribution of Soybean

There are vast history and reviews on soybean origin and domestication by several authors. One of the reviews by Hymowitz (1970) as cited by Hartman *et al*. (2011) reported that Soybean was initially grown as a food crop in Northeastern China about the 11th century B.C. Traditional accounts, on the other hand, say that domesticated soybeans were used as a food crop as early as 2300-2500 B.C. Soybean (*G. max* (L) Merril) is said to have originated in East Asia and was domesticated about the 9th century from wild soybean species (*G. soja* sieb and Zucc) (Sedivy *et al*., 2017). Soybean was first recorded in Sub-Saharan Africa by the Chinese traders around the 19th century (Khojely *et al*., 2018). It was introduced into countries like India, Myanmar, Vietnam, Korea, Malaysia, Japan, the Philippines, and Thailand around the 11th century A.D. (Hartman *et al*., 2011). By 1737 it was recorded in Europe in Linnaeus. In countries such as England and France, soybean was first recorded in 1790 and 1739, respectively, where it was grown for ornamental purposes (Hartman *et al*., 2011). In Ghana, it was first introduced in 1909 by the Portuguese Missionaries. It was not commonly used as a food crop, however, due to the difficulty of preparing it at the household level. Nonetheless, due to the demand from chicken farmers, the agro-processing industry, and human consumption, its cultivation has recently gained commercial relevance. Given the economic importance of soybeans and their limited genetic base, molecular genetics and genomics methods are becoming increasingly important in ensuring consistent gains in yield potential to fulfill the food and nutritional need (Xu *et al*., 2020). Brazil, the United States, and Argentina produce the majority of the world's soybeans. China is the top consumer of soybeans from the United States, Brazil, and Argentina (USD, 2020). So three large producers (Brazil, the United States, and Argentina) and one significant consumer drive the worldwide soybean market (China).

In 2020/21, Brazil, the United States of America, and Argentina, respectively were the leading producers of soybean with over 80% of the world's soybean production (Dohlman *et al*., 2022). In the same cropping season, 2019/20, the world's total soybean production volume was about 336.46 million metric tons with Brazil being the largest producer with about 126 million metric tons (Dohlman *et al*., 2022). The United States was the second-largest producer with production volumes of 96.67 million metric tons. The area under production in these two leading countries was 36.9 million hectares for Brazil and 30.33 million hectares for the United States (Dohlman *et al*., 2022). Ghana's production has surged dramatically in the recent decade, rising from 74,800 Mt in 2008 to 176,670 Mt in 2018, while the area under cultivation has climbed from 61,800 ha to 102,980 ha (MoFA, 2016). Ghana's Northern sector and Northern Volta areas produce the majority of soybeans. Northern Ghana produces the most among these geographical regions, as it is located within the Guinea savannah and Sahel agro-ecological zones (MoFA, 2016). The Northern region alone accounts for over 70% of total soybean land and 77 percent of total production (MoFA, 2016). The minor soybean production areas in Ghana include the Upper East, Upper West, Brong-Ahafo, and Volta Regions (Lawson et al., 2009). In Ghana, however, soybean production has increased since 2013. (MoFA, 2016). The expansion of production is attributed to poultry and small-scale processing companies, as well as rising food oil consumption (Gage *et al*., 2012).

2.3 Morphology of Soybean

Soybean as an annual crop shows an erect or twining growth habit (Bernard and Weiss, 1973). The leaves have three leaflets, which are shed at maturity. The plant has primary leaves which are opposing single-leaf and secondary leaves which are three-leaf alternate, and the compound leaves are mostly four leaflets (OECD, 2000). Flowers are small with small white or purple colour and grow in bunches from the leaf axils. The majority of soybean cultivars have tiny trichomes, however, glabrous varieties exist as well. The flower consists of tubules, Calyx,

sepals, corolla, petals, pistils, and stamens. Before pollination, the stamens create a ring at the base of the stigma and elongate, after which the elevated anthers form a ring around the stigma (Datta *et al*., 2005). Pods are short, hairy, and brown or grey in colour. The pod is straight or slightly curved, measures two to seven centimeters in length, and is made up of two halves of a single carpel linked by a dorsal and ventral suture. The seed shape, which is normally oval, varies amongst cultivars. The seeds are of different colours and sizes. The seed colour is diverse ranging from whitish-yellow, brown, black, green, and mottled. The varieties of soybeans are grouped as determinate, semi-determinate, and indeterminate (OECD, 2000). The determinate growth feature is that when the terminal buds become inflorescences on the axillary and terminal racemes, their vegetative activity ceases or cultivars stop growing in height at flowering or shortly thereafter while the stems continue to expand in width and the terminal buds usually become inflorescence (OECD, 2000). The indeterminate cultivars continue to grow in height throughout flowering and pod development stages; often the height is doubled after flowering or in other words cultivars continue to be nutritionally active throughout the flowering period (OECD, 2000). The semi-determinate type has an indeterminate stem and suddenly stops vegetative growth after the flowering period. The soybean plant shows the development of tap roots initially and later follows with secondary roots. The soybean roots have nodules that form a symbiotic relationship with nitrogen-fixing bacteria (rhizobia). The nodular root system consists of a tap root, from which a lateral root system grows. According to Carlson and Lersten, 2004 as cited by (Singh *et al*., 2007), soybean is a 99% self-pollinated species with only 1% likelihood of natural cross-pollination due to the position of the stigma and the anthers which contains the pollen grains.

2.4 Importance of Soybean

Soybean is an important annual legume crop with diverse uses. It contributes about 59% of the world's total oilseed production and 70% contribution to world protein consumption (Soystats,

2018). It also serves as a source of protein, oil, and therapeutic ingredients for many medically important chemical products (Marg & Mehta, 2019). Its oil is more desirable nutritionally due to its high linoleic acids content, low saturated fatty acid content, and high amount of Vitamin E which is a fat-soluble antioxidant that prevents cell damage, which may lead to chronic diseases such as cancer (Marg & Mehta, 2019). It contains all the eight essential amino acids and all the essential minerals (Marg & Mehta, 2019). Soybean has more protein (40%) as compared to 18% for fish or beef. The crop is widely grown for its high protein source (35-52%) and oil (14-24%). The majority of the underdeveloped countries in the tropics are currently interested in growing soybeans to meet rising protein, vegetable oil, and poultry feed demands. Soybean oil's neutral flavor and well-balanced fatty acid profile make it a versatile ingredient for a range of applications from food to dish dressings (Marg & Mehta, 2019). Soybean oil contains 23 percent monounsaturated fat, most of which is oleic acid. Polyunsaturated fats are considered healthy when consumed instead of saturated and trans fats since they help lower dangerous cholesterol levels. Omega-3 fatty acids, found in polyunsaturated fats, are essential fatty acids that the body cannot produce on its own. Omega-3 fatty acids are anti-inflammatory and help to prevent heart disease and arthritis. It also improves overall brain health and cognitive performance (Marg & Mehta, 2019).

In Sub-Saharan Africa (SSA), the discovery of the soybean as a high source of protein has led to the increase in its utilization and consumption of its products (Khojely *et al.*, 2018). It is commonly utilized by locals in Sub-Saharan Africa (SSA) for soymilk, oil, "dawadawa," soy yogurt, soy kebab, and a variety of other items. In Ghana, it is used in making local delicacies such as "gablee", "zimbegu", "tubaani" and "soya". A reference, Liu (2008), as cited by (Hartman *et al.*, 2011), indicated that 5% of the total oil of the soybean seed is used in the processing industry for cosmetic and hygiene products. Flour, oil, cookies, candies, milk, veggie cheese, lecithin, and a variety of other foods are made with soybean. Also, cake after

oil extraction, the haulm, and the husk after harvest can be used for livestock feed. The crop has the potential of providing a less expensive source of protein and improving the livelihood of smallholder farmers by supplementing family income through the selling of crop production for cash. Soybean benefits soil nitrogen enrichment by forming a symbiotic connection with rhizobia, a nitrogen-fixing bacteria. It is also beneficial in the management of Striga hemonthica, a lethal disease parasitic weed of cereal crops found in the Guinea savanna Agro-ecological zone of Northern Ghana that causes significant yield losses of millet, sorghum, and maize of up to 100%. Although soybean is not a Striga host plant, it does release chemicals that aid in the germination of Striga seeds. The germinated seeds will however vanish after a few days since they are unable to link their root system to the soybean in order to take nourishment and water.

2.5 Varieties of Soybean

There are a good number of high-yielding varieties that have been released by the CSIR-Savanna Agricultural Research Institute (CSIR-SARI) which adapt very well in Guinea Savanna and the forest zones of Ghana. These include; Jenguma, Afayak, Quarshie, Songda, Salintuya I, Salintuya II, Favour, and Suong Pungun. There yield potential range between 1.8 – 3.5 t/ha under good cultural practices, favorable conditions, and optimal plant population. They are all medium maturing varieties maturing between 115-120 days except Suong Pungun which is early maturing, within 90 days. They are all resistant to pod shattering which is a must-have attribute for any released variety. Soybean cultivars are classified according to adaption and are determined by latitude and day length. There are thirteen maturation groups (MG) of soybean in North America, ranging from MG 000 in the north (45° latitude) to MG X towards the equator (Datta *et al.*, 2005). Varieties are classified as early, medium, or late maturing within each maturity group.

Table 1: **Soybean varieties released in Ghana**

Variety	Origin/Source	Days to maturity	Yield potential (t/ha)
Salintuya - I	CSIR-SARI/ IITA	115-120	2.2
Salintuya - II	CSIR-SARI/ IITA	120-130	2.2
Jenguma	CSIR-SARI/ IITA	110-115	2.8
Afayak	CSIR-SARI/ IITA	110-115	2.4
Quarshie	CSIR-SARI/ IITA	110-115	2.4
Songda	CSIR-SARI/ IITA	115-120	2.2
Suongpungun	CSIR_SARI/IITA	85-92	1.8
Gyidie	CSIR-SARI/ IITA	80-90	3.2
Latara	CSIR-SARI/ IITA	110-115	3.2
Favour	CSIR-SARI/ IITA	115-118	3.5
Toondana	CSIR-SARI/ IITA	110-115	3.5
Anigye	CSIR-SARI/ IITA	101	3.4

Source: Catalogue of crop varieties released & registered in Ghana, 2019

2.6 Grain Yield of Soybean Reported So Far in Africa

With an average output of 2,290 kg ha^{-1}, South Africa is the largest soybean producer in Sub-Saharan Africa (SSA), followed by Zambia (1,940 kg ha^{-1}), Nigeria (960 kg ha^{-1}), and Uganda (600 kg ha^{-1}) (Khojely *et al*., 2018). Zimbabwe, Malawi, Ghana, Sudan, and Ethiopia are other nations with sizable production. Sub-Saharan African (SSA) soybean production has increased in area and yield over the past four decades, going from 20,000 ha at 13,000 tons (0.65 Mg ha^{-1}) in 1970 to 1.5 million ha at 2.3 million tons (1.53 Mg ha^{-1}) in 2016 (Khojely *et al*., 2018) However, smallholder farmers' average yields are low (1.0 t ha^{-1}) compared to the global average production of 2.8 Mg ha^{-1} (Khojely *et al*., 2018). (Purdy & Langemeier, 2018). Low

soil fertility, a lack of high-yielding cultivars, and little input use are the main causes of this (Pagano & Miransari, 2015). Over 80% of Ghana's soybean crop is grown in the northern savanna, with average yields of less than 0.8 Mg ha^{-1} in smallholder farms (Amanor-Boadu *et al*., 2015; Aidoo *et al*., 2014). This is in contrast to Ghana's average national yield of 1.65 Mg ha^{-1} (SRID-MOFA, 2016). Despite significant advancements in the development of high-yielding soybean cultivars (Tefera, 2011), low input farming practices (Tamimie, 2017), a lack of agronomic management expertise, and declining soil fertility continue to cause soybean yields to be relatively low. These issues make it difficult for smallholder farming systems to produce soybeans successfully and sustainably (Khojely *et al*., 2018).

2.7 Growth Requirements of Soybean

2.7.1 Soil and Moisture

Well-drained fertile soil with a pH of 5.8-7.0 is suitable for soybean cultivation. But it can be grown on a variety of soils such as sand and clay loam. Latitude 0-2000 m above sea level is best for soybean cultivation. For high nodulation of soybean in new areas of soybean production, Bradyrhizobium japonicum inoculation is required. Soybeans do not thrive in acidic soils and may require the addition of limestone (Awuni *et al*., 2020). Soybeans are frequently cycled with corn, cotton, and wheat. Soybeans thrive on soils with relatively high clay content, while they do not thrive on weak sands. Because of drought stress, soybeans perform poorly in sandy soils and soils with low water storage capacity, such as gravelly or shallow soils. Seed germination and plant establishment will be hampered on clay soils due to poor aeration (Idu *et al.*, 2003). Water stress is described as a lack of soil water necessary for plant growth and development, which might affect various metabolic processes in plant cells. The direct effects of drought stress on soybean physiological development are determined by the efficiency with which it uses water (Gebre & Earl, 2021; Zhao *et al*., 2020). Water use efficiency is a physiological trait connected to a plant's ability to cope with water stress that is

significant in soybean management. Grain yield is a function of transpired water, water use efficiency, and harvest index (Zhao *et al*., 2020).

2.7.2 Temperature and Rainfall

Soybean can grow at a variety of temperatures, but it thrives in warm, humid environments. Factors such as temperature, light, and moisture play a key role in influencing soybean germination (Kurt & Bozkurt, 2006). Soybean seed germinates when the soil temperature exceeds 10°C and emerges in 5-7 days if conditions are favorable. Soil temperatures at planting should be around 15^{o}C for quick and good germination and about 20^{o}C-25^{o}C is ideal for growth and development (Lamichhane *et al*., 2019). Soybean plant requires a well-distributed minimum rainfall of 400 mm for a period of 3-4 months after planting. Moisture content is one of the most important factors to consider before planting. Enough moisture but not high moisture content is required at planting for good germination. The high moisture content at the time of planting can cause the seed to rot, hence affecting germination. Soybeans, especially from flowering through pod maturity, require consistent rainfall. Increased drought stress can slow crop growth rate, reduce leaf area, and shoot dry matter which can lead to a reduction in soybean yield (Zhao *et al*., 2020). Drought stress during blooming and early pod development, according to (Cui *et al*., 2021; Gao *et al*., 2020) causes the biggest drop in the number of pods and seeds at harvest. Between emergence and the four-leaf stage, soybeans are prone to waterlogging (Morita *et al*., 2004). In comparison to other non-rice crops, soybean has high resilience to waterlogging after this stage. Soybeans can grow and produce grains practically throughout their life cycles, even when there is a lot of water (waterlog condition) (Kuswantoro, 2015). The water requirement of soybean grows during the vegetative stage, peaks at reproductive maturity, and subsequently decreases. Large fluctuations in soil water volume and distribution affect soybean output. According to (Ivanova *et al*., 2016), water plays two important functions in plants: it acts as a solvent and transport medium for plant nutrients,

and it also acts as an electron donor in photosynthetic reaction pathways. Soybean is highly susceptible to water stress, especially at the vegetative stage. Therefore it responds to regular irrigation by significantly increasing vegetative growth and production. According to (Wijewardana *et al*., 2019), lack of water in the soil affects cell formation and development, and leaf formation during the vegetative growth stages. Soybean soil moisture stress resistance has been measured using plant height, stem node, internode length, and leaf area expansion, among many other growth and developmental traits (Desclaux *et al*., 2000; Ku *et al*., 2013). Soybean is a versatile crop that thrives in a variety of climates and soil types. The Forest-Savanna Transition and Guinea-Savanna agro-ecological zones in Ghana, with well-drained fertile soils and annual rainfall of at least 700 mm dispersed throughout the growing season, are the finest settings for soybean growth (Lawson *et al*., 2009).

2.7.3 Photoperiod

Soybean is a quantitative short-day plant, which means it flowers faster under short-day sunlight (Bu *et al*., 2021). Therefore, the photoperiod and temperature are critical factors in selecting cultivar adaption zones. When it is planted at a time the short-day conditions are very close, the period to flowering and from flowering to pod set will be shorter than natural conditions (Zheng *et al*., 2003). Though soybean plants are very sensitive to dry conditions at flowering and pod settings, they require dry conditions for ripening. Planting should be carefully timed so that the crop gets enough moisture for growth, flowering, pod filling, and sufficient sunlight for seed maturity, pod drying, and harvesting. The latitude of an area that determines the day length is key in selecting a variety for planting since it can affect varietal maturity (Schoving *et al*., 2020). Soybeans when exposed to a day length shorter than the critical length at the early growth stages will grow stunted and mature early which can lead to low yield production.

2.8 Planting of Soybean

Soybean can be planted on flat, ridges or a well-prepared seedbed with a moderate tilth necessary to speed up germination. The planting time varies from region to region. The best time for planting in rainfall-dependent regions is when the rains are well established, thus from mid-May to July in the case of northern Ghana. Soybeans should be planted in rows for easy management. Depending on the type of soybean variety, row spacing can be as close as 35 cm by 5 cm between row and within plants, respectively. Close plant spacing provides adequate competition against weeds when the crop becomes well established (Toleikiene *et al*., 2021). They also reported that a minimum population of 250,000 plants per hectare is necessary for high yields. The yield of all legumes, including soybean, is a function of plants harvested in an area, pods per plant, seed weight, and seeds per pod (Toleikiene *et al*., 2021). Research results by (Lawson *et al*., 2009) showed that planting between 1 and 4 cm resulted in high crop emergence values ranging from 94.14 to 96.60 percent, and at below 4 cm depth, there was a significant drop in emergence. They observed that planting depth of 8 cm had the least crop emergence, with a crop emergence value of 19.58 percent. They again realized that when compared to conventional sowing on flat terrain, mounding and ridging the field significantly boosted crop emergence by 3.95 and 10.61 percent, respectively. Ridging resulted in the highest crop emergence and was statistically different from mounding.

Soybean seeds may fail to germinate when planted within 6-10 months after harvesting depending on the variety and the storage environmental conditions, especially under hot and humid conditions. Seed germination tests should be carried out before planting. The recommended class of seed for farmers is a certified seed and planting depth should be 3-5cm. Two seeds per hole are recommended to avoid seed wastage or seeds should be drilled within and later thinned out to one seedling per hole. Soybean seed germinates when the soil temperature exceeds 10°C and emerges in 5-7 days if conditions are favorable.

2.9 Fertilizer Application

There is less attention given to the need to apply fertilizer to soybean as compared to other crops in Ghana. However, many research reports are indicating that lack of essential nutrients in the soil such as nitrogen and phosphorous is one of the factors causing low soybean yields. In West Africa's Guinea Savannah, inadequate soil fertility is a key reason for low agricultural productivity (Kombiok *et al*., 2012). Nitrogen deficiency in the soil negatively affects the yield of soybean (Kinugasa *et al*., 2012). Nitrogen top treating during the blooming period of soybean can significantly increase soybean reproductive growth and grain yield (Zhou *et al*., 2019). Two sources of nitrogen (N): soil mineral nitrogen and nitrogen from the atmosphere via fixation in the root nodules are required to meet the soybean nitrogen demand (Rymuza *et al*., 2020). For high soybean production, high quantities of nitrogen must be sustained for a length of time by nitrogen fixation, however chemical fertilizer application can severely restrict nodule formation and nitrogen-fixing (Ohyama *et al*., 2017). Soybean yield can be increased slightly by applying phosphate fertilizer to phosphorus-deficient soils. When phosphorous fertilizer was applied at 60 kg ha^{-1} in the Guinea Savanna Agro-ecological Zones of Ghana, (Adjei-Nsiah *et al*., 2018) observed a 390 kg increase in soybean yield. NPK recommendation for soybean in all ecologies in Ghana is N-P_2O_5-K_2O: 20-60-30 + 0.8Zn per hectare (Tetteh *et al*., 2017). However, for a profitable cost-effective program, a soil test is required to be carried out before fertilizer application. Phosphorous deficiency in the soil can limit atmospheric nitrogen fixation by the root nodules.

2.10 Diseases, Pests, and Control Measure

There are so many diseases that affect soybean in the world. The widespread soybean introduction and the increase in the production area have caused an increase in the number of soybean diseases (Hartman *et al*., 2011). Continuous cultivation of soybean on a piece of land can lead to disease-build up which can result in economic losses. The need to produce crops

that are pathogen free often necessitates various inputs, including the identification of problems that impair the crop's health. A healthy crop starts with strong seed quality that is pathogen-free and is highly viable (Hartman & Murithi, 2014). Based on where the pathogen affects the plant, soybean diseases are classed as leaf diseases, stem diseases, pod and seed diseases, and root diseases (Hartman & Murithi, 2014; Hartman *et al*., 1992)). Bacterial Pustule, Brown Spot, Cercospora Leaf Blight, Frog-eye Leaf Blight, Red-Leaf Blotch, and Viruses are some of the most frequent soybean leaf diseases (Hartman & Murithi, 2014). Charcoal Rot, Green Stem, Anthracnose, Pod and Stem Blight, Sclerotium Blight, and Sclerotinia Stem Blight are among the stem diseases on their list. Phomopsis Seed Decay, Purple Seed Stain, and Seed Mottling disease are examples of soybean Pod and Seed diseases (Hartman *et al*., 2011). Charcoal Rot, Sudden Death Syndrome, and Root-Knot Nematode were all cited again as root diseases. Hartman *et al*. (2011) and Hartman *et al*. (1999) estimated losses caused by disease to be at 11%. In Ghana, the major insect pests of soybean are the defoliators, *Spodoptera spp*, *Zonocerus variegatus, Sylepta derogate,* and a complex of pod sucking bugs including *Nezara viridula* L, *Aspavia amigera* F, and *Riptortus dentipes* F (Abudulai *et al*., 2012). They also reported that soybean disease pathogens and pests can cause damage to any part of the plant. And that conventional chemical control has been the principal recourse for the control of pests on soybean worldwide. Products such as Endosulfan, Thiometon, Carbaryl, Trichlorfon, and Cypermethrin are among the chemicals recommended for insect control especially in large-scale production (Abudulai *et al*., 2012). According to Hartman and Hill (2010), as cited by Hartman *et al*. (2011) the recommended management practices that can help reduce pest and disease losses include the planting of resistant varieties, crop rotation, appropriate fertilization, irrigation and drainage, field scouting, use of disease-free seeds and pesticide application.

2.11 Biological Nitrogen Fixation by Soybean

The biological nitrogen fixation process was discovered by Hermann Hellriegel and Wilfarth in 1886 (Franche *et al*., 2009). N_2-fixing organisms, often known as diazotrophs, come in a broad variety of forms (Unkovich *et al*., 2008). Some organisms can fix N_2 in a free-living state, while others can only fix N_2 in the presence of plants. Because of its role in plant growth and development, nitrogen is critical for soybean production, and its fixed form, ammonia (NH3) is needed as an essential component of DNA and proteins (Singh, Nelson, *et al*., 2007). It is also needed for the biosynthesis of chlorophyll which is a main component of photosynthesis (Bano & Sheikh, 2016).

Two sources of nitrogen (N): Soil mineral nitrogen and nitrogen from the atmosphere via fixation in the root nodules are the main sources required to meet the soybean nitrogen demand (Rymuza *et al*., 2020). Biological nitrogen fixation is a high-energy process that necessitates the use of 16 ATP molecules to break down a single N2 molecule. Also, For NH4+ assimilation and transport, an extra 12 ATP molecules are required, for a total of 28 ATP molecules (Soumare *et al*., 2020). Crop demands, soil (soil type, texture, organic matter content), the type of farming practices utilized by farmers, and the availability or absence of effective rhizobia strains in the production of legumes all influence the amount to which N deficit arises. Rhizobia is a broad term that refers to a group of soil bacteria that induce new organs, known as nodules, to grow on the roots of certain legumes. Nitrogen gas (N_2) in the atmosphere accounts for about 80% of the earth's atmosphere (Bano & Sheikh, 2016). However, for this amount of nitrogen to be available for plant use, it has to be converted from the inert gas form (N_2) into its usable form called ammonia (NH_3) through a natural process known as biological nitrogen fixation (Soumare *et al*., 2020). Prokaryotes, archaea, and bacteria are the nitrogen-fixing microorganisms that are involved in biological nitrogen fixation (Soumare *et al*., 2020). Examples of the groups of bacteria that are involved in this process include the free-living

bacteria such as Azotabacter, Bacillus, and Azospirillum or Clostridium; symbiotic bacteria such as Rhizobium which is associated with legumes; Cyanobacteria which is in association with cycads and Frankia associated with actinorhizal plants (Ininbergs *et al*., 2011; Ravikumar *et al*., 2007). For Archaea, nitrogen fixation is restricted only to groups that can produce methane, Methanogens (Welte, 2018). The bacteria grow, develop into bacteroids, and fix N_2, which is transformed to ammonium by nitrogenase, a prokaryote-only enzyme. Plants are provided with ammonium, which in turn provides bacteria with carbon sources. These gram-negative bacteria can be found in the soil or used to treat seedlings or soil. In some areas, the presence of suitable rhizobia in the soil happens naturally, whereas, in others, this is not the case. The free-living bacterium tends to grow well in the rhizosphere where they are stimulated by the flow of carbon from the plant root. Existing mineral nitrogen in the soil limits biological nitrogen fixation (BNF). Therefore, root nodule formation which results in BNF is initiated when nitrogen levels in the soil are low. According to (Franche *et al*., 2009), soybean root and the bacteria use cell signaling for association and developing nodules and the steps of nodulation are as follows: The association is initiated by the soybean root. It initiates by sending out a signal (Flavonoid) to attract the rhizobia which is compatible with the legume root. The attracted rhizobia around the root hair will then secrete nodulation factors, which cause the root hair to curl and facilitate selective adsorption to the plant. The invasion is facilitated by the invagination of the wall of the root hair into a tube called the infection thread. Once the bacteria enter the root, both the plant and the bacteria cells multiply and this initiates the development of the nodule. An enzyme complex called nitrogenase possessed by the nodule-forming bacteria enables the Rhizobia to fix the nitrogen. Vascular tissues are developed for nodules for the exchange of nutrients. Biotic and abiotic factors such as the cultivar, rhizobium, weather, and agricultural conditions are responsible for nitrogen uptake

(Rymuza *et al*., 2020). Rhizobial activity is the main determining factor in the contribution of N from BNF in soybean, which ranges from 50 to 60% (Salvagiotti *et al*., 2008)

2.12 Nitrogen Nutrition in Soybeanm

By symbiotically fixing N_2, legumes are known to increase soil fertility (Vanlauwe *et al*., 2010). However, the nitrogen (N) requirement for soybean plants is very high (Bellaloui, *et al*., 2015). Biologically fixed N_2 and mineral N fertilizer are the two main sources of nitrogen for soybean plants (Salvagiotti *et al*., 2008). These two sources may be complementary or antagonistic to one another, depending on the environmental conditions or phases of development (Basal & Szabó, 2020). One advantage of fixed N_2 is that plants use it right away, with no possible losses brought on by the environment (Basal & Szabó, 2020). Also, chemical N-fertilizer is significantly more expensive than commercial inoculants (Basal & Szabó, 2020). Although some researchers claimed that inoculated soybean does not require the application of N fertilizer (Basal & Szabó, 2020), other researchers claimed the opposite (Ray *et al*., 2006; Lindström *et al*., 2010), suggesting that fixed N_2 supplies soybean plants with, on average, 50–60% of the N they need (Salvagiotti *et al*., 2008). Additionally, the process of inoculation can strengthen the plant's tolerance to abiotic stressors (Gurska *et al*., 2009). The N_2-fixation process has been shown to be inhibited by high rates of N fertilizer, yet early stages of soybean development can benefit from a relatively low dose because the process has not yet begun (Caliskan *et al*., 2008). Herridge, (1982) have reported that soybean can contribute about 337kgNha^{-1}. The percentage of nitrogen that derives via symbiotic fixation in soybeans in most soils with moderate nitrate levels is around 50% (Hardarson *et al*., 1984; Bergersen *et al*., 1985) but can reach 75% in sandy loamy soils (Matheny and Hunt, 1983). During pod fill and at the end of blooming, nitrogen fixation is at its maximum rate (Sogut, 2006; Caliskan *et al*., 2008). The primary source of nitrogen for seed growth seems to be the nitrogen ingested between the beginning of pod development (stage R3) and the beginning of maturity (stage R7)

(Warembourg & Fernandez, 1985; Zapata *et al*., 1987). Increased seed production and seed protein content can result from better N fixing (Fabre and Planchon, 2000).

2.13 Importance of Nitrogen to Crop Production

Nitrogen is a vital limiting ingredient for plant development and production, as well as one of the most significant nutrients for crop growth. Because it's necessary for chlorophyll formation which is a vital pigment for photosynthesis, and amino acids, the basic building blocks of proteins, it's worth mentioning (Nelson *et al*., 2008). Although it makes up 78 percent of the atmosphere, it is not directly accessible to plants. Plants utilise it in the form of nitrate or ammonium ions, which they absorb through their roots. It is a source of protein and nucleic acids that are carried from older to younger tissues, which explains why a nitrogen-deficient plant's older leaves would yellow first, due to the death of chloroplasts and the absence of the green pigment, chlorophyll. Nitrogen fertilizer application increases biomass and protein yields and concentrations in plant tissue (Blumenthal *et al*., 2008). They also observed that the amino acid composition of protein, and thus its nutritional quality, is frequently influenced by nitrogen. An abundance of nitrogen in grains reduces the relative amount of lysine and threonine, lowering the protein's biological usefulness (Blumenthal *et al*., 2008). Increased nitrogen levels improve kernel integrity and strength, resulting in improved grain milling qualities. When nitrogen fertilizer is applied to oilseed crops, protein levels rise while oil concentration falls (Blumenthal *et al*., 2008). Nitrogen fertilizer has a mixed effect on oil composition and quality. (Blumenthal *et al*., 2008) when compared unfertilized soybeans to fertilized soybeans at a rate of 179 kg ha^{-1} nitrogen application on clay soil they observed a reduction in seed protein by 1.05 percent. However, compared to unfertilized soybeans, N application at 179 kg ha^{-1} increased oil content by 0.7 percent on clay soil (Kaur *et al*., 2017). They further observed that nitrogen application reduced the amount of stachyose in both soil textures, although fatty acids had a mixed reaction to nitrogen. When compared to properly

nourished plants, inadequate nitrogen supply frequently causes stunted development, low protein levels, low yields, and inefficient water use. All of these factors contribute to greater disease susceptibility. Excess nitrogen, on the other hand, can be harmful to crop growth and quality, as well as have negative environmental consequences. Insufficient nitrogen causes stunted and slower plant growth, which also reduces the quantity of protein in the seed and plant (Silva & Uchida, 2000). Furthermore, nitrogen deficiency can influence crop standability as grain fill occurs, since a deficient plant would suck nitrogen from the leaves and stalk for grain fill, weakening the stalk and generating standability issues. To ensure enhanced efficiency and profitability for the farmer, soil nitrogen management should be included in a soil fertility program.

2.14 Factors Affecting legumes Biological Nitrogen Fixation

Nitrogen fixation of legumes is a chemical process which can be affected by soil pH, soil water, available nitrogen content and temperature (Liu *et al*., 2011). Essential nutrients such as potassium and phosphorus when present in very low levels affect directly or indirectly the nodule growth and the metabolic activities of the nitrogenase (Khosro Mohammadi, 2012).

2.14.1 Soil pH

In soils, pH is commonly cited as the primary determinant of prokaryotic community structure. The pH of the soil is among the important factors that influence the process of legume biological nitrogen fixation. There are many scientific research reports that have extensively looked at the role soil pH plays in the process of legume biological nitrogen fixation. Research findings by (Lammel *et al*., 2018) reported that low and high soil pH can indirectly affect crop growth and development by affecting the availability of nutrients needed by crop for its growth and development. In acidic soils, elements such as phosphorus (P), magnesium (Mg), calcium (Ca), molybdenum (Mo), and potassium (K) are few, while the iron (Fe), aluminum (Al), hydrogen (H), copper (Cu), and manganese (Mn) ions are abundant (Bakari *et al*., 2020; Keino

et al., 2015). Soybean growth is inhibited by soil pH levels of less than 5.2 and more over 6.5, resulting in low yields (Bakari *et al*., 2020). In acidic soils, large amounts of aluminum and low levels of phosphorus limit the activity of symbiotic nitrogen-fixing bacteria. Soil pH below 5.0 inhibits soybean nodulation due to the toxicity of Al and Fe ions, resulting in poor nodule production and function (Lin *et al*., 2012). They also suggested that acidic soils inhibit organic matter breakdown, nutrient cycling by microbes, and reduced nutrient absorption by plant roots, and root elongation restriction, among other issues. Soybeans require a lot of nutrients, with P and K being the most important (Keino *et al*., 2015). Acidic soils have a lot of Al and Fe ions in their solution, which causes P sorption and makes it unavailable to plants (Keino *et al*., 2015). Low soil pH limits soybean nodulation and BNF, which has been ascribed to a low P concentration at pH 5.5 due to sorption by Al and Fe (Kisinyo *et al*., 2014). They discovered that liming the soil increases the availability of essential cations, lowers the amount of harmful levels of Al, and increases the supply of P, raising the pH. On the market, there are a number of lime products to choose from, with agricultural lime being the most prevalent. According to (Nekesa *et al*., 2011) liming works well both on its own and in combination with fertilizers to increase production. Farmers in Ghana are limited in their use of lime due to cost, accessibility, labor costs, and inadequate information about the advantages of liming. Liming can improve crop yield and increase microbial activity in acid soils (Kisinyo *et al*., 2014).

2.14.2 Temperature

Soils with high temperatures in parts of the tropics are a severe hindrance to legume crops' biological nitrogen fixing (Michiels *et al*., 1994). Too high or too low soil temperature suppresses legume BNF via controlling nodule formation, nodule development, and nitrogenase activity. Soil temperature in the surrounding soil is one of the governing factors mifor nodulation and nodule formation (Liu *et al*., 2011). In various legume species, including soybean, clover, pea, guar, peanut, cowpea, and beans, higher soil temperatures have been

demonstrated to have a significant impact on bacterial infection and N2 fixation. For clover and pea, the temperature required for N2 fixation is 30°C, while soybean, guar, peanut, and cowpea require temperatures between 35 and 40°C (Michiels *et al*., 1994). Temperature dependence of nodulation and symbiotic nitrogen fixation has been shown to depend on, in addition to the plant cultivar, the nodulating strain (Liu *et al*., 2011; Michiels *et al*., 1994). Several bean-nodulating rhizobia have recently been reported that can nodulate beans and fix atmospheric nitrogen under a 40/23°C (day/night) temperature regime (Liu *et al*., 2011). The lowest temperature required for nitrogen fixation varies per species, ranging from 2 to 10 degrees Celsius, with intemperate legumes often having higher minimum temperatures than temperate legumes (Liu *et al*., 2011). Nitrogenase activity is strongest in most legumes around 12–35°C and peaks at 20–25°C (Liu *et al*., 2011).

2.14.3 Soil Water

Through nodule development, nodule activity, and gas permeability, the amount of water in the root zone influences nitrogen fixation (Maekawa *et al*., 2011). Nitrogen fixation is inhibited by a lack of water in the soil, and this inhibition is amplified as drought stress worsens. Furthermore, waterlogging can significantly limit N fixation by inhibiting the development and activity of nodules (Maekawa *et al*., 2011).

2.14.4 Nitrogen Concentration in the Root Zone

Soil mineral nitrogen at the plant roots was shown to inhibit legume nodulation on numerous occasions (Reinprecht *et al*., 2020; Wilker *et al*., 2019), nodule formation (Khosro Mohammadi, 2012), and nitrogenase activity (Liu *et al*., 2011; Weisany *et al*, 2013). In most cases, the degree of soil mineral N inhibition of N-fixation increases as the amount of soil mineral N increases. Under specific conditions, it has been revealed that a precise concentration of mineral N in the root zone, known as “starting N,” stimulates nodule development and N fixing more than non-mineral N. Furthermore, depending on the cultivar and growth

conditions, the amount of "starting N" necessary to create legume BNF varies significantly (Keino *et al*., 2015). External N application time in reference to the legume stage of growth, on the other hand, has an effect on nodule formation and N fixation. (Keino *et al*., 2015). This is largely due to the fact that nodules are already mature when N is applied.

2.15 Quantification of Legume Biological Fixation

To make certain right control and completely recognize the advantages of the legume-rhizobium symbioses, it's far important with the intention to quantify the quantity of nitrogen constant and having measured the effectiveness of atmospheric N-fixation, the macro or micro-symbionts in addition to agronomic elements may be manipulated with the objective to enhance biological nitrogen fixation. Plant-associated N-fixation contributes approximately 50-70 million metric tonnes per year to the global agricultural N budget, according to (Herridge *et al*., 2008; Unkovich *et al*., 2008), so increasing or sustaining that level of input requires a significant increase in scientific research to optimize and apply the various N-fixing systems. They also stated that conducting trials to establish treatment impacts on N-fixation or on-farm surveys to determine activity at a regional or country level is impossible until the technique can be effectively and accurately defined. Furthermore, studies have shown that tropical grasses such as sugarcane produce fixed nitrogen inputs in the range of 10-65 kg N/ha per year, but there are few conclusive data to suggest that bacteria associated with non-legumes fix significant amounts of N in temperate agriculture (Herridge *et al*., 2008). The yearly N_2 fixation by soybean is projected to be 5.7, 4.6, and 3.4 Tg in the United States, Brazil, and Argentina, respectively (Herridge *et al*., 2008). Any plant breeding program aimed at enhancing N-fixation must consequently use a suitable technique for quantifying N-fixation (Unkovich *et al*., 2008). Legume BNF can be directly measured, calculated using yield or empirical approaches, or simulated using crop models. The acetylene reduction/hydrogen increase assay, nitrogen

balance, nitrogen difference, 15N isotope, and ureides procedures had all been well-reviewed and applied to detect N fixation thus far (Herridge *et al*., 2008; Unkovich *et al*., 2008).

2.15.1 The Total Nitrogen Difference Technique

The difference between the total N yield of a nodulated (N-fixing) plant and that of a non-nodulated (Non-fixing) plant, preferably of the same species, is referred to as the N difference technique. The accuracy of the estimates in this method is determined by the structural and functional similarities of the two root systems; thus, the two crops must have the same growth cycle, rooting habit, root system, and other characteristics in order to ensure that they take up the same amount of nutrient from the soil, which is one of the method's principles. This method is based on the assumption that nitrogen-fixing plants and non-fixing plants consume the same amount of soil mineral nitrogen. This approach can be employed successfully in soils with low N supplies, especially if the N_2-fixing plants derive high amounts of N from the atmosphere. According to Chalk *et al*. (1998) and Herridge *et al*. (1995) as cited by Herridge *et al*. (2008), the N difference technique may be less beneficial in moderate-to-high N soils due to differences in root shape and rooting depth between N_2-fixing and non-fixing plants, which can result in varied capacities to exploit soil N. It's also of limited use for on-farm surveys when non-nitrogen-fixing plants aren't readily available. However, this technique has the benefit of being a straightforward, low-cost method that can be used when only dry matter measurements and total N analyses are available.

2.15.2 Nitrogen Balance Technique

The total N-balance technique is based on the principle that if an N_2 fix is added to the plant/soil system, it will accumulate N over time and the N_2 fixation can be credited with a net positive N balance in the system under consideration. The method seeks to quantify the difference between N input and N loss within the study period. Sainju, (2017) determined the total N balance by using the formula below:

$$\textbf{Nitrogen balance} = \text{N inputs} - \text{N outputs} - \text{changes in the soil total N} \quad (1)$$

$$\boldsymbol{N\ inputs} = N\ fertilization\ (inorganic\ N\ fertilizer) + N\ fertilization\ (organic) + atmospheric\ N\ depositions + biological\ N\ fixation + irrigation + crop\ seed \quad (2)$$

$$\boldsymbol{N\ outputs} = crop\ N\ removal\ (biomass\ and\ grain) + N\ losses(leaching\ etc) \quad (3)$$

$$\boldsymbol{Changes\ in\ soil\ N} = soil\ total\ N\ at\ the\ end\ of\ experiment - soil\ total\ N\ at\ the\ begining\ of\ the\ experiment \quad (4)$$

The main advantage of this technique is that it's a straightforward method. Measurements of N_2 fixation, on the other hand, maybe understated due to N losses from the system throughout the research period due to ammonia volatilization, denitrification, leaching, and other processes. This suggests that the N balance method is not suitable in field research due to the difficulties of quantifying various inflows and outflows of N. It isn't a precise indicator of N fixation (Unkovich *et al*., 2008). For accuracy, N balance requires evaluation of as many potential N inputs and outputs as possible for a long period of time which can increase the cost of labor (Herridge *et al.*, 2008).

2.15.3 Acetylene Reduction Essay Technique

The widely discussed biological nitrogen fixation enzyme, nitrogenase has the ability to also reduce acetylene (C_2H_2) to ethylene (C_2H_4). The acetylene method is based on the enzyme nitrogenase's ability to convert acetylene to ethylene. It's predicated on the idea that acetylene can easily replace N2, and that nitrogenase activity measured under these circumstances may be linked to the rate of N fixing (Hardy *et al*., 1968; Herridge *et al*., 2008; Schöllhorn & Burris, 1967; Unkovich *et al*., 2008). The process entails incubating samples in an acetylene-filled gas-tight chamber. The ethylene production is then monitored using a gas chromatograph (Hardy *et al*., 1968; Schöllhorn & Burris, 1967; Unkovich *et al*., 2008). The total amount of nitrogen fixed can be calculated using the amount of ethylene produced as a measure of nitrogenase or relative N fixing activity (Hardy *et al*., 1968; Schöllhorn & Burris, 1967; Unkovich *et al*.,

2008). They reported that the procedure is straightforward, affordable, and sensitive enough to detect nitrogenase. However, the rate of N fixation determined in the incubation vessel cannot be generalized beyond that. They also reported that ethylene generated after 30 minutes can cause nitrogenase levels to drop.

2.15.4 The ^{15}N Isotope Method

The ^{15}N isotope method compares non-fixing with nitrogen-fixing plants cultivated in soil containing ^{15}N in the form of labeled urea, ammonia, or nitrate (Unkovich *et al*., 2008). Nitrogen-fixing plants acquire nitrogen from two sources: air and soil, and consequently have a lower isotope ^{15}N content than non-nitrogen-fixing plants, which solely absorb labeled soil N. The ^{15}N isotope methods are based on the assumption that the difference in terms of 15N composition of the air and soil is very small in comparison to the difference between them. It's also assumed that during the absorption and utilization of plant-available soil nitrogen, there'll either be no discrimination or the same discrimination that can be explained (Unkovich *et al*., 2008). The percent excess of ^{15}N atoms in non-fixing and N-fixing plants is used to compute the amount of plant nitrogen derived via N-fixation (Boddey *et al*., 2001). Because it requires advanced equipment and specialized abilities, the ^{15}N approach produces the most exact findings but is also the most expensive.

2.15.5 The Ureide Method

The Ureide method relies on determining the composition of nitrogen compounds or solutes in plant tissue and xylem sap. In plant tissues and xylem sap, the relative quantities of N solutes: allantoin, allantoic acid, amino compounds, and nitrate reflect the sources of N assimilated by the legume. They can be used to determine the plant's reliance on N_2 fixation (Percent Nitrogen Derived from the Atmosphere) once calibrated (Vigna *et al*., 1978).

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Experimental Material

The experimental materials used in this study were lines that were developed by the soybean improvement program section of the CSIR-SARI using the locally commercialized varieties developed by the said institution and some lines from the Illinois University/USDA (Table 2)

Table 2: **Genotypes used in this study and their parental**

Cross	Genotype
Jenguma × Afayak	SAR-JEN/AFA-18-5
Jenguma × FT Cristaline	SAR-JEN/USL-18-5
Jenguma × FT Cristaline	SAR-JEN/USL-18-6
Jenguma × FT Cristaline	SAR-JEN/USL-18-7
Saliintuya II × Suong Pungun	SAR-SL2/SPG-18-4
Saliintuya II × FT Cristaline	SAR-SL2/USL-18-2
Salintuya II × Afayak	SAR-SL2/AFA-18-1
Quarshie × Afayak	SAR-QUA/AFA-18-1
Quarshie × Afayak	SAR- QUA/AFA-18-6
Salintuya I × Afayak	SAR-SL1/AFA-18-1
Salintuya I × FT Cristaline	SAR-SL1/USL-18-2
Quarshie × Afayak	SAR-QUA/AFA-18-3
Salintuya II × FT Cristaline	SAR-SL2/USL-18-1
Salintuya I × FT Cristaline	SAR-SL1/USL-18-3
Quarshie × Afayak	SAR- QUA/AFA-18-4
Jenguma × FT Cristaline	SAR-JEN/USL-18-3
Jenguma × Suong Pungun	SAR-JEN/SPG-18-1
Salintuya II × Suong Pungun	SAR-SL2/SPG-18-5
Salintuya I × FT Cristaline	SAR-SL1/USL-18-1
Jenguma × FT Cristaline	SAR-JEN/USL-18-4
Jenguma	CHECK
Afayak	CHECK

Favour	CHECK
FT Cristaline	CHECK

Table 3: **Major traits of importance of the parents used.**

Parent	Special characteristics of cultivar
Jenguma	High yielding, Medium maturing and Non-shattering
Afayak	High yielding, Striga resistant, Non-shattering medium maturing
Favour	High protein, High biomass, and High yielding
SoungPungun	Early maturity and Good yielding
Salintuya I	High and stable yields, Good grain quality
Salintuya II	Late maturity, Good grain quality, and Stable yields
Quarshie	Stable yields and intermediate shattering
FT Cristaline	Medium maturity, High yielding and Non-shattering

Source: CSIR-SARI

3.2 Experimental Site

The site for this study was at the CSIR-Savanna Agricultural Research Institute experimental fields in Nyankpala (9^{o} 23’ 54.08” N; 0^{o} 58’ 58.57” W, 102 m asl), Northern Ghana in the Guinea Savannah zone during the 2020 cropping season. The site has a uni-modal annual rainfall which ranges between 900-1100 mm and usually lasts from May-October. The texture of the soil in this site is sandy loam (

Table 4). The average monthly minimum and maximum temperatures in this site are 23^{o}C and 35^{o}C, respectively. The soils are named Nyankpala series and classified under savannah Ochrosols, Plinthic luvisols (Vaccari & Panza, 2001)

3.3 Experimental Design

For each treatment, the experimental design was Randomized Complete Block with three replicate plots (genotype). Plot size was 2.4 × 5m with each plot containing 4 rows. The treatments were sown without rhizobium inoculation. The spacing between rows and within plants was 60 and 5cm, respectively. The treatment consisted of 20 advanced breeding lines and 5 checks (Table 2).

3.4 Method used for Soil Sampling and analysis

The field had been harrowed and plowed. On level soil, planting took place in the second week of July. Sub-samples were taken in a diagonal manner from 0-20 cm depth on the field with a soil auger before planting and during harvesting. The extracted sub-samples were then combined and air-dried to generate the composite sample. The composite soil sample was then transferred to the laboratory and sieved for the physic-chemical properties of the soil in the experimental area using a 1mm size mesh sieve. According to the techniques used by Klute (1986) and Page *et al*. (1982), some physical and chemical characteristics of the experimental field soil were identified (

Table 4)

3.4.1 Soil pH

The Electrometric method of determining the soil pH meter was used to measure pH in a 1:2.5 ratio of soil to water suspension.

3.4.2 Soil Total Nitrogen

The Kjeldahl method was used to determine the total nitrogen content of the soil.

3.4.3 Phosphorus

The Bray-1 extraction method was employed to determine the soil available phosphorus by using dilute acid fluoride for its extraction.

3.4.4 Potassium

The ammonium acetate extraction method of extraction was employed to determine the available potassium in the experimental field.

Table 4**: The Physico-chemical properties of the soil samples**

Soil Property	**Soil test values**	**Interpretation**
Physical		
Soil texture (%)		
Sand	79.68	-
Silt	16.52	-
Clay	3.8	-
Classification	-	sandy loam
Chemical		
pH	6.33	moderately acidic
Nitrogen (%)	0.136	low
Phosphorus (mg/kg)	8.47	low
Potassium (mg/kg)	63	low

3.5 Agronomic Practices

The experimental field was ploughed and harrowed. The seeds were planted by dibbling on flat soil. Weeds were controlled manually with a hoe.

3.6 Data Collected

The data collected were days to 50% flowering, nodules per plant and nodule dry weight per plant, shoot biomass dry matter per plant, Photosynthetic Active Radiation (PAR), leaf stomata conductance, leaf area index, leaf transpiration rate, photosynthetic rate, height at maturity, pod clearance and number of pods per plant, days to maturity, grain yield per plot and one hundred seed weight.

3.6.1 Days to 50% flowering

Days to 50% is achieved when half of the plant population per treatment plot is having one or more flowers. The field was closely monitored after planting and date recorded when half of the plants in a plot had one or more flowers.

3.6.2 Nodule count and dry weight

Eight (8) plants were carefully dug out from within each plot the rows bordering the 2 middle rows at full podding stage (R3 stage). The soil on the roots of the 8 plants dug out was then washed off under running tap water. The nodules after washing were then detached and all the nodules were counted and the number recorded. The nodules were then put in a well-labeled paper bag and oven-dried at 60^{o}c for 24hr and the dry weight was recorded. The average nodule weight per plant was then obtained by dividing the total by the total number of the plants harvested.

3.6.3 Photosynthetic Active Radiation (PAR)

The amount of light accessible for photosynthesis in the 400-700 nanometer wavelength range is referred to as photosynthetic active radiation. It fluctuates based on the time and latitude of the day and changes seasonally. This data was collected using a Ceptometer.

3.6.4 Stomatal conductance

This is a measurement of the degree of stomata opening and closing which can be used to determine the water condition of a plant. It was recorded by using a Ceptometer.

3.6.5 Leaf transpiration rate

This refers to the quantity of water lost per unit time from the leaf into the atmosphere. This was measured using a Ceptometer.

3.6.7 Photosynthetic rate

This can be referred to as the rate of oxygen production per unit mass or area of green plant tissue or per unit weight of total chlorophyll. Ceptometer was again used to determine this parameter.

3.6.8 Days to maturity

Maturity is reached when 95% of the plant pods have transformed from Yellow to Tan or Grey. Visual observation was employed and when it was observed that about 95% of the plants' pods turned yellow to tan or grey, the date was recorded and the days calculated using the planting date.

3.6.9 Plant height at maturity

The length of the plant's main stems (not petioles and leaves) at the time of maturity. This parameter was recorded on 6 randomly selected plants within the 2 middle rows per treatment plot. The measuring tape was used to measure and the unit of measurement was centimeters.

3.6.10 Pod clearance

Length of the distance between the first pod on the stem of each plant and the ground level measured at maturity. This was done on 6 plants using a rule.

3.6.11 Number of pods per plant

Six separate plants' pods were counted, and the total number of pods collected for each plant was recorded. The average number of pods among the six plants was used to compute the number of pods per plant.

3.6.12 Grain yield per plot

The seed after threshing was uniformly dried and the weight of the seed of the 2 middle rows (net plots) of each plot was measured with a good balance scale. At the time of weighing, a moisture meter was utilized to assess the seed moisture percent.

3.6.13 Grain weight

This data was obtained by selecting 100 seeds at random and weighing them. The weight of these seeds was then recorded in grams to represent the grain size of the various treatments.

3.6.14 Shoot biomass sampling

At podding, the plants' shoots were collected from a 2 m^2 area of each plot and the fresh weight was recorded. The samples were then put in paper bags differently and sent to the laboratory for drying. They were oven-dried at 60^{o}c for 72hr and the dry weight of the shoots was recorded in kilograms. The reference plant (maize) which was planted adjacent to the soybean field, was sampled and processed in the same manner as the soybean. For the purpose of determining N fixation, a 500g sub-sample was used.

3.7 Measurement of N-fixation

In this study, the N difference technique was employed to quantify the amount of N_2 fixed and N contribution by the genotypes. This technique compares the total N of the N_2 - fixing species (genotypes) with that of a neighboring non N_2 -fixing species with the assumption that the difference between the two is due to N_2 fixation ((Unkovich *et al.*, 2008). Total N in the shoots of both the soybean and maize was analyzed by using the Kjeldahl procedure.

The following formulas were used to calculate; the amount of N-fixed, N derived from the atmosphere, % N-fixed and N fixed (kg/ha).

Amount of N_2- fixed = $N_{legume} - N_{maize}$ (1)

Soil N uptake = Total N - N_2-fixed (2)

% N-fixed = $\frac{(Nleg - Nref)}{Nleg} \times 100$ (3)

N fixed (kg/ha) = $\frac{\%Nleg}{100} \times BW_{leg}$ (kg/ha) - $\frac{\%Nref}{100} \times BW_{ref}$ (kg/ha)

Where;

$\%N_{leg}$ = Percent of N-fixed by the legume

$\%N_{ref}$ = Percent of N-fixed by the non-legume (maize)

BW = Biomass weight

3.8 Measurement of water use efficiency

Water shortage is a significant limiting factor, especially when reproductive development is taking place (Oya *et al*. 2004). Stabilizing yield can be achieved by increasing soybean's ability to withstand drought. A complex physiological process known as drought tolerance results in a quantitative change in composition or the synthesis of new substances such as ureides, amides, and acetylene reduction activity (ARA) and N_2 concentration (Sinclair *et al*. 2007). Other morphological indicators of drought tolerance include nodule formation, canopy wilting, and water usage efficiency (WUE) (Bazzer & Purcell, 2020).

The water use efficiency of the genotypes was determined by using the ratio of photosynthetic rate to the rate of transpiration.

$$\boldsymbol{Water\ Use\ Efficiency\ (WUE)} = \frac{Phosynthetic\ rate}{Transpiration\ rate} \quad (1)$$

The photosynthetic rate and transpiration rate parameters were determined with the use of ceptometer.

3.9 Statistical and genetic analysis

All data collected on the parameters were subjected to statistical analysis of variance (ANOVA) among the genotypes using GenStat® (12^{th} Edition) statistical package (VSN International, Hemel Hempstead, UK.), and treatment means were compared using Fisher test Least Significance Difference (LSD) at 5% probability level.

3.9.1 Heritability estimate

The variance components were analyzed using the restricted maximum likelihood method in the lme4 package in R (Bates *et al*., 2015). Heritability estimates were calculated using the following formula by Burton, (1952) and Sharma, (1988) as cited by Ene *et al*. (2016) on the population heritable traits to ascertain the amount of variations in the traits that are due to

genetic factors as opposed by environmental factors. These genetic variance components include; genotypic variance, phenotypic variance, genotypic coefficient variation, phenotypic coefficient of variation, genetic advance, genetic advance a percentage of the mean and K = selection intensity differential.

$$Genotypic\ variance\ (Vg) = \frac{\text{Genotype mean square} - \text{Error mean square}}{\text{Number of replications}} \quad (1)$$

$$Phenotypic\ variance\ (Vp) = Genotypic\ variance\ (Vg) + \frac{Environmental\ variance}{Number\ of\ replications} \quad (2)$$

$$Genotypic\ coefficient\ of\ variation, Vgc\ (\%) = \frac{\sqrt{Vg}}{Grand\ Mean} \times 100 \quad (3)$$

$$Phenotypic\ coefficient\ of\ variation, Vpc\ (\%) = \frac{\sqrt{Vp}}{x} \times 100 \quad (4)$$

$$Heritability\ (H^2) = \frac{Vg}{Vp} \quad (5)$$

Genetic advance (GA) = $K\sqrt{PV} \times H^2$ (6)

Genetic advance mean (GAM) = $\frac{GA}{Grand\ Mean} \times 100$ (7)

Where; Vp = Phenotypic variance, Vg = genetic variance, H2 = Broad sense heritability K = Selection intensity differential.

CHAPTER FOUR

4.0 RESULTS

Farmers in Ghana's guinea savanna agro-ecology face a number of challenges, including low soil nutrients, particularly N and P (Table 4) irregular rainfall, and insufficient improved varieties (Pagano & Miransari, 2015). For evidence to support this study, several parameters were considered for data. These include; Days to 50% flowering, number of nodules per plant and nodule dry weight per plant, shoot biomass dry matter per plant, Photosynthetic Active Radiation (PAR), leaf stomata conductance, leaf area index, leaf transpiration rate, photosynthetic rate, height at maturity, pod clearance and number of pods per plant, plants harvested, days to maturity, grain yield per plot and One hundred seed weight.

4.1 Grain yield

The genotypes had statistically significant differences (P = 0.03), according to the ANOVA (Figure 1). The highest grain yield (1502 kg/ha) was obtained by genotype SAR-SL2/SPG-18-4, while the lowest grain yield (811 kg/ha) was reported by genotype SAR-JEN/USL-18-6.). Apart from twelve of the genotypes that produced grain yield significantly lower than the genotype SAR-SL2/SPG-18-4, all other treatment means were not significantly different from the genotype SAR-SL2/SPG-18-4.

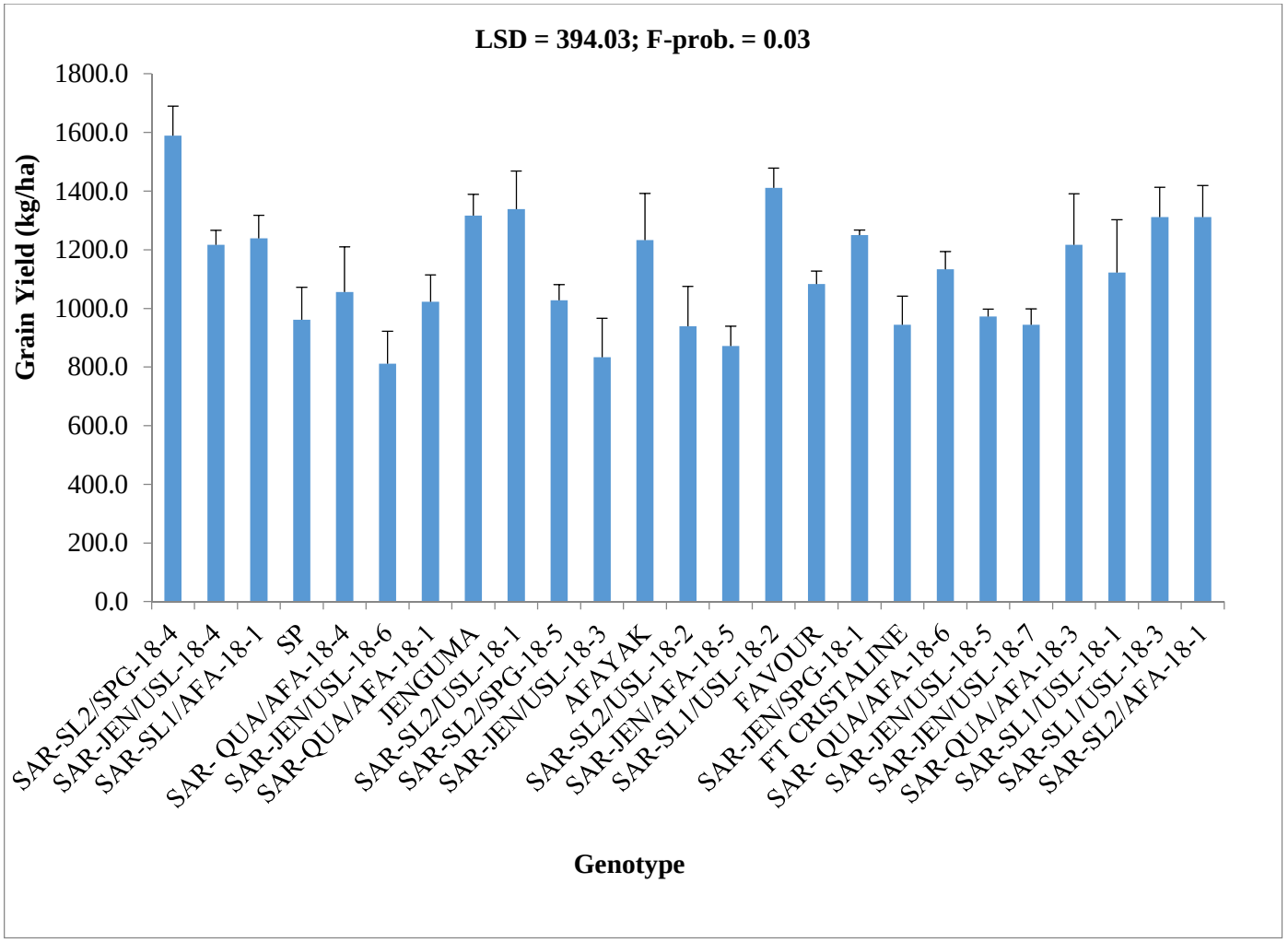

Figure 1: Mean grain yield of twenty-five soybean genotypes

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.2 One hundred seed weight

The result for 100 seed weight from ANOVA revealed that there were statistically significant differences (P = 0.001) among the genotypes. Genotype SAR-SL2/SPG-18-4 recorded the highest 100 seed weight (14 g), while genotype SAR-JEN/AFA-18-5 recorded the lowest seed weight (9.3 g). One hundred seed weight for genotype SAR-SL2/SPG-18-4 was significantly higher than eighteen of the genotypes. However, there was no significant difference between the genotype SAR-SL2/SPG-18-4 and all the other genotypes. Genotype SAR-JEN/AFA-18-5 was significantly lower than only eighteen of the treatments but there was no significant difference between its mean and that for six of the genotypes.

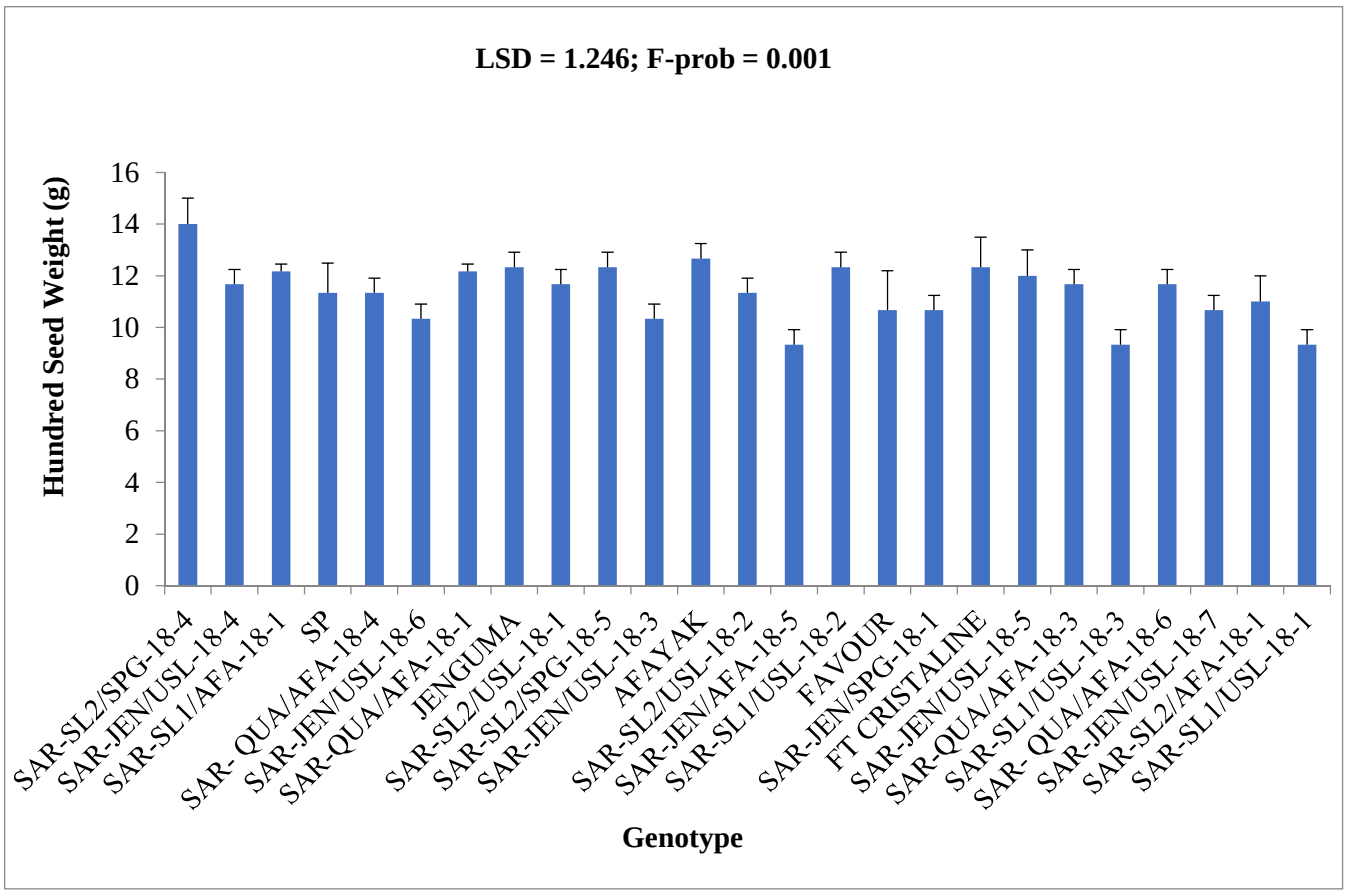

Figure 2: Mean One hundred seed weight of twenty-five soybean genotypes

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.3 Pod per plant and Pod clearance

From the ANOVA, there was no statistically significant difference (P = 0.366) in the mean values of all genotypes for the number of pods per plant. The genotype SAR-SL2/USL-18-1 produced the most pods per plant (86), followed by genotypes SAR-QUA/AFA-18-4 (84) and SAR-QUA/AFA-18-3 (78). The lowest pod per plant was produced by genotype SAR-JEN/USL-18-3 (53) (Table 5). Similar to pod per plant, there were no significant mean differences (*P* = 0.355) among all the genotypes. The mean pod clearance was 6.77 cm. Genotype SAR-SL1/USL-18-1 recorded the highest pod clearance (9.39 cm) followed by genotype SAR-JEN/USL-18-3 (8.83 cm) (Table 5).

4.4 Effective nodules per plant and dry weight

The results from the ANOVA showed that genotype differences did not significantly affect the number of nodules produced per plant and the nodule dry weigh (Table 6). However, genotype FT Cristaline produced the highest nodule number per plant (56.5), while variety Suong Pungun produced the lowest number of nodules per plant (21.9). But, the nodule number for the genotype FT Cristaline was not significantly different (*P* = 0.886) than those for all other genotypes. The highest nodule weight per plant was recorded for genotype FT Cristaline (0.66 g) followed by genotype SAR-JEN/USL-18-6 (0.60), while the lowest was recorded for genotype SAR-SL2/USL-18-1 (0.167). Again the nodule weight for the genotype FT Cristaline was not significantly different (*P* = 0.0839) than those for the other treatments (Table 6).

Table 5**: Mean pod per plant and pod clearance of twenty-five soybean genotypes**

Genotype	Pod per Plant	Pod Clearance
SAR-SL2/SPG-18-4	61	8.06
SAR-JEN/USL-18-4	61	5.66
SAR-SL1/AFA-18-1	64	5.96
SP	65	6.7
SAR- QUA/AFA-18-4	84	7.46
SAR-JEN/USL-18-6	62	7.8
SAR-QUA/AFA-18-1	54	7.23
JENGUMA	76	6.00
SAR-SL2/USL-18-1	86	6.9
SAR-SL2/SPG-18-5	59	5.93
SAR-JEN/USL-18-3	53	8.83
AFAYAK	73	6.63
SAR-SL2/USL-18-2	74	5.26
SAR-JEN/AFA-18-5	63	7.13
SAR-SL1/USL-18-2	72	6
FAVOUR	70	7.19
SAR-JEN/SPG-18-1	69	6.22
FT CRISTALINE	65	5.46
SAR- QUA/AFA-18-6	72	6.02
SAR-JEN/USL-18-5	64	6.82
SAR-JEN/USL-18-7	68	8.52
SAR-QUA/AFA-18-3	78	6.19
SAR-SL1/USL-18-1	59	9.39
SAR-SL1/USL-18-3	72	4.46
SAR-SL2/AFA-18-1	54	7.42
Grand Mean	67	6.77
CV (%)	21.4	28
LSD (0.05)	23.73	3.137
F-Prob.	0.366	0.355

Note: CV = coefficient of variation, LSD = least significant difference, F-pro. = fisher probability

Table 6**: Mean nodule number and nodule dry weight of twenty-five soybean genotypes**

Genotype	Nodule per Plant	Nodule Dry Weight (g)
SAR-SL2/SPG-18-4	47.3	0.445
SAR-JEN/USL-18-4	31.9	0.361
SAR-SL1/AFA-18-1	53.9	0.501
SP	21.9	0.351
SAR- QUA/AFA-18-4	30.9	0.261
SAR-JEN/USL-18-6	49.3	0.601
SAR-QUA/AFA-18-1	45.3	0.451
JENGUMA	35.9	0.344
SAR-SL2/USL-18-1	12.9	0.167
SAR-SL2/SPG-18-5	42.6	0.357
SAR-JEN/USL-18-3	31.7	0.325
AFAYAK	51.4	0.565
SAR-SL2/USL-18-2	31.7	0.399
SAR-JEN/AFA-18-5	42.4	0.299
SAR-SL1/USL-18-2	28.7	0.332
FAVOUR	39	0.449
SAR-JEN/SPG-18-1	45.7	0.465
FT CRISTALINE	56.5	0.66
SAR- QUA/AFA-18-6	23.5	0.187
SAR-JEN/USL-18-5	27	0.282
SAR-JEN/USL-18-7	42.5	0.337
SAR-QUA/AFA-18-3	37.3	0.275
SAR-SL1/USL-18-1	30.8	0.343
SAR-SL1/USL-18-3	32.8	0.393
SAR-SL2/AFA-18-1	37	0.449
Grand Mean	37.2	0.384
CV (%)	62.5	64.9
LSD (0.05)	38.51	0.4128
F-Prob.	0.886	0.839

Note: CV = coefficient of variation, LSD = least significant difference, F-pro. = fisher probability

4.5 Photosynthetic Rate

The result for ANOVA for the photosynthetic rate of the genotypes demonstrates that there was a statistically significant difference (P = 0.001) among the genotypes (Figure 3). Genotype SAR-SL2/USL-18-2 recorded the highest rate of photosynthesis, while genotype SAR-SL1/AFA-18-1 recorded the lowest rate. Genotype SAR-SL2/USL-18-2 photosynthetic rate was significantly higher than those for nineteen of the genotypes but there was no significant difference between the genotype SAR-SL2/USL-18-2 and all the other five genotypes (Figure 3). Genotype SAR-SL1/AFA-18-1was significantly lower than all the genotypes except for genotype FT Cristaline. However, there were no statistically significant differences between the mean photosynthetic rate of genotype FT Cristaline and genotypes, SAR-QUA/AFA-18-3, SAR- JEN/USL-18-7and QUA/AFA-18-4.

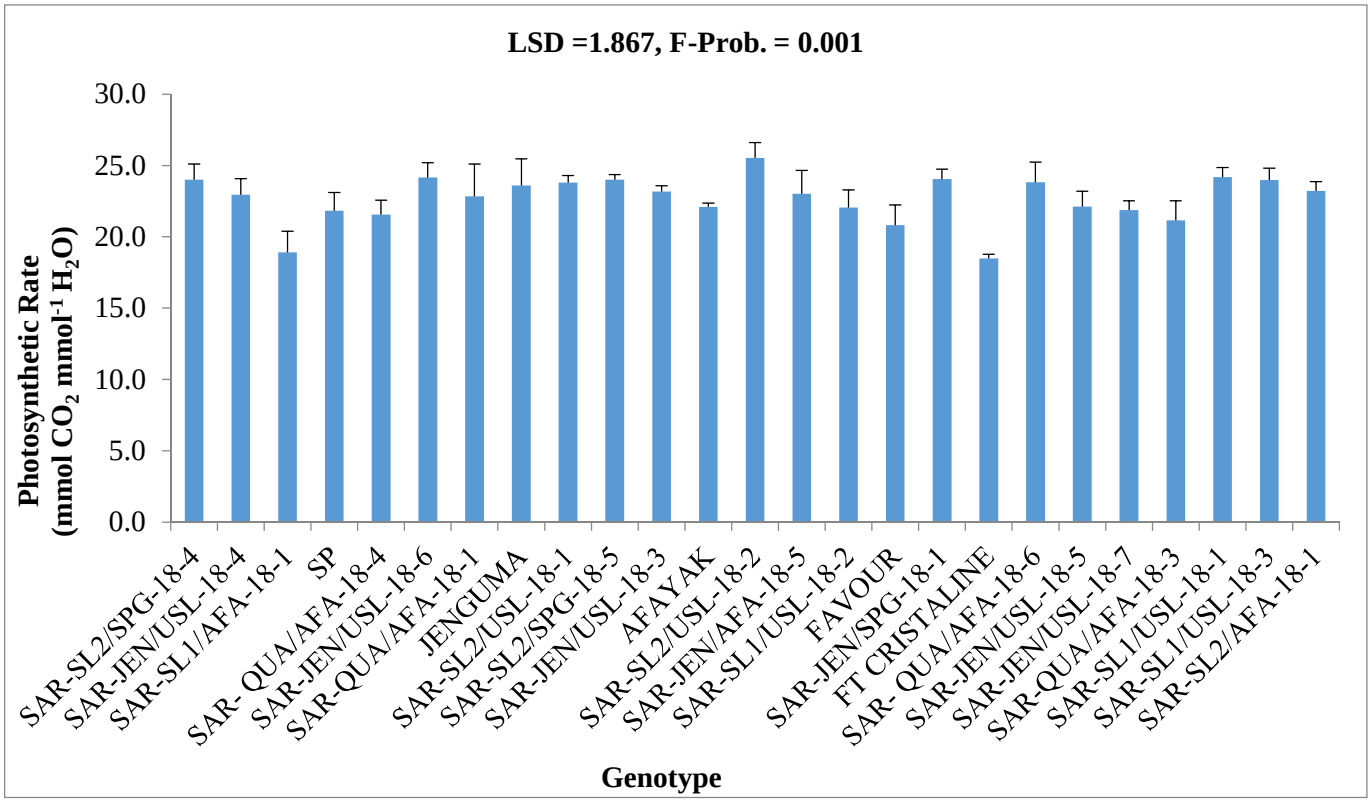

Figure 3: Mean photosynthetic rate of twenty-five soybean genotypes

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.6 Leaf area index

The result from the ANOVA showed that there was no statistically significant difference (P = 0.995) among all the genotypes (Table 7). The highest leaf area index was recorded for variety Afayak (2.406 m^2), while the lowest was recorded by genotype FT Cristaline (1.83 m^2).

4.7 Stomata conductance

ANOVA for stomata conductance of the twenty-five treatments showed that there was no statistically significant difference (P = 0.111) between their mean values. Although treatment Favour recorded the highest mean value (0.837) for the stomata conductance and treatment FT Cristaline recorded the lowest mean value (0.547), statistically their mean values were not different (Table 7).

4.8 Leaf transpiration rate

There were no significant mean differences (P = 0.059) observed among the treatments when the data for leaf transpiration rate was subjected to ANOVA (Table 7). The average leaf transpiration rate was 31.26 (Table 7). Genotype SAR-SL2/SPG-18-4 recorded the highest transpiration rate (32.044) at the time of measurement, whiles genotype SAR-SL1/USL-18-2 recorded the lowest (31.048).

Table 7**: Mean leaf area index, leaf transpiration rate, and Stomata conductance of twenty-five soybean genotypes**

Genotype	Leaf Area Index(m^2)	Leaf Transpiration Rate	Stomata Conductance ($mmol^{-2}s^{-2}$)
SAR-SL2/SPG-18-4	1.884	32.044	0.645
SAR-JEN/USL-18-4	2.051	31.200	0.723
SAR-SL1/AFA-18-1	2.186	31.671	0.555
SP	1.886	31.053	0.790
SAR- QUA/AFA-18-4	2.086	31.398	0.689
SAR-JEN/USL-18-6	1.914	31.757	0.679
SAR-QUA/AFA-18-1	1.856	30.968	0.712
JENGUMA	2.206	31.356	0.707
SAR-SL2/USL-18-1	2.007	31.537	0.711
SAR-SL2/SPG-18-5	2.007	30.760	0.801
SAR-JEN/USL-18-3	2.258	31.404	0.693
AFAYAK	2.406	31.600	0.717
SAR-SL2/USL-18-2	2.229	31.142	0.765
SAR-JEN/AFA-18-5	2.014	31.546	0.703
SAR-SL1/USL-18-2	2.043	31.048	0.778
FAVOUR	2.040	30.766	0.837
SAR-JEN/SPG-18-1	2.374	30.867	0.768
FT CRISTALINE	1.830	31.546	0.547
SAR- QUA/AFA-18-6	2.143	31.398	0.758
SAR-JEN/USL-18-5	2.131	30.890	0.799
SAR-JEN/USL-18-7	2.264	31.114	0.671
SAR-QUA/AFA-18-3	2.039	31.525	0.742
SAR-SL1/USL-18-1	1.990	31.561	0.695
SAR-SL1/USL-18-3	2.006	30.824	0.811
SAR-SL2/AFA-18-1	2.159	31.288	0.689
Grand Mean	2.074	31.264	0.719
CV (%)	22.4	1.4	13.7
LSD (0.05)	0.7678	0.7286	13.7
F-Prob.	0.995	0.059	0.111

Note: CV = coefficient of variation, LSD = least significant difference, F-pro. = fisher probability

4.9 Photosynthetic Active Radiation

There was a statistically highly significant difference (P = 0.001) between the treatment means. It was observed that the highest photosynthetic active radiation was recorded by genotype SAR-SL1/USL-18-3 (87.0 µmol.s^{-1}) and this was significantly higher than for twenty-one of the genotypes. The lowest photosynthetic active radiation was recorded in genotype SAR-SL1/USL-18-2 (65.0 µmol.s^{-1}), but this was significantly lower than those of twenty-two genotypes. All other treatment differences were not significantly significant (Figure 4).

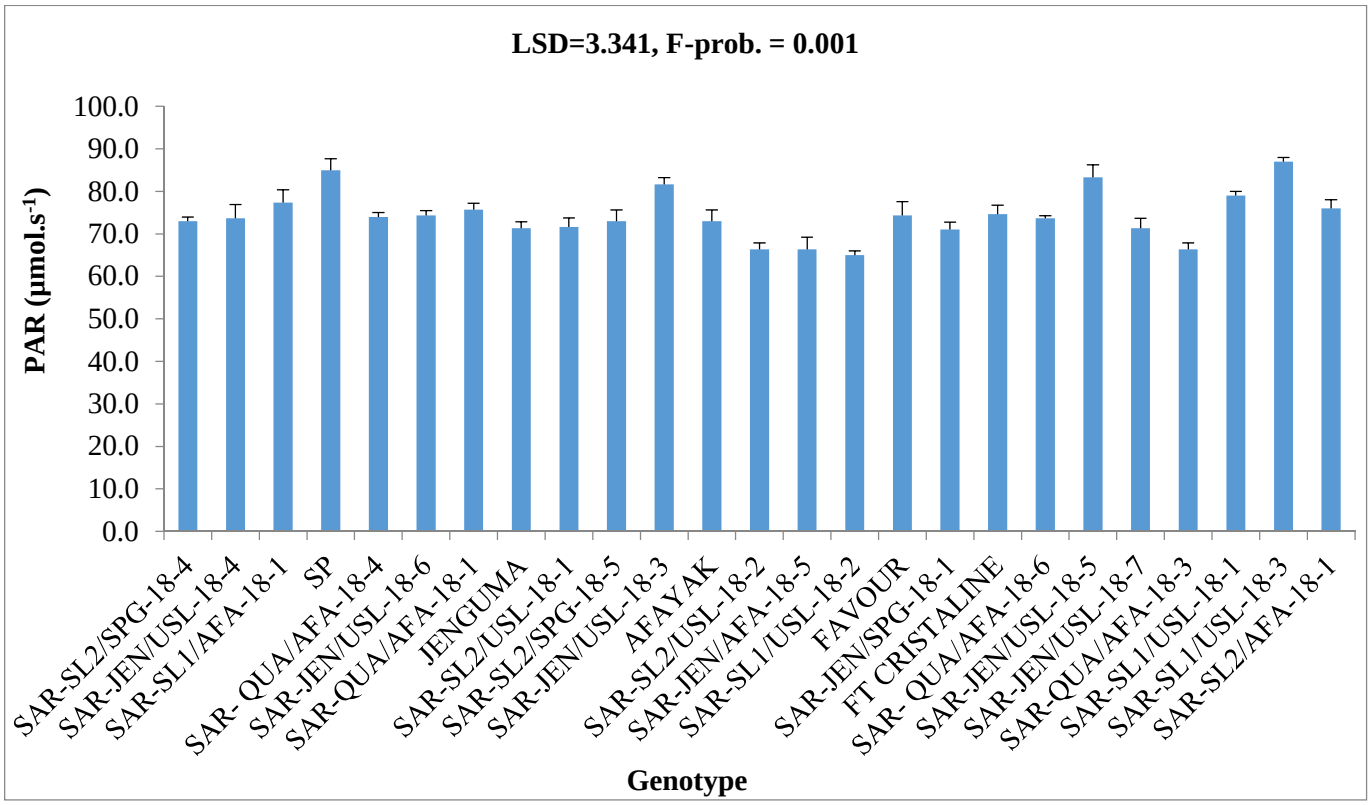

Figure 4**: Mean Photosynthetic Active Radiation (PAR) of twenty genotypes**

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.10 Days to 50% flowering

Genotypes, SAR-JEN/USL-18-6, SAR-JEN/AFA-18-5, SAR-JEN/SPG-18-1, and SAR-JEN/USL-18-7 took the highest number of days (53 days) each to attain 50% flowering, while variety Suong Pungun took the lowest number of days (43 days). The genotypes, SAR-JEN/USL-18-6, SAR-JEN/AFA-18-5, SAR-JEN/SPG-18-1, and SAR-JEN/USL-18-7 were significantly different (P = 0.001) than all the other treatments (Figure 5). The treatment Suong Pungun which recorded the lowest number of days to 50% flowering was significantly lower than all the other treatments. There was also a significant difference between the second-lowest genotype SAR-SL2/AFA-18-1 and eighteen of the genotypes but it was statistically similar to five of the genotypes (Figure 5).

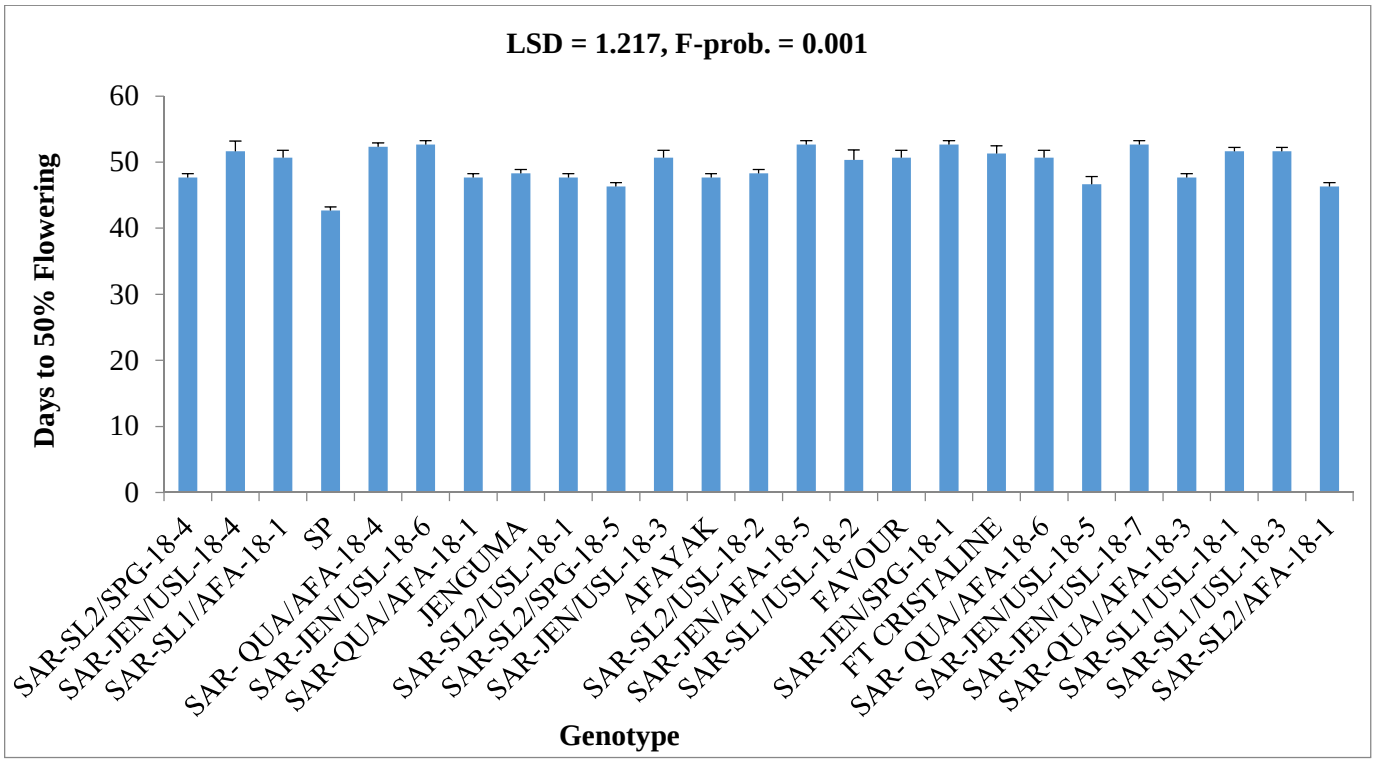

Figure 5: Mean 50% Flowering Days of twenty-five soybean genotypes

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.11 Days to Maturity

Genotypes that took the longest number of days to maturity were SAR-SL2/SPG-18-4, SAR-SL1/AFA-18-1, SAR-JEN/USL-18-4, SAR-QUA/AFA-18-1, SAR-SL1/SPG-18-5, SAR-JEN/USL-18-3, SAR-SL1/USL-18-2, FAVOUR, FT CRISTALINE, SAR-QUA/AFA-18-6, SAR-SL1/USL-18-1, and SAR-SL2/AFA-18-1 (120 days) each. They were significantly higher (P = 0.001) than thirteen of the genotypes. Similar to the days to 50% flowering, the lowest number of days to maturity was recorded by the treatment SuongPungun (103 days) and it was again significantly lower than all the other treatments (Figure 6). However, there were no significant differences between the second-lowest treatment SAR-JEN/USL-18-7 and eleven of the genotypes, but there were statistically significant differences between genotype SAR-JEN/USL-18-7 and all the other treatments (Figure 6)

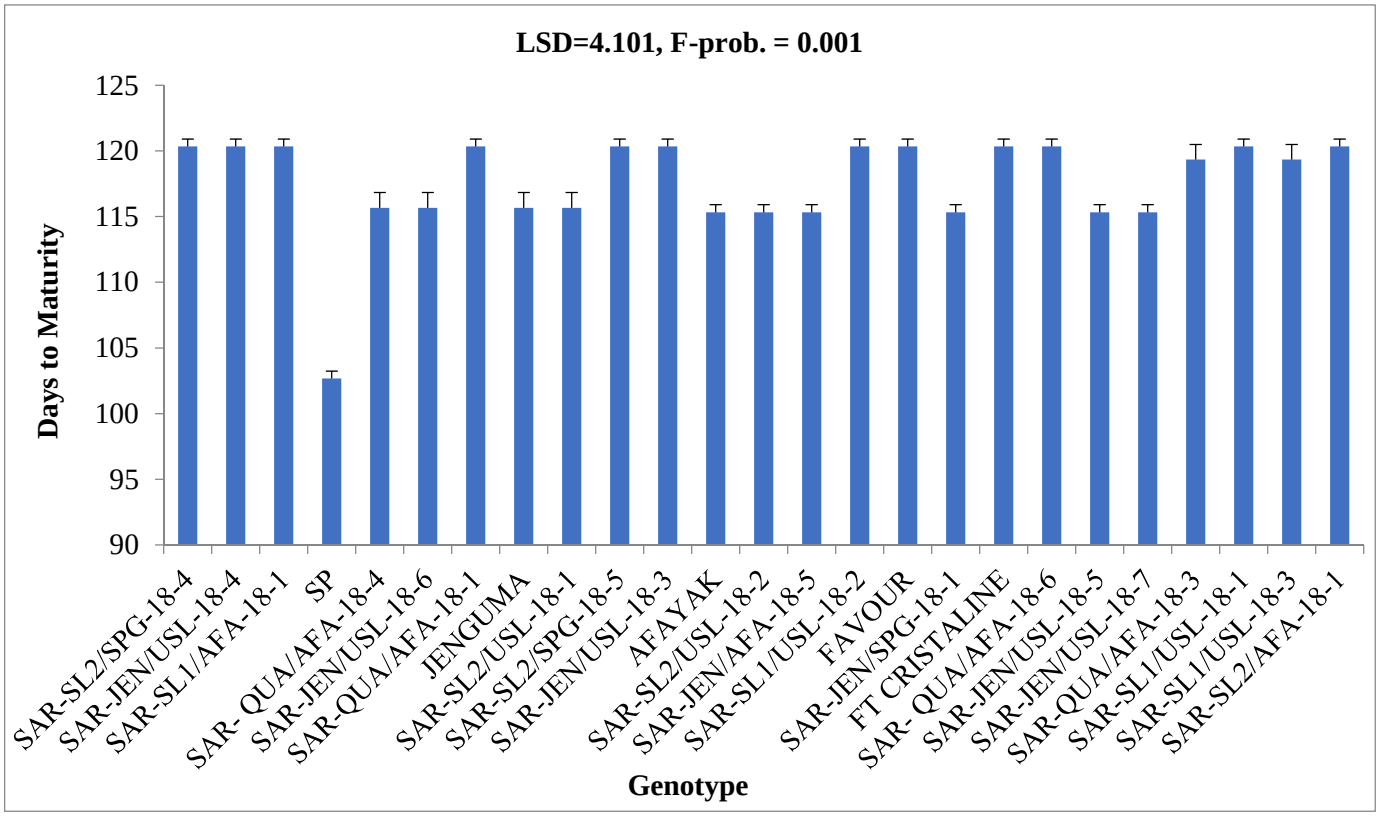

Figure 6: Mean Maturity Days of twenty-five soybean genotypes

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.12 SPAD Chlorophyll meter readings

The fact available from the ANOVA for chlorophyll content of the genotypes is presented in table 7. The result clearly shows that there was no significant difference in the means of all the treatments (P = 0.403). The average chlorophyll content was 38.31 (Table 8). The highest SPAD Chlorophyll meter readings were recorded by the genotype SAR-JEN/USL-18-3 (42.85) and the lowest value was recorded by genotype JENGUMA (35.79).

Table 8**: Mean SPAD Chlorophyll and Height of twenty-five soybean genotypes**

Genotype	Chlorophyll Content	Height at Maturity (cm)
SAR-SL2/SPG-18-4	39.04	48.49
SAR-JEN/USL-18-4	38.51	52.19
SAR-SL1/AFA-18-1	39.37	42.95
SP	36.51	49.19
SAR- QUA/AFA-18-4	38.14	50.52
SAR-JEN/USL-18-6	37.92	48.21
SAR-QUA/AFA-18-1	38.55	46.44
JENGUMA	35.79	44.77
SAR-SL2/USL-18-1	39.15	56.51
SAR-SL2/SPG-18-5	40.15	47.41
SAR-JEN/USL-18-3	42.85	45.57
AFAYAK	38.79	51.17
SAR-SL2/USL-18-2	36.39	52.27
SAR-JEN/AFA-18-5	37.09	47.5
SAR-SL1/USL-18-2	36.45	52.27
FAVOUR	37.15	46.22
SAR-JEN/SPG-18-1	39.85	41.95
FT CRISTALINE	37.93	47.79
SAR- QUA/AFA-18-6	39.03	45.92
SAR-JEN/USL-18-5	38.25	53.62
SAR-JEN/USL-18-7	39.2	53.65
SAR-QUA/AFA-18-3	38.35	49.75
SAR-SL1/USL-18-1	36.6	46.22
SAR-SL1/USL-18-3	38.8	49.75
SAR-SL2/AFA-18-1	37.95	51.79
Grand Mean	38.31	48.67
CV (%)	6.5	11.6
LSD (0.05)	4.124	9.343
F-Prob.	0.407	0.346

Note: CV = coefficient of variation, LSD = least significant difference, F-pro. = fisher probability

4.13 Plant height at maturity

The ANOVA result for plant height at maturity revealed that there was no genotypic effect on the height at maturity (P = 0.346). The average value for the plant height at harvest was 48.68 cm. Although there were no significant differences among the treatment means; genotype SAR-SL2/USL-18-1 recorded the highest mean value (56.51 cm) while genotype SAR-JEN/SPG-18-1 recorded the lowest height (41.95 cm) (Table 8).

4.14 Biomass Weight

There were statistically significant differences (P = 0.001) among the mean values of the genotypes for biomass weight when the data was subjected to the ANOVA. Genotypes, SAR-SL2/SPG-18-4 and FT Cristaline recorded the highest biomass weight (8133.3 Kg/ha) and (7226.7) respectively, while genotype SAR-SL2/USL-18-1 recorded the lowest. Genotype SAR-SL2/SPG-18-4 biomass weight was significantly higher than fifteen of the genotypes but there was no significant difference between the genotype SAR-SL2/SPG-18-4 and the rest of the genotypes (Figure 7)

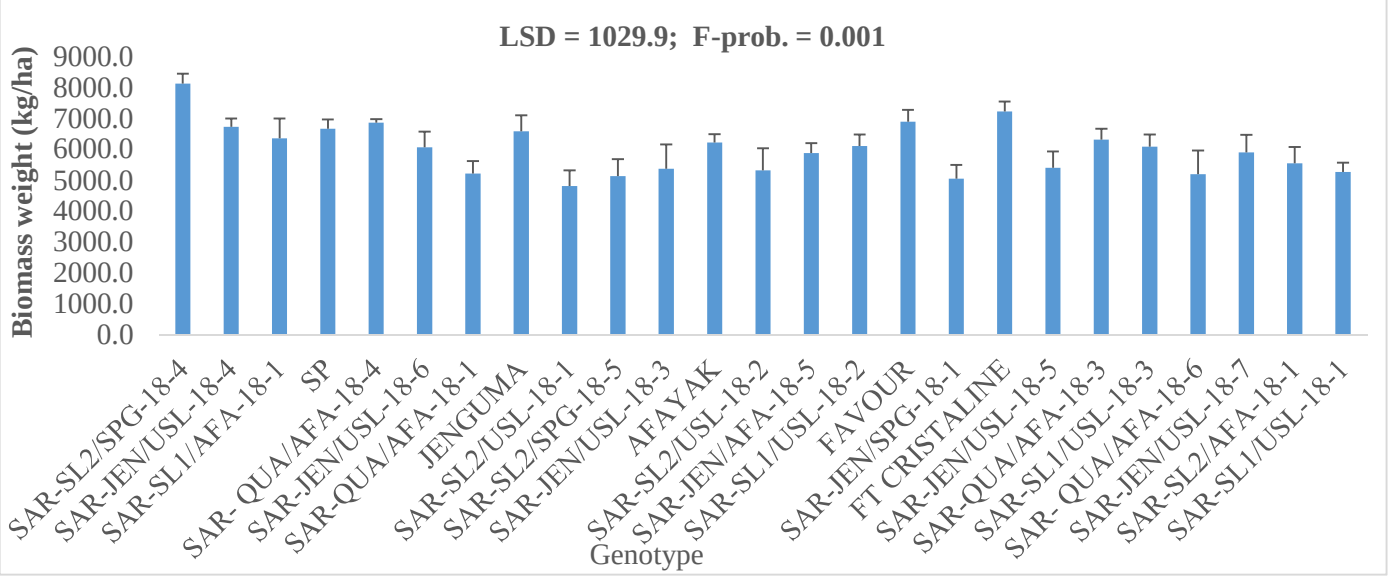

Figure 7: **Mean Biomass weight of twenty-five soybean genotypes**

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.15 Amount of N- fixed

The ANOVA of the data on N-fixed revealed that there were statistically significant differences (P = 0.001) among the genotypes means (Figure 8). Favour produced the highest amount of N-fixed (370.5 kg N/ha), followed by SAR-SL2/SPG-18-4 (256.8 kg/ha) and FT Cristaline (133.3 kg N /ha), while genotype SAR-SL2/USL-18-1 recorded the lowest amount of N-fixed per hectare (53.6 kg N/ha). The N-fixed by favour was statistically significantly different than all the other genotypes (Figure 8).

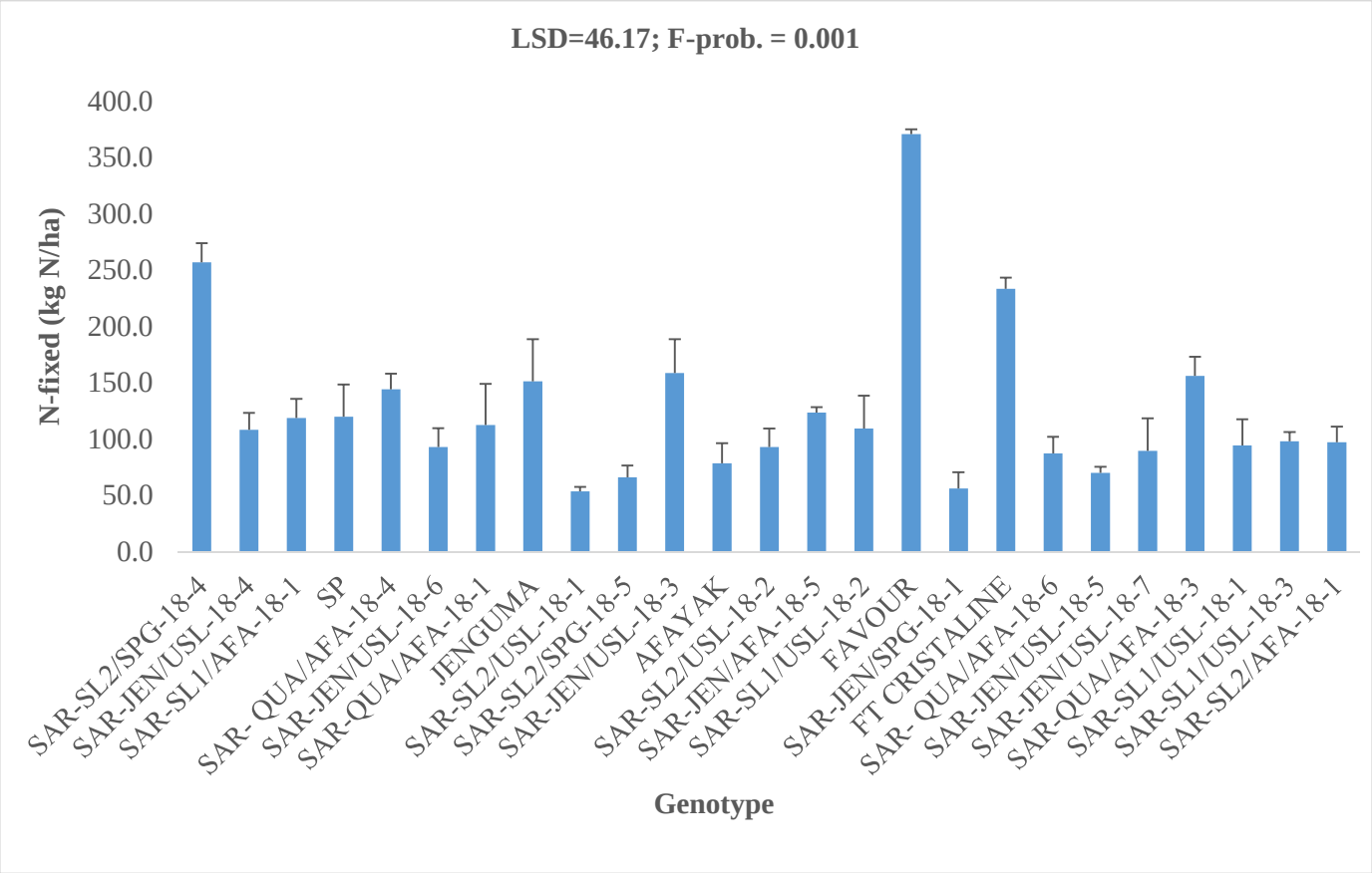

Figure 8**: Mean N-fixation of twenty-five soybean genotypes**

Note: LSD = least significant difference, F-prob. = fisher probability value = Error Bars, standard error mean

4.16 Soil N Uptake

There was a highly significant difference (P = 0.001) in the mean values of the genotypes for N uptake (Figure 9). The greatest amount of soil mineral N was taken up by variety Favour (468 kg/ha) and the lowest soil N uptake was recorded by genotype SAR-SL2/USL-18-1 (34 kg/ha). The N uptake of favour was significantly higher than all the other treatments (Figure 9). The second highest soil mineral nitrogen (N) uptake was recorded by the genotype, SAR-JEN/USL-18-3 (277 kg/ha). However, there was no significant difference between the mean N uptake of the genotype SAR-JEN/USL-18-3 and FT Cristaline.

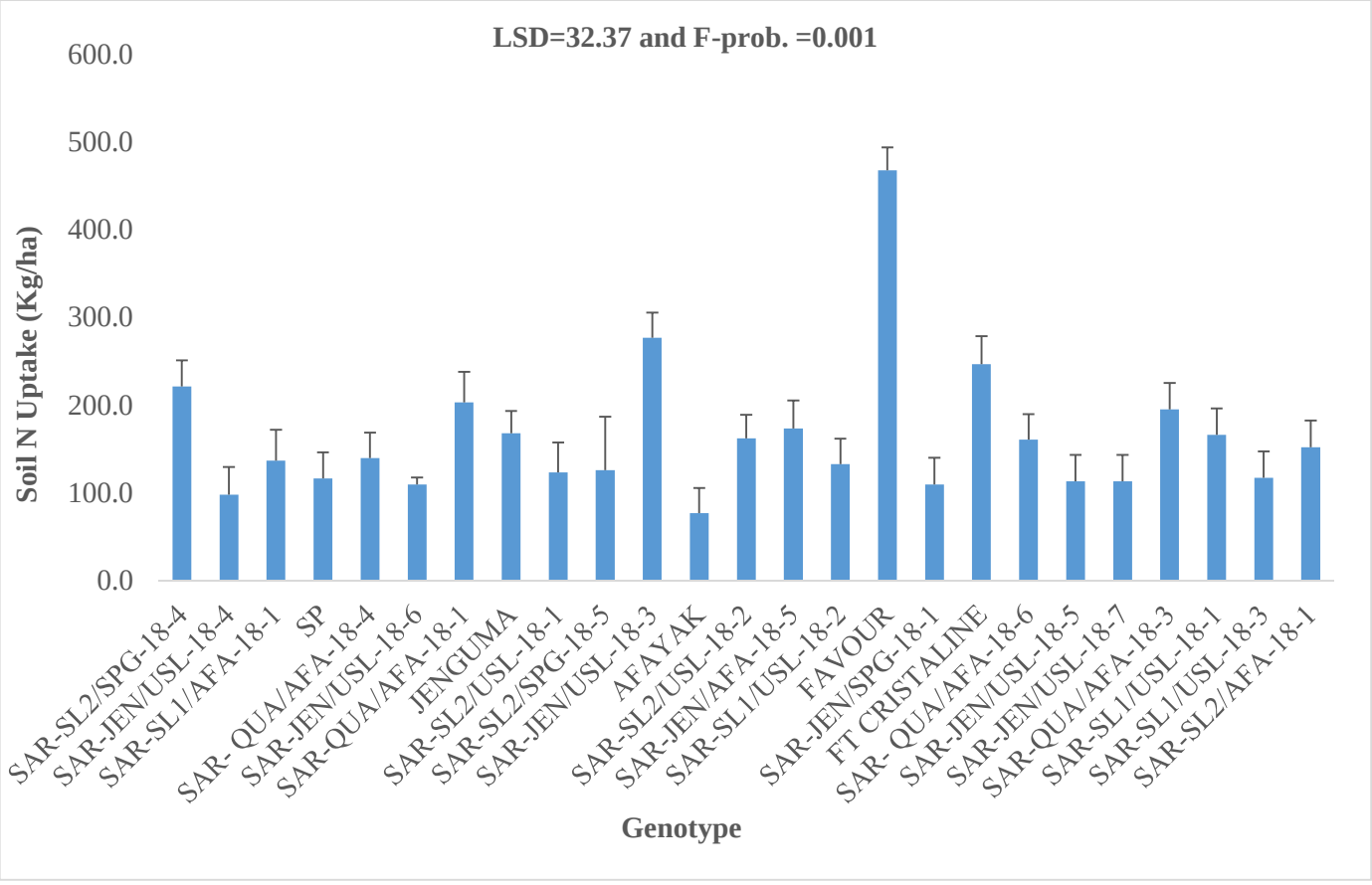

Figure 9: **Mean soil N Uptake of twenty-five Soybean genotypes**

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.17 Percent of N derived from the atmosphere

The ANOVA revealed that there were statistically significant differences (P = 0.001) among the genotypes for their mean percent N derived from the atmosphere (Figure 10). The percent mean Ndfa by genotype favour was significantly different from all the other genotypes. Genotype, Afayak recorded the least percent Ndfa.

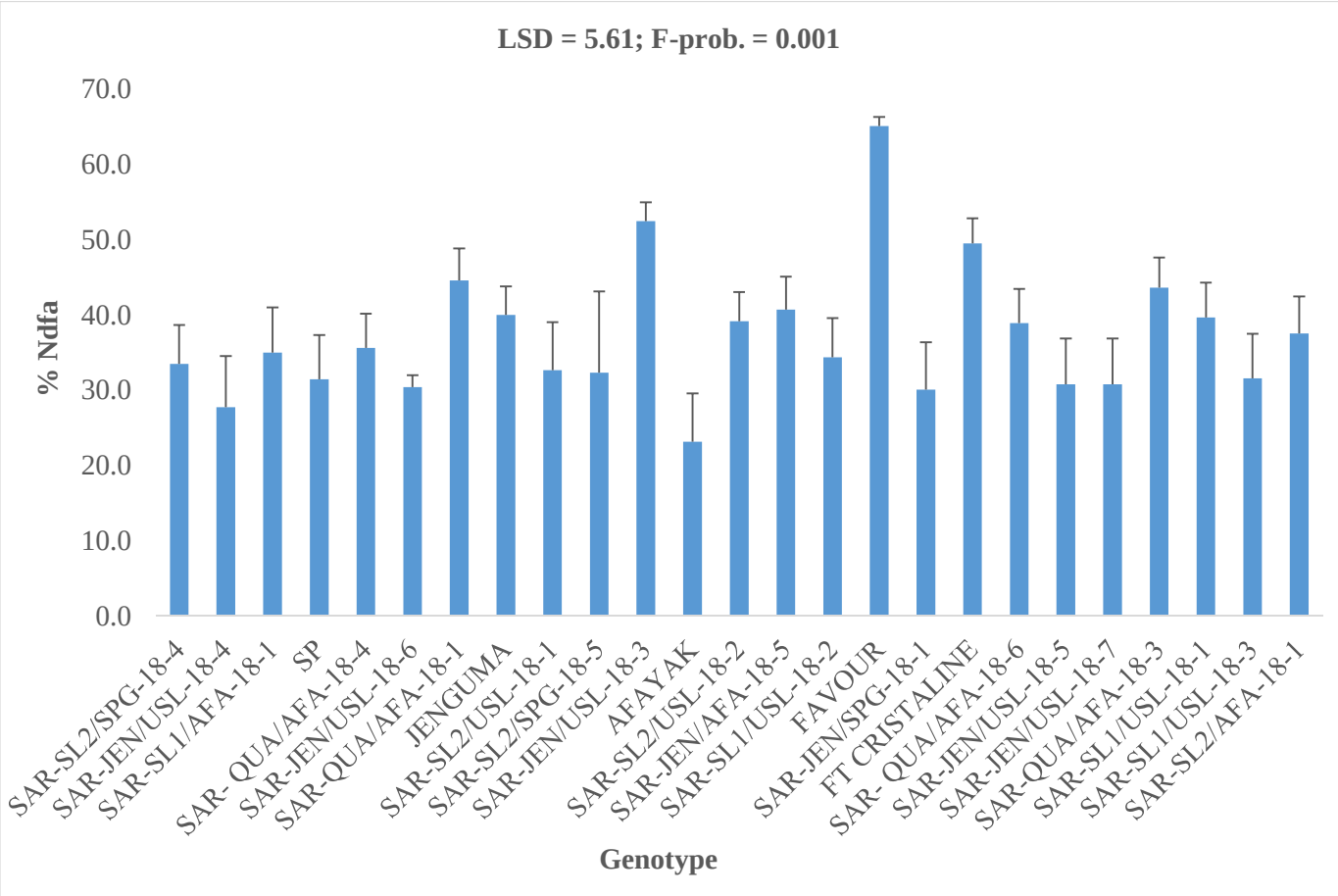

Figure 10**: Mean percent N- fixed of twenty-five genotypes**

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.18 Photosynthetic Water Use-efficiency

Statistically, there were significant differences (P = 0.05) among the genotypes mean for photosynthetic water-use efficiency at the time of data collection (Figure 11). The genotype SAR-SL2/USL-18-2 showed the highest photosynthetic water use efficiency rate at the time of data collection, while genotype FT Cristaline showed the lowest water use efficiency. However, there was no statistically significant difference between the mean photosynthetic water use efficiency of genotype SAR-SL2/USL-18-2 and six of the genotypes, JENGUMA, SAR-SL1/USL-18-3, SAR-SL1/USL-18-1, SAR-QUA/AFA-18-6, SAR-SL2/SPG-18-5, and SAR-JEN/USL-18-6 (Figure 11).

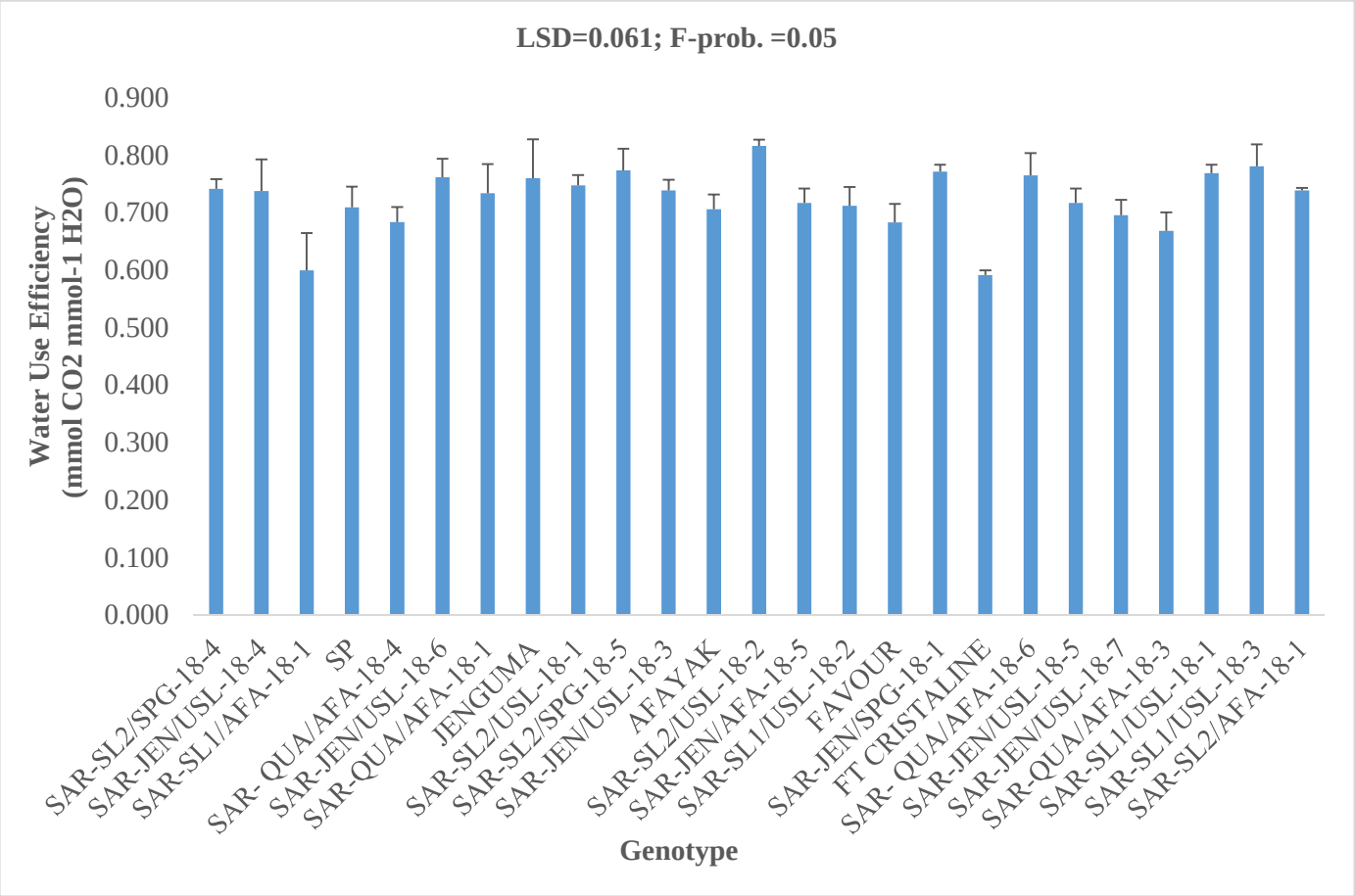

Figure 11: **Mean photosynthetic water use efficiency of twenty-five genotype**

Note: LSD = least significant difference, F-prob. = fisher probability value, Error Bars = standard error mean

4.19 Estimates of heritability and genetic parameters

The estimate of heritability of the variance components as presented in Table 9 below showed the traits 50% flowering, percent nitrogen, nitrogen derived from the atmosphere, and nitrogen uptake by the genotypes recording the highest heritability of (0.98 or 98%) each, followed by the amount of nitrogen fixed (0.95 or 95%), and 100 seed weight (0.87 or 87%). The lowest Broad sense heritability was recorded by the trait, nodule number (0.00002 or 0.002%). The genotypic variance in all the traits observed ranged from 0.00002 (Nodule per plant) to 545916.00 (Shoot biomass weight). In this study, it was observed that the Phenotypic Coefficient of Variation values was higher than the Genotypic Coefficient Variation values. However, the differences between these two were not significant. The phenotypic variation values ranged from 0.47 (Shattering score) to 847977.78 (Shoot biomass). The highest genotypic coefficient of variation was observed in trait amount of N-fixed (54.84) while the lowest was recorded by nodule number per plant (0.002). The phenotypic coefficient of variation values ranges from 2.74–58.65. The highest expected genetic gain was 1601.58 and the selection intensity differential value was 2.06. The estimated heritability values were very high in most of the parameters with a significant variation between the parameters.

Table 9**: Estimates of genetic parameters of twenty-five soybean genotypes**

Genetic Parameter	GV	RV	GM	H^2	PV	PCV	GCV	EGA	%GA
BW (kg/ha)	545916.00	302061.78	6015.73	0.84	847977.78	15.31	12.28	1601.58	26.62
%N leg	0.62	0.03	4.14	0.98	0.65	19.50	18.98	1.63	39.45
N-fixed (kg/ha)	4733.87	681.34	125.47	0.95	5415.21	58.65	54.84	144.65	115.29
%NDFA	0.62	0.03	4.01	0.98	0.65	20.17	19.63	1.63	40.79
N Uptake (Kg/ha)	6181.14	344.17	400.56	0.98	6525.31	20.17	19.63	163.37	40.79
50% Flowering	6.90	0.45	49.56	0.98	7.05	5.36	5.30	5.35	10.80
Leaf Area Index	0.58	0.34	3.26	0.84	0.69	25.58	23.37	1.43	44.00
Nodules Per Plant	0.00	424.76	37.19	0.00	141.59	32.00	0.00	0.00	0.00
PAR	28.02	24.33	74.79	0.78	36.13	8.04	7.08	9.60	12.84
Chlorophyll Content	0.75	5.01	38.31	0.31	2.42	4.06	2.26	0.99	2.59
Maturity Days	8.59	5.33	117.56	0.83	10.37	2.74	2.49	5.50	4.68
Pod Clearance	0.50	3.27	6.77	0.31	1.58	18.59	10.40	0.81	11.99
Height at Maturity	6.91	27.14	48.67	0.43	15.96	8.21	5.40	3.56	7.32
Pods per Plant	55.45	173.74	67.09	0.49	113.37	15.87	11.10	10.73	15.99
Grain Yield (kg/ha)	22210.26	52086.58	1125.11	0.56	39572.45	17.68	13.25	230.00	20.44
100SDW (g)	1.13	0.51	11.36	0.87	1.30	10.02	9.34	2.04	17.94
Shattering Score (1-5)	0.34	0.39	1.96	0.72	0.47	34.95	29.73	1.02	52.10

Where; VG=Genetic variance, RV=Residual variance, GM=Grand mean, VP=Phenotypic variance, and H^2=Broad sense heritability, PCV=Phenotypic coefficient of variation, GCV=Genotypic coefficient of variation, EGA=Expected genetic advance, %GA=Genetic advance as a percentage of the mean, K=Selection intensity differential

CHAPTER FIVE

5.0 DISCUSSION

This research was conducted to determine the nitrogen-fixing potential, water-use efficiency, and grain yield of twenty elite lines and five varieties (Checks) of soybeans using the N difference technique. The results showed the poor fertility nature of these soils, particularly, low nitrogen (N) and phosphorus (P). Farmers in this region often rely heavily on chemical nitrogen fertilizers to achieve high production which is expensive and can affect the soil health, environment, and agricultural sustainability. A research finding by (Sanginga et al., 2002) on the contribution of nitrogen by promiscuous soybeans to maize-based cropping in the moist savanna of Nigeria revealed that soybean significantly contributed N to cropping systems in Nigeria and double yield of the following maize.

The N difference technique was used to quantify the N fixation of soybean elite lines. A similar technique was used by Sarkodie-Addo *et al*. (2007) in soybean to determine the nitrogen fixation potential of medium maturing soybean lines, by Simunji *et al*. (2019) in cowpea (*Vigna unguiculata* L. Walp.) to evaluate cowpea genotypes for biological nitrogen fixation in maize – cowpea crop rotation and by Oteng-Frimpong & Dakora (2018) in groundnut to select groundnut (*Arachis hypogaea* L) genotypes for improved nitrogen fixation.

5.1 Effect of Genotype on Nodulation and Nodule Dry Weight

Even though there was no Rhizobium inoculation, there was a substantial amount of nodulation exhibited by all the soybean genotypes. This result suggests that there were indigenous bacteria, Bradyrhizobia available in the soil. A report by Delamuta *et al*. (2013) suggested that *Bradyrhizobium spp* is a native of the tropics and is the main symbiont of cowpea and many other legumes such as groundnut, Bambara groundnut, and Soybean. Although there were no significant differences among the genotypes for both the number of nodules produced and nodule dry weight, Genotype FT Cristaline produced the highest nodule number per plant,

while genotype SuongPungun produced the lowest number of nodules per plant. Similar to nodule number, the highest nodule weight per plant was recorded for genotype FT Cristaline, while the lowest was recorded for genotype SAR-SL2/USL-18-1 (Table 6). It was interesting to observe that genotype FT Cristaline which produced the highest number of nodules was the same genotype that recorded the highest nodule dry weight. This result was in contrast with what Sarkodie-Addo *et al*. (2007) reported. They observed significant differences in both the number of nodules produced per plant and nodule dry weight with a negative correlation between the two when medium maturing soybean lines were evaluated for their nitrogen fixation potentials. Also, significant differences in nodule production and nodule dry weight among varieties have been reported in legumes such as cowpea by (Egbe & Egbo, 2011) and groundnut by (Moji *et al*., 2020).

5.2 Shoot Biomass and N fixation of soybean genotypes

The result showed statistically significant differences (P = 0.001) among the genotypes for biomass weight and N fixation. Even though it was genotype SAR-SL2/SPG-18-4 that produce the highest grain and most biomass, its amount N fixed was lower than the N fix by favour (Figure 7 and 8). This findings were contrast with what (Belane & Dakora, 2010) reported. They suggested that a higher amount of nitrogen fixed caused increased deposition of other mineral elements in the plant shoot, contributing to increased performance in terms of growth and grain yield when compared with low nitrogen-fixing genotypes. Their study area was on symbiotic N fixation in 30 field grown cowpea genotypes in the Upper West Region of Ghana measured using ^{15}N natural abundance. The relatively significant performance exhibited by the genotypes in terms of shoot biomass produced can be attributed partly to their ability to fix a greater amount of nitrogen.

5.3 Grain yield and N_2 fixation of soybean genotypes

Grain yield result showed that the genotype effect significantly affected the grain yield (P = 0.03) and the amount of N fixed (P = 0.001) by the genotypes. This result suggests that the genotypic effect was responsible for the significant differences observed since all the genotypes were treated the same and planted under the same environment. Similar research findings have been reported by (Sarkodie-Addo et al., 2007) in Soybean, by (Oteng-Frimpong & Dakora, 2018) in groundnut, and *(Berchie et al*., 2010) in Bambara groundnut. Herridge, (1982) have reported that soybean can contribute about 337kgN ha^{-1}. The percentage of nitrogen that derives via symbiotic fixation in soybeans in most soils with moderate nitrate levels is around 50% (Hardarson *et al*., 1984; Bergersen *et al*., 1985) but can reach 75% in sandy loamy soils (Matheny and Hunt, 1983). In this study, the percent of N derived from biological was about 65% (Figure 10) which falls within the reported figure (50-75%). The finding was supported by the soil physicochemical analyses (Table 4). The highest mean N fixation recorded in this study was 370 kg ha^{-1}. Although genotype, favour was the treatment that recorded the highest amount of N-fixed, its grain yield was significantly lower than the genotype SAR-SL2/SPG-18-4 which produced the largest grain yield. The grain yield of the genotype, favour was even lower than the genotype, SAR-SL2/USL-18-1 which fixed the lowest amount of N. The negative association observed from this result could be partly due to the inability of the genotype favour to translate its greatest amount of N-fixed into a grain yield production. This result was in contrast with what Samago *et al.* (2018); Sarkodie-Addo *et al.* (2007) reported. They observed a positive correlation between N-fixed and grain yield in common bean (Phaseolus vulgaris L.) and soybean (*G. max* (L) Merril) respectively. Samago *et al*. (2018) observed this when they assessed common bean varieties′ response to Rhizobium inoculation and phosphorus application. Sarkodie-Addo *et al.* (2007) recorded this when they evaluated soybean medium maturing lines for their nitrogen fixation potentials. The genotype SAR-

SL2/SPG-18-4 was the superior genotype in terms of grain yield with a mean yield of 1502 kg/ha. Even though it's mean grain yield was less than the Sub-Saharan African (SSA) lead soybean producer, South Africa average output of 2,290 kg ha^{-1} (Khojely *et al*., 2018), its average value was higher than second larger producer, Zambia (1,940 kg ha^{-1}) and small farmer average of (1000 kg ha^{-1}) (Khojely *et al*., 2018). This results suggest that the genotype has the potential of increasing the small farmer production.

5.4 Estimates of genetic parameters

With the exception of a few features, heritability estimates were generally high in most of the traits observed. For variables such as 50 percent flowering, percent nitrogen, NDFA, N uptake, N fixed, and 100 seed weight, there was substantial broad-sense heritability observed among the genotypes, implying that the phenotypes strongly reflect the genotypes. The findings of this study matched those of other studies (Datta *et al*., 2005; Jain *et al*., 2018). Also, this study observed a high PCV and GCV which are required in a breeding program for crop improvement. Although the PCV was observed to be higher than the GCV, the difference was not significant. This result was in line with what (Baraskar et al., 2014) reported. He observed higher values for PCV than the GCV when he carried out a study on genetic variability, heritability and genetic advance in soybean. This suggests the role the environment played in the expression of the characters. The highest PCV and GCV were observed by traits N-fixed (Kg/ha) and shattering score. The N-fixed trait was also among the traits with the highest heritability values which probably will make it ideal for selection. GCV and Genetic advances, which are important genetic parameters for selection was observed to be high for shattering, which suggest that the trait is core to be considered in a breeding program.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Generally, the native rhizobium, Brydirhizobium supported the nodulation of the soybean genotypes. Even though there were no significant differences among the genotypes for nodulation. The genotypes showed statistically significant variability for Amount of N-fixed, percent nitrogen derived from the atmosphere, water use efficiency and grain yield. Addition of the high N fixing lines in to integrated farming system will help enrich the poor soils in the Guinea Savannah agro-ecology and ensure sustainable agriculture. The significant variation could be attributed to genotypic differences among the genotypes. This study illustrated a high heritability amongst the selected traits and high phenotypic coefficient of variation and genotypic coefficient of variation which is required in breeding program for crop improvement. Therefore, the genotypes can be used as breeding lines in crop improvement programmes.

6.2 Recommendations

i. Genotypes with high nitrogen fixation could be used in integrated farming system schemes to improve soil fertility which will indirectly reduce the cost of production and also maintain beneficial eco-systems.

ii. Genotypes that showed increased performance for water use efficiency with contrast performance in fixing nitrogen can be used as breeding lines in breeding programs that have the objective of enhancing water-use efficiency in high nitrogen-fixing genotypes.

iii. Genotypes that perform creditably well in terms of grain yield could be evaluated in multi-location and if perform the same, should be selected for release as variety.

REFERENCES

Abudulai, M., Salifu, A. B., Opare-Atakora, D., Haruna, M., Denwar, N. N., & Baba, I. I. Y. (2012). Yield loss at the different growth stages in soybean due to insect pests in Ghana. *Archives of Phytopathology and Plant Protection*, *45*(15), 1796–1809. https://doi.org/10.1080/03235408.2012.706744

Adjei-Nsiah, S., Alabi, B. U., Ahiakpa, J. K., & Kanampiu, F. (2018). Response of grain legumes to phosphorus application in the Guinea savanna agro-ecological zones of Ghana. *Agronomy Journal*, *110*(3), 1089–1096. https://doi.org/10.2134/agronj2017.11.0667

Ahiabor, B. (2011). Innovations as Key to the Green Revolution in Africa. *Innovations as Key to the Green Revolution in Africa*, *September*. https://doi.org/10.1007/978-90-481-2543-2

Aidoo, R., Mensah, J. O., Opoku, A., & Abaidoo, R. C. (2014). Factors influencing soybean production and willingness to pay for inoculum use in Northern Ghana. American Journal of Experimental Agriculture, 4, 290–301. https://doi.org/10.9734/AJEA/2014/6011

Amanor-Boadu, V., Zereyesus, Y., Ross, K., Ofori-Bah, A., Adams, S., Aseidu-Dartey, J., Salin, M. (2015). Agricultural production survey for the northern regions of Ghana: 2013–2014 results. Retrieved from https://www.agmanager.info/sites/default/files/APS_Report_2016_Final.pdf

Anna Siczek, A. Siczek, & Jerzy Lipiec, J. Lipiec. (0000). Soybean nodulation and nitrogen fixation in response to soil compaction and surface straw mulching. *Soil & tillage research*, *114*, 50-56. doi: 10.1016/j.still.2011.04.001

Awuni, G. A., Reynolds, D. B., Goldsmith, P. D., Tamimie, C. A., & Denwar, N. N. (2020). Agronomic and economic assessment of input bundle of soybean in moderately acidic Savanna soils of Ghana. *Agrosystems, Geosciences & Environment*, *3*(1), 1–15. https://doi.org/10.1002/agg2.20085

Bakari, R., Mungai, N., Thuita, M., & Masso, C. (2020). Impact of soil acidity and liming on soybean (*Glycine max*) nodulation and nitrogen fixation in Kenyan soils. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science*, *70*(8), 667–678. https://doi.org/10.1080/09064710.2020.1833976

Bano, S., & Sheikh, M. (2016). Biological Nitrogen Fixation to Improve Plant Growth and Productivity. *International Journal of Agriculture Innovations and Research*, *4*(February), 597–599. http://files/515/Bano et al. - 2016 - Biological Nitrogen Fixation to Improve Plant Grow.pdf%0Ahttps://www.researchgate.net/publication/292982251_Biological_Nitrogen_Fixation_to_Improve_Plant_Growth_and_Productivity

Baraskar, V. V., Kachhadia, V. H., VachhanI, J. H., Barad, H. R., Patel, M. B., & Darwankar, M. S. (2014). Genetic variability, heritability and genetic advance in soybean [*Glycine max* (L.) Merrill]. *Electronic Journal of Plant Breeding*, *5*(4), 802–806.

Basal, O., & Szabó, A. (2020). Physiomorphology of Soybean as Affected by Drought Stress and Nitrogen Application. *Scientifica*, *2020*. https://doi.org/10.1155/2020/6093836

Bates D, Mächler M, Bolker B, Walker S (2015). "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software*, **67**(1), 1–48. doi:10.18637/jss.v067.i01.

Bazzer, S. K., & Purcell, L. C. (2020). Identification of quantitative trait loci associated with canopy temperature in soybean. *Scientific Reports*, *10*(1), 1-11.

Bergersen, F.J., Turner, G.L., Gault, R.R., Chase, D.L., Brockwell, J. (1985). The natural abundance of ^{15}N in an irrigated soybean crop and its use for the calculation of nitrogen fixation. *Australian Journal of Agricultural Research* **36**, 411-423.

Bernard, R.L. and M.G. Weiss 1973 Qualitative Genetics. Soybeans, Production and Uses. B.E. Caldwell (ed.). Agronomy Series, American Society of Agronomy, Madison, Wisconsin, USA. pp. 117-154.

Belane, A. K., & Dakora, F. D. (2010). Symbiotic N2 fixation in 30 field-grown cowpea (Vigna unguiculata L. Walp.) genotypes in the Upper West Region of Ghana measured using 15N natural abundance. *Biology and Fertility of Soils*, *46*(2), 191–198. https://doi.org/10.1007/s00374-009-0415-6

Bellaloui, N., Bruns, H. A., Abbas, H. K., Mengistu, A., Fisher, D. K., & Reddy, K. N. (2015). Effects of row-type, row-spacing, seeding rate, soil-type, and cultivar differences on soybean seed nutrition under us Mississippi Delta conditions. *PloS one*, *10*(6), e0129913.

Berchie, J. N., Sarkodie-Addo, J., Adu-Dapaah, H., Agyemang, A., Addy, S., Asare, E., & Donkor, J. (2010). Yield evaluation of three early maturing bambara groundnut (*Vigna subterranea* L. Verdc) Landraces at the CSIR-Crops Research Institute, Fumesua-Kumasi, Ghana. *Journal of Agronomy*, *9*(4), 175–179. https://doi.org/10.3923/ja.2010.175.179

Blumenthal, J. D. M., Baltensperger, D. D., Cassman, K. G., Mason, S. C., & Pavlista, A. D. (2008). Importance and Effect of Nitrogen on Crop Quality and Health. *Nitrogen in the Environment*, 51–70. https://doi.org/10.1016/B978-0-12-374347-3.00003-2

Boddey, R. M., Polidoro, J. C., Resende, A. S., Alves, B. J. R., & Urquiaga, S. (2001). Use of the 15N natural abundance technique for the quantification of the contribution of N2 fixation to sugar cane and other grasses. *Australian Journal of Plant Physiology*, *28*(9), 889–895. https://doi.org/10.1071/pp01058

Bu, T., Lu, S., Wang, K., Dong, L., Li, S., Xie, Q., Xu, X., Cheng, Q., Chen, L., Fang, C., Li, H., Liu, B., Weller, J. L., & Kong, F. (2021). A critical role of the soybean evening complex in the control of photoperiod sensitivity and adaptation. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(8), 1–10. https://doi.org/10.1073/pnas.2010241118

Caliskan, S., Ozkaya, I., Caliskan, M. E., & Arslan, M. (2008). The effects of nitrogen and iron

fertilization on growth, yield and fertilizer use efficiency of soybean in a Mediterranean-type soil. *Field Crops Research*, *108*(2), 126–132. https://doi.org/10.1016/j.fcr.2008.04.005

Chen, Y., & Nelson, R. L. (2004). Genetic Variation and Relationships among Cultivated, Wild, and Semiwild Soybean. *Crop Science*, *44*(1), 316. https://doi.org/10.2135/cropsci2004.0316

Cui, Y., Ning, S., Jin, J., Jiang, S., Zhou, Y., & Wu, C. (2021). Quantitative lasting effects of drought stress at a growth stage on soybean evapotranspiration and aboveground BIOMASS. *Water (Switzerland)*, *13*(1). https://doi.org/10.3390/w13010018

Datta, A., Hossain, M., & Rahman, L. (2005). Genetic parameters, inter-relationship and path-coefficient analysis in soybean (*Glycine max* (L.) Merrill). *Journal of the Bangladesh Agricultural University*, *3*(2), 225–230. https://doi.org/10.22004/ag.econ.276479

Delamuta, J. R. M., Ribeiro, R. A., Ormeño-Orrillo, E., Melo, I. S., Martínez-Romero, E., & Hungria, M. (2013). Polyphasic evidence supporting the reclassification of Bradyrhizobium japonicum group Ia strains as Bradyrhizobium diazoefficiens sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *63*(PART9), 3342–3351. https://doi.org/10.1099/ijs.0.049130-0

Desclaux, D., Huynh, T. T., & Roumet, P. (2000). Identification of soybean plant characteristics that indicate the timing of drought stress. *Crop Science*, *40*(3), 716–722. https://doi.org/10.2135/cropsci2000.403716x

Dohlman, Erik & Hansen, James & Boussios, David, 2022. "**USDA Agricultural Projections to 2031**," USDA Miscellaneous 323859, United States Department of Agriculture. DOI:10.22004/ag.econ.323859

Dukhnytskyi, B. (2019). World agricultural production. *Ekonomika APK*, *7*, 59–65. https://doi.org/10.32317/2221-1055.201907059

Egbe, O. ., & Egbo, C. (2011). Nodulation , nitrogen fixation and harvest index of extra- short- and short-duration cowpea varieties intercropped with maize at Otobi , Benue state , Nigeria. *Journal of Animal and Plant Science*, *10*(3), 1315–1324.

Ene, C. O., Ogbonna, P. E., Agbo, C. U., & Chukwudi, U. P. (2016). Studies of phenotypic and genotypic variation in sixteen cucumber genotypes. *Chilean Journal of Agricultural Research*, *76*(3), 307–313. https://doi.org/10.4067/S0718-58392016000300007

Fabre, F., & Planchon, C. (2000). Nitrogen nutrition, yield and protein content in soybean. *Plant Science*, *152*(1), 51-58.

Franche, C., Lindström, K., & Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and Soil*, *321*(1–2), 35–59. https://doi.org/10.1007/s11104-008-9833-8

Gage et al., 2012. (2012). The Market for Maize , rice , Soy , and warehousing in Northern Ghana. *Enabling Agricultural Trade*, *January*, 58.

Gao, X. B., Guo, C., Li, F. M., Li, M., & He, J. (2020). High soybean yield and drought adaptation being associated with canopy architecture, water uptake, and root traits. *Agronomy*, *10*(4). https://doi.org/10.3390/AGRONOMY10040608

Gebre, M. G., & Earl, H. J. (2021). Soil Water Deficit and Fertilizer Placement Effects on Root Biomass Distribution, Soil Water Extraction, Water Use, Yield, and Yield Components of Soybean [*Glycine max* (L.) Merr.] Grown in 1-m Rooting Columns. *Frontiers in Plant Science*, *12*(March), 1–17. https://doi.org/10.3389/fpls.2021.581127

GenStat. 2009. GenStat for Windows, 12th Edition. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk.

Gomes, M. da M., Quinhones, M. S., Engelhardt, E., Lerner, R. M., Andrés, A., Pereira, P. M. C. de M., Miguens, S., Nacional, S., Ministério da Educação e Ciência, Berger, J., Dubowy, C., Sehgal, A., Rosbash, M., Benzer, S., Picco, S., Villegas, L., Tonelli, F.,

Merlo, M., Rigau, J., … Doğan, Z. (2013). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. *Intech*, *15*(1), 13. https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics%0Ahttps://doi.org/10.32725/jab.2004.016

Guignard, M. S., Leitch, A. R., Acquisti, C., Eizaguirre, C., Elser, J. J., Hessen, D. O., Jeyasingh, P. D., Neiman, M., Richardson, A. E., Soltis, P. S., Soltis, D. E., Stevens, C. J., Trimmer, M., Weider, L. J., Woodward, G., & Leitch, I. J. (2017). Impacts of nitrogen and phosphorus: From genomes to natural ecosystems and agriculture. *Frontiers in Ecology and Evolution*, *5*(JUL). https://doi.org/10.3389/fevo.2017.00070

Gurska, J., Wang, W., Gerhardt, K. E., Khalid, A. M., Isherwood, D. M., Huang, X. D., Glick, B. R., & Greenberg, B. M. (2009). Three year field test of a plant growth promoting rhizobacteria enhanced phytoremediation system at a land farm for treatment of hydrocarbon waste. *Environmental science & technology*, *43*(12), 4472–4479. https://doi.org/10.1021/es801540h

Hardarson, G., Zapata, F. & Danso, S.K.A. (1984). Effect of plant genotype and nitrogen fertilizer on symbiotic nitrogen fixation by soybean cultivars. *Plant Soil* **82**, 397–405 (1984). https://doi.org/10.1007/BF02184277

Hardy, R. W. F., Holsten, R. D., Jackson, E. K., & Burns, R. C. (1968). The acetylene - ethylene assay for N2 fixation. *Plant Physiol*, *43*(1968), 1185–1207. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1086994/pdf/plntphys00487-0017.pdf

Hartman, G. L., West, E. D., & Herman, T. K. (2011). Crops that feed the World 2. Soybean-worldwide production, use, and constraints caused by pathogens and pests. *Food Security*, *3*(1), 5–17. https://doi.org/10.1007/s12571-010-0108-x

Hartman, G., & Murithi, H. M. (2014). Field Guide to African Soybean Diseases and Pests. *Soybean Innovation Laboratory*, *May*, 1–33. http://soybeaninnovationlab.illinois.edu

Herridge, D. F., Peoples, M. B., & Boddey, R. M. (2008). Global inputs of biological nitrogen fixation in agricultural systems. *Plant and Soil*, *311*(1–2), 1–18. https://doi.org/10.1007/s11104-008-9668-3

Idu, M., Omonhinmin, C. A., & Ogboghodo, A. I. (2003). Effect of soil types on the seed germination of *Helianthus annuus* L. *Agricultural Science Digest*, *23*(2), 101–103.

Ininbergs, K., Bay, G., Rasmussen, U., Wardle, D. A., & Nilsson, M. C. (2011). Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses. *New Phytologist*, *192*(2), 507–517. https://doi.org/10.1111/j.1469-8137.2011.03809.x

Ivanova, N., Gugleva, V., Dobreva, M., Pehlivanov, I., Stefanov, S., & Andonova, V. (2016). Water, Plant and Soil Relation under Stress Situations. *Intech*, *i*(tourism), 13.

Jain, R. K., Joshi, A., Chaudhary, H. R., Dashora, A., & Khatik, C. L. (2018). Study on genetic variability, heritability and genetic advance in soybean[*Glycine max* (L.) Merrill]. *Legume Research*, *41*(4), 532–536. https://doi.org/10.18805/LR-3874

Kaur, G., Serson, W. R., Orlowski, J. M., McCoy, J. M., Golden, B. R., & Bellaloui, N. (2017). Nitrogen sources and rates affect soybean seed composition in Mississippi. *Agronomy*, *7*(4), 1–10. https://doi.org/10.3390/agronomy7040077

Keino, L., Baijukya, F., Ng'etich, W., Otinga, A. N., Okalebo, J. R., Njoroge, R., & Mukalama, J. (2015). Nutrients limiting soybean (*Glycine max*) growth in acrisols and ferralsols of Western Kenya. *PLoS ONE*, *10*(12). https://doi.org/10.1371/journal.pone.0145202

Khojely, D. M., Ibrahim, S. E., Sapey, E., & Han, T. (2018). History, current status, and prospects of soybean production and research in sub-Saharan Africa. *Crop Journal*, *6*(3), 226–235. https://doi.org/10.1016/j.cj.2018.03.006

Khosro Mohammadi. (2012). Effective factors on biological nitrogen fixation. *African Journal of Agricultural Research*, *7*(12), 1782–1788. https://doi.org/10.5897/ajarx11.034

Kim, M. Y., Van, K., Kang, Y. J., Kim, K. H., & Lee, S. H. (2011). Tracing soybean domestication history: From nucleotide to genome. *Breeding Science*, *61*(5), 445–452. https://doi.org/10.1270/jsbbs.61.445

Kinugasa, T., Sato, T., Oikawa, S., & Hirose, T. (2012). Demand and supply of N in seed production of soybean (Glycine max) at different N fertilization levels after flowering. *Journal of Plant Research*, *125*(2), 275–281. https://doi.org/10.1007/s10265-011-0439-5

Kisinyo, P. O., Opala, P. A., Gudu, S. O., Othieno, C. O., Okalebo, J. R., Palapala, V., & Otinga, A. N. (2014). Recent advances towards understanding and managing Kenyan acid soils for improved crop production. *African Journal of Agricultural Research*, *9*(31), 2397–2408. https://doi.org/10.5897/ajar2013.8359

Klute, A. (1986) Water retention: Laboratory methods. In: Klute, A., Ed., Methods of Soil Analysis, Part 1, Physical and Mineralogical Methods, ASA and SSSA, Madison, 635-662.

Kofsky, J., Zhang, H., & Song, B. H. (2018). The untapped genetic reservoir: The past, current, and future applications of the wild soybean (Glycine soja). *Frontiers in Plant Science*, *9*(July). https://doi.org/10.3389/fpls.2018.00949

Kombiok, J. Buah, S. S., & M., J. (2012). Enhancing Soil Fertility for Cereal Crop Production Through Biological Practices and the Integration of Organic and In- Organic Fertilizers in Northern Savanna Zone of Ghana. *Soil Fertility*, *January 2012*. https://doi.org/10.5772/53414

Kuo, S. 1996. Phosphorus. p. 894-895. In: Methods of Soil Analysis, Part 3—Chemical Methods. Soil Science Society of America, Madison, WI, USA.

Kurt, O., & Bozkurt, D. (2006). Effect of temperature and photoperiod on seedling emergence of flax (Linum usitatissimum L.). *Journal of Agronomy*, *5*(3), 541–545. https://doi.org/10.3923/ja.2006.541.545

Kuswantoro, H. (2015). Agronomical characters of some soybean germplasm under waterlogging condition. *Journal of Agronomy*, *14*(2), 93–97. https://doi.org/10.3923/ja.2015.93.97

Lamichhane, J. R., Constantin, J., Schoving, C., Maury, P., Debaeke, P., Aubertot, J. N., & Dürr, C. (2019). Analysis of soybean germination, emergence, and prediction of a possible northward expansion of the crop under climate change. *BioRxiv*, 1–28. https://doi.org/10.1101/632976

Lammel, D. R., Barth, G., Ovaskainen, O., Cruz, L. M., Zanatta, J. A., Ryo, M., de Souza, E. M., & Pedrosa, F. O. (2018). Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. *Microbiome*, *6*(1), 7–9. https://doi.org/10.1186/s40168-018-0482-8

Lawson, I. Y. D., Mensah, E. A., & Yeboah, E. N. (2009). Improving the establishment and yield of soybean through planting depth and land preparation methods in Northern Ghana. *West African Journal of Applied Ecology*, *14*(July). https://doi.org/10.4314/wajae.v14i1.44706

Lin, M. H., Gresshoff, P. M., & Ferguson, B. J. (2012). Systemic regulation of soybean nodulation by acidic growth conditions. *Plant Physiology*, *160*(4), 2028–2039. https://doi.org/10.1104/pp.112.204149

Lindström, K., Murwira, M., Willems, A., & Altier, N. (2010). The biodiversity of beneficial microbe-host mutualism: the case of rhizobia. *Research in microbiology*, *161*(6), 453–463. https://doi.org/10.1016/j.resmic.2010.05.005

Liu, Y., Wu, L., Baddeley, J. A., & Watson, C. A. (2011). Models of biological nitrogen fixation of legumes. A review. *Agronomy for Sustainable Development*, *31*(1), 155–172. https://doi.org/10.1051/agro/2010008

Maekawa, T., Shimamura, S., & Shimada, S. (2011). Effects of short-term waterlogging on

soybean nodule nitrogen fixation at different soil reductions and temperatures. *Plant Production Science*, *14*(4), 349–358. https://doi.org/10.1626/pps.14.349

Marg, R. A. K., & Mehta, M. (2019). CLINICAL ADVANTAGES AND VERSATILITY OF SOYBEAN OIL AND PRODUCTS. *GJRA*, *8*(7), 32–34.

Matheny, T.A. & Hunt, P.G. (1983). Effects of Irrigation on Accumulation of Soil and Symbiotically Fixed N by Soybean Grown on a Norfolk Loamy Sand1. Agronomy Journal. 75. 10.2134/agronj1983.00021962007500050001x.

Michiels, J., Verreth, C., & Vanderleyden, J. (1994). Effects of temperature stress on bean-nodulating Rhizobium strains. *Applied and Environmental Microbiology*, *60*(4), 1206–1212. https://doi.org/10.1128/aem.60.4.1206-1212.1994

MoFA. (2016). Agriculture in Ghana, facts and figures. Ministry of Food and Agriculture, Statistics, Research and Information Directorate (SRID). *Statistics, Research and Information Directorate (SRID), October 20*(25th), 3137–3146. https://www.agrofood-westafrica.com/fileadmin/user_upload/messen/agrofood-Westafrica/Brochure/AGRICULTURE-IN-GHANA-Facts-and-Figures-2015.

Moji, K. T., Jibrin, M. J., Umar, F. G., & Abubakar, U. D. (2020). Genotypic Effect of Groundnut on Nodulation, N Fixation and N Balance in The Two Savannahs of Nigeria. *Nigerian Journal of Soil Science*, *June*, 25–35. https://doi.org/10.36265/njss.2020.300104

Morita, S., Abe, J., Furubayashi, S., Lux, A., & Tajima, R. (2004). Effects of waterlogging on root system of soybean. *New Directions for a Diverse Planet: Proceedings of the 4th International Crop Science Congress Brisbane, Australia*, *26*(November 2017). http://www.regional.org.au/au/asa/2004/poster/2/7/4/743_morita.htm

Nekesa, A. O., Okalebo, J. R., Othieno, C. O., Thuita, M. N., & Bationo, A. (2011). Innovations as Key to the Green Revolution in Africa. *Innovations as Key to the Green Revolution in Africa, January 2011*. https://doi.org/10.1007/978-90-481-2543-2

OECD [Organisation for Economic Co-operation and Development]. Consensus document on the biology of *Glycine max* (l.) merr. (soybean) - PDF (51.2 kb). [2000].

Ohyama, T., Tewari, K., Ishikawa, S., Tanaka, K., Kamiyama, S., Ono, Y., Takahashi, Y. (2017). *Role of Nitrogen on Growth and Seed Yield of Soybean and a New Fertilization Technique to Promote Nitrogen Fixation and Seed Yield*. IntechOpen.

Oldroyd, G. E. D., Murray, J. D., Poole, P. S., & Downie, J. A. (2011). The rules of engagement in the legume-rhizobial symbiosis. *Annual Review of Genetics*, *45*(November 2010), 119–144. https://doi.org/10.1146/annurev-genet-110410-132549

Oteng-Frimpong, R., & Dakora, F. D. (2018). Selecting elite groundnut (*Arachis hypogaea* L) genotypes for symbiotic N nutrition, water-use efficiency and pod yield at three field sites, using 15 N and 13 C natural abundance. *Symbiosis*, *75*(3), 229–243. https://doi.org/10.1007/s13199-017-0524-1

Oya, T., Nepomuceno, A. L., Neumaier, N., Farias, J. R. B., Tobita, S., & Ito, O. (2004). Drought Tolerance Characteristics of Brazilian Soybean Cultivars—Evaluation and characterization of drought tolerance of various Brazilian soybean cultivars in the field—. *Plant Production Science*, *7*(2), 129-137.

Plahar, W. A. (2011). OVERVIEW OF THE SOYBEAN INDUSTRY IN. *Scientific Reports*, *233*(0). http: //www. wishh. org/%0Aworkshops/intl/ghana/ghana06/plahar-06.pdf

Purdy, R., & Langemeier, M. (2018). International benchmarks for soybean production. Retrieved from https://farmdocdaily. illinois.edu/2018/06/international-benchmarks-soybean production.html

Ray, J. D., Fritschi, F. B., & Heatherly, L. G. (2006). Large applications of fertilizer N at planting affects seed protein and oil concentration and yield in the Early Soybean Production System. *Field Crops Research*, *99*(1), 67-74.

Ravikumar, S., Kathiresan, K., Liakath Alikhan, S., Prakash Williams, G., & Anitha Anandha

Gracelin, N. (2007). Growth of Avicennia marina and Ceriops decandra seedlings inoculated with halophilic azotobacters. *Journal of Environmental Biology*, *28*(3), 601–603.

Reinprecht, Y., Schram, L., Marsolais, F., Smith, T. H., Hill, B., & Pauls, K. P. (2020). Effects of Nitrogen Application on Nitrogen Fixation in Common Bean Production. *Frontiers in Plant Science*, *11*(August), 1–19. https://doi.org/10.3389/fpls.2020.01172

Rymuza, K., Radzka, E., & Wysokiński, A. (2020). Nitrogen uptake from different sources by Non-GMO soybean varieties. *Agronomy*, *10*(9). https://doi.org/10.3390/agronomy10091219

Sainju, U. M. (2017). Determination of nitrogen balance in agroecosystems. *MethodsX*, *4*(July), 199–208. https://doi.org/10.1016/j.mex.2017.06.001

Salvagiotti, F. Cassman, K.G. Specht, J.E. Walters, D.T. Weiss, A. Dobermann, A. (2008). Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research*, *108*(1), pp. 1-13.

Samago, T. Y., Anniye, E. W., & Dakora, F. D. (2018). Grain yield of common bean (*Phaseolus vulgaris* L.) varieties is markedly increased by rhizobial inoculation and phosphorus application in Ethiopia. *Symbiosis*, *75*(3), 245–255. https://doi.org/10.1007/s13199-017-0529-9

Sanginga, N., Okogun, J., Vanlauwe, B., & Dashiell, K. (2002). N. Sanginga*, J. Okogun, B. Vanlauwe & K. Dashiell. *Plant and Soil*, *241*(November 2000), 223–231.

Sarkodie-Addo, J., Adu-Dapaah, H., Ewusi-Mensah, N., & Asare, E. (2007). Evaluation of medium-maturing soybean (*Glycine max* (L) Merrill) lines for their nitrogen fixation potentials. *Journal of Science and Technology (Ghana)*, *26*(2). https://doi.org/10.4314/just.v26i2.32986

Saryoko, A., Homma, K., Lubis, I., & Shiraiwa, T. (2017). Plant development and yield

components under a tropical environment in soybean cultivars with temperate and tropical origins. *Plant Production Science*, *20*(4), 375–383. https://doi.org/10.1080/1343943X.2017.1356203

Schöllhorn, R., & Burris, R. H. (1967). Acetylene as a competitive inhibitor of N-2 fixation. *Proceedings of the National Academy of Sciences of the United States of America*, *58*(1), 213–216. https://doi.org/10.1073/pnas.58.1.213

Schoving, C., Stöckle, C. O., Colombet, C., Champolivier, L., Debaeke, P., & Maury, P. (2020). Combining Simple Phenotyping and Photothermal Algorithm for the Prediction of Soybean Phenology: Application to a Range of Common Cultivars Grown in Europe. *Frontiers in Plant Science*, *10*(January), 1–14. https://doi.org/10.3389/fpls.2019.01755

Sedivy, E. J., Wu, F., & Hanzawa, Y. (2017). Soybean domestication: the origin, genetic architecture and molecular bases. *New Phytologist*, *214*(2), 539–553. https://doi.org/10.1111/nph.14418

Silva, J., & Uchida, R. (2000). Essential Nutrients for Plant Growth : *Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture*, 31–55.

Simunji, S., Munyinda, K. L., Lungu, O. I., Mweetwa, A. M., & Phiri, E. (2019). Evaluation of Cowpea (*Vigna unguiculata* L.walp) Genotypes for Biological Nitrogen Fixation in Maize-cowpea Crop Rotation. *Sustainable Agriculture Research*, *8*(1), 82. https://doi.org/10.5539/sar.v8n1p82

Sinclair, T. R., Purcell, L. C., King, C. A., Sneller, C. H., Chen, P., & Vadez, V. (2007). Drought tolerance and yield increase of soybean resulting from improved symbiotic N2 fixation. *Field crops research*, *101*(1), 68-71.

Singh, R. J. (2019). Cytogenetics and genetic introgression from wild relatives in soybean. *Nucleus (India)*, *62*(1), 3–14. https://doi.org/10.1007/s13237-019-00263-6

Singh, R. J., Chung, G. H., & Nelson, R. L. (2007). Landmark research in legumes. *Genome*,

50(6), 525–537. https://doi.org/10.1139/G07-037

Soumare, A., Diedhiou, A. G., Thuita, M., & Hafidi, M. (2020). Exploiting Biological Nitrogen Fixation : A Route. *Plants*, 1–22.

Soystats. (2018). A reference guide to important soybean facts and figures. *American Soybean Association*, 1–36.

Tahsin Sogut (2006) Rhizobium inoculation improves yield and nitrogen accumulation in soybean (*Glycine max*) cultivars better than fertiliser, New Zealand Journal of Crop and Horticultural Science, 34:2, 115-120, DOI: 10.1080/01140671.2006.9514395

Tamimie, C. A. (2017). Determinants of soybean adoption and performance in northern Ghana (Master's thesis). Graduate College of the University of Illinois at Urbana-Champaign. Retrieved from https://www.ideals.illinois.edu/bitstream/handle/2142/97450/TAMIMIE-THESIS-2017.pdf? Sequence=1&isAllowed=y

, D. F. M., Quansah, G. W., Adjei, E. O., & Fening, J. O. (2017). New Fertilizer Recommendations for Ghana. *Research Brief*, *1*(003), 1–3.

Tefera, H. (2011). Breeding for promiscuous soybeans at IITA. In A. Sudaric (Ed.), Soybean molecular aspects of breeding (pp. 147–162). Rijeka, Croatia: InTech.

Thomas, G.W. 1996. Soil pH and soil acidity. p. 475-490. In: Methods of Soil Analysis, Part 3—Chemical Methods. Soil Science Society of America, Madison, WI, USA.

Toleikiene, M., Slepetys, J., Sarunaite, L., Lazauskas, S., Deveikyte, I., & Kadziuliene, Z. (2021). Soybean Development and Productivity in Response to Organic Management above the Northern Boundary of Soybean Distribution in Europe. *Agronomy*, *11*(2), 214. https://doi.org/10.3390/agronomy11020214

Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, B., Giller, K., Alves, B., & Chalk, P. (2008). Measuring plant-associated nitrogen fixation in agricultural systems. *Measuring Plant-Associated Nitrogen Fxation in Agricultural Systems*, 132–188.

https://doi.org/10.1071/AR9940119

U.S. Department of Agriculture. 2019. USDA Agricultural Projections to 2028. U.S. Department of Agriculture Long-term Projections Report OCE-2019-1.

Vaccari, F., & Panza, G. F. (2001). *Abdus Salam International Centre for Theoretical Physics*. *July 1991*, 1–24.

Vanlauwe, B., Bationo, A., Chianu, J., Giller, K. E., Merckx, R., Mokwunye, U., Ohiokpehai, O., Pypers, P., Tabo, R., Shepherd, K. D., Smaling, E. M. A., Woomer, P. L., & Sanginga, N. (2010). Integrated Soil Fertility Management: Operational Definition and Consequences for Implementation and Dissemination. Outlook on Agriculture, 39(1), 17–24. https://doi.org/10.5367/000000010791169998

Vigna, C., Walp, L. J., Herridge, D. F., Atkins, C. A., Pate, J. S., & Rainbird, R. M. (1978). *Ailantoin and Allantoic Acid in the Nitrogen Economy of the*. 495–498.

Vitousek, P. M., Menge, D. N. L., Reed, S. C., & Cleveland, C. C. (2013). Biological nitrogen fixation: Rates, patterns and ecological controls in terrestrial ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *368*(1621). https://doi.org/10.1098/rstb.2013.0119

Vollmann, J. (2016). Soybean versus other food grain legumes: A critical appraisal of the United Nations International Year of Pulses 2016. *Bodenkultur*, *67*(1), 17–24. https://doi.org/10.1515/boku-2016-0002

Warembourg, F.R. and Fernandez, M.P. (1985) Distribution and remobilization of symbiotically fixed nitrogen in soybean (*Glycine max*). Physiologia Plantarum, 65, 281-286. doi:10.1111/j.1399-3054.1985.tb02396.x

Warncke, D. and J.R. Brown. 1998. Potassium and other basic cations, p. 31-33. In: Recommended Chemical Soil Test Procedures for the North Central Region. NCR Publication No. 221. Missouri Agricultural Experiment Station, Columbia, MO,

USA.

Weisany, W., & , Yaghoub Raei, K. H. A. (2013). Role of Some of Mineral Nutrients in Biological Nitrogen\nFixation. *Bulletin of Environment, Pharmacology and Life Sciences*, *2*(4), 77–84. http://www.bepls.com/march_2013.html

Welte, C. U. (2018). Revival of Archaeal Methane Microbiology. *MSystems*, *3*(2), 1–4. https://doi.org/10.1128/msystems.00181-17

Wijewardana, C., Alsajri, F. A., Irby, J. T., Krutz, L. J., Golden, B., Henry, W. B., Gao, W., & Reddy, K. R. (2019). Physiological assessment of water deficit in soybean using midday leaf water potential and spectral features. *Journal of Plant Interactions*, *14*(1), 533–543. https://doi.org/10.1080/17429145.2019.1662499

Wilker, J., Navabi, A., Rajcan, I., Marsolais, F., Hill, B., Torkamaneh, D., & Pauls, K. P. (2019). Agronomic Performance and Nitrogen Fixation of Heirloom and Conventional Dry Bean Varieties Under Low-Nitrogen Field Conditions. *Frontiers in Plant Science*, *10*(July), 1–21. https://doi.org/10.3389/fpls.2019.00952

Xu H, Zhang L, Zhang K and Ran Y (2020) Progresses, Challenges, and Prospects of Genome Editing in Soybean (*Glycine max*). Front. Plant Sci. 11:571138. doi: 10.3389/fpls.2020.571138

Yee-Shan Ku, Wan-Kin Au-Yeung, Yuk-Lin Yung, Man-Wah Li, Chao-Qing Wen, X. L. and H.-M. L., & Additional. (2013). Drought Stress and Tolerance in Soybean. *Intech*, *i*, 1–31. http://dx.doi.org/10.5772/52945

Zapata, F., Danso, S.K., Hardarson, G., & Fried, M. (1987). Time Course of Nitrogen Fixation in Field-Grown Soybean Using Nitrogen-15 Methodology1. *Agronomy Journal*, *79*, 172-176.

Zhao, W., Liu, L., Shen, Q., Yang, J., Han, X., Tian, F., & Wu, J. (2020). Effects of Water Stress on Photosynthesis, Yield, and Water Use Efficiency in Winter

Wheat. *Water*, *12*(8), 2127. https://doi.org/10.3390/w12082127

Zheng, S. H., Maeda, A., & Fukuyama, M. (2003). Genotypic and Environmental Variation of Lag Period of Pod Growth in Soybean. *Plant Production Science*, *6*(4), 243–246. https://doi.org/10.1626/pps.6.243

Zhou, H., Yao, X., Zhao, Q., Zhang, W., Zhang, B., & Xie, F. (2019). Rapid Effect of Nitrogen Supply for Soybean at the Beginning Flowering Stage on Biomass and Sucrose Metabolism. *Scientific Reports*, *9*(1), 1–13. https://doi.org/10.1038/s41598-019-52043-6

APPENDIX

ANALYSIS OF VARIANCE (ANOVA)

Appendix 1 : ANOVA table for days to 50% flowering

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	13.04	6.52	12.06	
Rep*Block stratum	12	101.44	8.453	15.630	
Treatment	24	408.53	17.022	31.48	<0.001
Residual	36	19.466	0.540		
Total	74	542.480			
LSD (5%)	1.217				
CV (%)	1.5				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 2 : ANOVA table for plant height at maturity

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	153.84	76.92	2.42	
Rep*Block stratum	12	428.23	35.69	1.12	
Treatment	24	877.65	36.57	1.15	0.346
Residual	36	1145.94	31.83		
Total	74	2605.67			
LSD (5%)	9.434				
CV (%)	11.6				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 3 : ANOVA table for grain yield

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	95785	47893	0.85	
Rep*Block stratum	12	873978	72831	1.29	
Treatment	24	72831	108698	1.92	0.037
Residual	36	2038370	56621		
Total	74	5616874			
LSD (5%)	394.03				
CV (%)	21.1				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 4: ANOVA table for nodule dry weight

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	0.136	0.068	1.10	
Rep*Block stratum	12	0.157	0.013	0.21	
Treatment	24	1.012	0.042	0.68	0.839
Residual	36	2.237	0.062		
Total	74	3.54			
LSD (5%)	0.4128				
CV (%)	64.9				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 5 : ANOVA table for nodule per plant

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	1322.9	661.5	1.22	
Rep*Block stratum	12	3020.5	251.7	0.47	
Treatment	24	8096.3	337.3	0.62	0.886
Residual	36	19465.7	540.7		
Total	74	31905.4			
LSD (5%)	38.51				
CV (%)	62.5				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 6: ANOVA table for pod clearance

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	12.467	6.234	1.74	
Rep*Block stratum	12	76.012	6.334	1.77	
Treatment	24	98.083	4.087	1.14	0.355
Residual	36	129.177	3.588		
Total	74	315.739			
LSD (5%)	3.137				
CV (%)	28.0				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 7 : ANOVA table for pod per plant

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	250.4	125.2	0.61	
Rep*Block stratum	12	3554.3	296.2	1.44	
Treatment	24	5552.7	231.4	1.13	0.366
Residual	36	7394.9	205.4		
Total	74	16752.3			
LSD (5%)	23.73				
CV (%)	21.4				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 8 : ANOVA table for shattering score

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	0.320	0.16	0.37	
Rep*Block stratum	12	9.760	0.813	1.89	
Treatment	24	29.333	1.222	2.84	0.002
Residual	36	15.466	0.429		
Total	74	54.880			
LSD (5%)	1.085				
CV (%)	33.4				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 9 : ANOVA table for leaf area index

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	0.7042	0.3521	1.64	
Rep*Block stratum	12	3.4529	0.2877	1.34	
Treatment	24	1.8661	0.0778	0.36	0.995
Residual	36	7.7385	0.2150		
Total	74	13.761			
LSD (5%)	0.767				
CV (%)	22.4				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 10 : ANOVA table for photosynthesis rate

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	11.045	5.523	4.34	
Rep*Block stratum	12	48.967	4.081	3.21	
Treatment	24	155.127	6.464	5.09	<.001
Residual	36	45.759	1.271		
Total	74	260.898			
LSD (5%)	1.867				
CV (%)	5.0				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 11 : ANOVA table for stomata conductance

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	0.039	0.019	2.07	
Rep*Block stratum	12	0.188	0.015	1.63	
Treatment	24	0.361	0.015	1.56	0.111
Residual	36	0.347	0.009		
Total	74	0.937			
LSD (5%)	0.162				
CV (%)	13.7				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 12 : ANOVA table for weight biomass weight

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	3887435	1943717	5.02	
Rep*Block stratum	12	18145280	1512107	3.91	
Treatment	24	28983040	1207627	3.12	0.001
Residual	36	13926080	386836		
Total	74	64941835			
LSD (5%)	1029.9				
CV (%)	10.3				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.

Appendix 13 : ANOVA table for nitrogen fixed

Source of variation	d.f	s.s	m.s	v.r	F.pr.
Rep stratum	2	4367.2	2183.6	2.81	
Rep*Block stratum	12	38990.8	3249.2	4.18	
Treatment	24	322923.7	13455.2	17.31	<.001
Residual	36	27980.7	777.2		
Total	74	394262.4			
LSD (5%)	46.17				
CV (%)	22.2				

Note: df = degrees of freedom, ss = sums of squares, ms = mean squares, LSD (5%) = least significant different at 5% level of significant, CV (%) = coefficient of variation in percent, F.pr = fisher probability value.