

**GENETIC VARIABILITY STUDIES OF SOME NIGERIAN SOYBEAN (GLYCINE MAX
(L) MERRIL) ACCESSIONS**

BY

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ABSTRACT

Soybean is a very important leguminous seed crop; known for its highly valued protein and oil owing to its use in food, feed and industrial applications. The cultivation of soybean could be of great impact in curbing food insecurity and its improvement could be of great benefit to humanity. Genetic variability is the basic requirement for crop improvement as it provides wider scope for selection. Selection of traits through morphological and molecular means increases the frequency for favourable alleles which can be explored in breeding programme for any crop. Therefore, study on genetic variability among soybean was undertaken to evaluate the genetic variation among some accessions of soybean for effective selection, utilization and improvement. Twenty accessions of soybeans were collected from the gene bank of International Institute of Tropical Agriculture (IITA), Ibadan. The accessions were evaluated for agro- morphological parameters using randomized complete block design (RCBD) with three replicate. Soybean accessions were further evaluated for genetic diversity using simple sequence repeat (SSR) marker, pollen parameters was also used to characterize the accessions. There are significant ($P < 0.05$) wide ranges of variability observed in all the morphological characters assessed, with specific accessions being favoured by different trait(s). Accessions TGX-2016-4E recorded the highest plant height (46.17cm), TGX-1485-1D recorded highest pod production (216.47) and TGX-2027-1E recorded the highest seed yield per plant (1167.67). Wide variability was also observed for qualitative traits such as leaflet size, leaflet shape, pubescence, pubescence density, pubescence colour, pubescence type, cotyledon colour, corolla colour, seed shattering and mature pod colour. The phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the traits. Moderate to high broad sense heritability ranging from 32.99 to 95.37 was obtained and the genetic advance as percentage mean ranges from 0.16%-58.89%. Most of the accessions exhibit high pollen viability up to 84.60%; except TGX-2010-11F

which had 26.70%. Molecular diversity of 11 selected genotypes from the initial twenty accessions using simple sequence repeat (SSR) DNA marker generated 150-320 base pair with six primers. Genetic similarity among the genotypes varies from 0.17-4.09 with an average gene diversity of 0.28. Clustered dendrogram of the 11 genotypes revealed two major clade (TGX- 1987-62F and TGX-2011-6F). The high genetic variability obtained for both morphological and molecular characterizations coupled with high heritability and genetic advance in most parameters studied indicate that the genotypes could be selected for those heritable traits and used as tool for crop improvement.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Soybean [*Glycine max* (L) Merrill] is a member of Papilionancae family and believed to have originated in North Eastern China and distributed in Asia, USA, Brazil and Argentina (Chandrawat *et al.*, 2017). The crop has a fairly wide range of climatic condition for adaptation and mostly cultivated on rain fed land (Chandrawat *et al.*, 2017). Soybean is aptly called as “Golden bean” or “Miracle crop” of the 20th century and is one of the most important oil seed crop in the world (Adoloju *et al.*, 2009) followed by cotton, sunflower canola, palm oil and peanut. It is also very important crop for rotation with cereals like maize and sorghum because of biological nitrogen fixation that is important in improving soil fertility (Abush *et al.*, 2017) and is considered a strategic crop in fighting the world’s food shortage and malnutrition problems and most food aids to displaced and malnourished people are fortified with soybean (Abush *et al.*, 2017).

Soybean is grown in many parts of the world and is a primary source of vegetable oil and protein used in food, feed and industrial applications (Endres, 2001). Soybean was domesticated in the Eleventh (11th) century BC around northeast China (Hymowitz and Shurtleff, 2005). It may have been introduced to Africa in the Nineteenth (19th) century by Chinese traders along the east coast of Africa (Giller and Dashiell, 2006). Reports indicate that soybean was cultivated in Tanzania in 1907 and Malawi in 1909 (Giller and Dashiell, 2006). African countries with the largest area of production are Nigeria (650,000 ha), South Africa (245,000 ha), Uganda (147,000 ha), Malawi (79,480 ha), and Zimbabwe (69,900 ha)

(Ishaq and Ehirim, 2014).

Nigeria is the largest producer of soybeans in Africa (Agada, 2015). Soybeans were first introduced into Nigeria in 1908 (Fennel, 1966) but the first successful cultivation was in 1937 with the Malayan variety which was found suitable for commercial production in Benue State (Oyekan, 1985). The producing areas of Central Nigeria have been responsible for a large proportion of the domestic requirement for this cheap source of plant protein. Today, soybean has made a successful incursion into diet of many Nigerians, particularly children and nursing mothers.

Nutritional value of soybean lies in protein (40-42 %) and oil content (18-22 %) thereby containing twice the protein of meat or poultry and contain all eight essential amino acids needed for childhood development (Malik *et al.*, 2006). In Nigeria markets, soybean cost about one-fifth as much as other forms of protein, including dairy and fish and are easier to store and transport. They also fix atmospheric nitrogen which reduces the need for farmers to purchase fertilizers. Soybean is also medicinal and is extremely useful for treatment of malnutrition, particularly among children and fight against disease such as heart disease, cancer diabetes, high blood pressure, stroke, ulcer as well as loss of body mass among people living with HIV/AIDS (Agada, 2015). Concerted efforts are greatly needed towards improvement of this crop due to its high demand lately. Unfortunately, many factors, both biotic and abiotic, heavily hinder the production of this crop in Nigeria as a whole. Thus, there is need to further assess the available land races in order to exploit some hidden genetic variability for improvement of the crop in the future.

Genetic variability is the basic requirement for crop improvement as this provides wider scope for selection (Pushpa and Ketoswara, 2013). Thus effectiveness of selection is dependent upon

the nature, extent and magnitude of genetic variability present in material and extent to which it is heritable. The success of any crop improvement depends on the nature and magnitude of genetic variability present in the crop along with in-depth understanding of the underlying gene action and genetic architecture of traits related to yield. The knowledge of nature and magnitude of genetic variability is of immense value for planning efficient breeding programme to improve the yield potentials of genotypes (Pushpa and Ketoswara, 2013). The role of genetic diversity for crop improvement programmes has been emphasized by Sujata and Basavaraja (2011). Genetic divergence among parents is essential since the crossing programme involving diverse parents is likely to produce high heterotic effects and also more variability could be expected in the segregating generations.

1.2 Statement of the Research Problem

Despite the economic importance of soybean as source of oil and other pulses, the crop has been facing some challenges of agronomic inferiority particularly among accessions compared to commercial cultivars. Inadequate knowledge of the scope, nature of the alleles and their relationship with those already introgressed into commercial cultivars has been reported to be limiting its genetic diversity (Kaudzu *et al.*, 2017). In Nigeria, the genetic diversity of this crop has not been fully studied and poorly understood, as well as improper documentation of research finding for exploitation in its breeding programme, thus narrowing its genetic base. Major focus on genetic diversity on soybean has been on Random Amplified Polymorphic DNA and Restriction Fragment Length Polymorphism, limited works have been done using Simple Sequence Repeat marker to determine genetic

variability among the accessions. Proper attention is yet to be given to pollen characteristics, such as pollen viability, as important trait in the characterization of soybean genotypes.

1.3 Justification for the Study

Soybean is a very important leguminous seed crop; known for its highly valued protein and oil owing to its use in food, feed and industrial application. It enriches the soil by fixing nitrogen in symbiosis with bacteria and is ranked number one in the world among the major oil crops (Rajkumar *et al.*, 2010). The cultivation of soybean will be of great impact in curbing food insecurity and its improvement will be of great benefit to humanity. Before planning any breeding programme assessment of genetic variability is a necessary step (Bhagasara *et al.*, 2017). Soybean exhibits genetic variability for agro morphological traits and yield components. Morphological traits can be used to assess phenotypic variation in growing environment and are also used as tool for the indirect analysis of genetic variability and diversity (Ravindra *et al.*, 2017). Thus for soybean to assume this prominence, the genetic amelioration work will pay a key role. The development of superior varieties is based on presence and extent of genetic variability (Rajkumar *et al.*, 2010).

Genetic parameter estimates have been recognized as veritable tool for selecting important traits in various crops. Suliystyo and Majeya (2018) opined that any character with high heritability value and genetic advance as percentage of mean (GAM) is a good character to be selected. Pollen Viability has over time been recongnised as a source of high yield and gene for hybridization. Thus according to Olaoye *et al.* (2014), high viability of pollen usually results in high yield.

Molecular characterization of the crop will provide better understanding of the selective impacts of breeding practices as well as broaden the genetic base of the crop for its maximum utilization (Tantaswat *et al.*, 2011). Among different DNA markers, Restriction fragment length polymorphism (RFLPs), Random Amplified polymorphic DNA (RAPDs), Amplified fragment length polymorphism (AFLPs), Single Nucleotide polymorphism (SNPs) and simple sequence repeat (SSRs) have been used extensively in soybean. SSR have been shown to produce the highest polymorphism compared to RFLPs, AFLPs and RAPDs and have much greater ability to identify unique alleles in elite and parental soybean germplasm than other markers (Tantaswat *et al.*, 2011). Thus it is on these premises that this research is set in order to achieve the goal, which will eventually lead to improvement of the crop in the future.

1.4 Aims and Objective of Study

The aim of this study is to evaluate the genetic variation among accessions of soybean for effective selection, utilization and improvement.

The objectives were to:

- i. determine the morphological variation among some accessions of soybean.
- ii. identify appropriate traits of soybean for effective selection.
- iii. determine the variation in some pollen parameter among the accessions
- iv. determine the genetic diversity among some accession of soybean using SSR

Molecular markers.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Origin and Domestication of Soybean (*Glycine max*)

Soybean is a leguminous vegetable of the pea family that grows in tropical, sub-tropical and temperate climates. They are crucial crop in eastern Asia, and also remains a major crop in China, Japan and Korea. Soybean is perhaps the world oldest food crop and for centuries; nutritionally speaking, they provide meat, milk, cheese, bread and oil for the people of Asia.

The first domestication of soybean has being traced to the Eastern half of North China in the Eleventh Century B.C or perhaps a bit earlier (Herbert *et al.*, 2009). Soybeans were first introduced into Europe in the early 18th century and to the British colonies in North America in 1765 where it was first grown for hay. Soybeans were introduced to Africa from China in the late 19th century. It is now wide spread across the continent. The crop was introduced into Nigeria in 1908 (Fernel, 1966). In Nigeria, most soybean product occurs in the middle belt zone consisting of Benue states and the adjoining states where small scale farmers have being growing soybeans for about 50years (Odeleye *et al.*, 2007). The total annual product of soybean was estimated at 500,000 mt from 650,000 hectares in Nigeria (FAO, 2017).

The wild ancestors of the soybeans are *Glycine soja* previously called *G. ussuriensis*, a legume native to Central China. According to the ancient Chinese myth in 2853B.C. The legendary emperor Shennong of Central China proclaimed that five plants were scared; soybeans, rice, wheat, barley and millet. Cultivation of soybean was long confined chiefly to East Asia, but gradually spread to other countries during the 20th century (Herbert *et al.*, 2009).

Soybean is commonly considered one of the oldest cultivated crops, native to North and Central China (Hymowitz, 1998). The first recording of soybean was in a series of book known as *Pen Ts'ao Kong Mu* written by the emperor Sheng Nung in the year 2838 B.C. in which various plants in China are described. Historical and geographical evidence suggests that soybeans were first domesticated in the eastern half of China between 17th and 11th century B.C (Hymowitz, 1998).

Soybeans were introduced into several countries such as India, Japan, Indonesia, the Philippines, Vietnam, Thailand, Cambodia, Malaysia, Burma, Taiwan and Nepal. Soybean was first introduced into the United States in 1765 by Samuel Bowen, a sailor who had visited China (Hymowitz and Harlan, 2004), into Canada in 1893 where production began in Ontario as a hay crop. Soybeans first arrived in South America in Argentina in 1882 (Shurtleff and Aoyagi, 2012). The plant arrives in Africa in Egypt in 1857 (Shurtleff and Aoyagi, 2012). Wild soybean was discovered in North Eastern Austria in 1970 by explorers Bank and Solander. In 1804, the first soybeans product was sold in Sydney

For many years, soybean acreage increased slowly. There were only 1.8 million acres in the United States in 1924, when the first estimate became available at that time; most of the crop was used for hay. Before World War II, the United States imported more than 40% of its edible fat and oil. Disruption of trade route during the war resulted rapid expansion of soybean acreage in the United States as the country looked for alternatives to these imports. Soybean was successful because there was immediate need for soybean oil and meal.

Following World War II, soybean production moved from the Southern United States into the Corn Belt. The major soybean producing states of Iowa, Illinois, Minnesota, Indiana, Ohio,

Missouri and Nebraska produced 67% of its total in 2003; the Southern and Southeastern States of Arkansas, Mississippi, North Carolina, Kentucky, Alabama and Georgia produced 14% (Doyle, 2006). A record of 2.9 million bushel soybean crop was produced in 2001 on 74.1 acres with an average per acre yield of 39.6 bushel. The leading soybean states are Iowa and Illinois. In 2003, Iowa had 10.6million acres of soybeans while Illinois had 10.3 million. The highest state yield ever achieved was 505 bushel per acre produced by Iowa farmers in 1994 (FAO, 2008).

Soybean nitrogen requirement are more in a complex manner as the crop is capable of utilizing both soil Nitrogen and atmospheric Nitrogen through Nitrogen fixation (Milic *et al.*, 2003). The relative Nitrogen supply from this source can change widely depending on soil Nitrogen supply (Varco, 1999; Gan *et al.*, 2002). Nitrogen is one of the most important nutrient elements affecting the yield of soybean (Penal and Wiese, 1987).

2.2 Classification of Soybeans

According to Merrill (1972) soybeans can be classified as

Kingdom	Plantae
Order	Fabales
Family	Fabaceae
Sub-family	Faboideae
Genus	<i>Glycine</i>
Species	<i>G. max</i>

2.3 Morphology and Biology of Soybeans

Soybean varies in growth and habit, the height of the plant varies from less than 0.2 to 2.0 m (0.66 to 6.6 feet). The pods stem and leaves are covered with fine brown or gray hair. The primary leaves are unifoliate, opposite and ovate. The secondary leaves are trifoliate, alternate and compound leaves with four or more leaflet per leaf and the leaflet are 6 – 15 cm (2.4- 5.9 inc) long and 2-7 cm (0.79-2.8 inc) broad. The leaves fall before the seeds are matured. The nodulated root system consists of tap root from which emerges a lateral root system (Acquaah, 2007). The inconspicuous self- fertile flower are borne in the axil of the leaf and are white, pink, or purple in colour, the flower consist of a tubular calyx of five (5) sepals, corolla of five (5) petal, one pistil and nine (9) fused stamen with a single posterior stamen (Acquaah, 2007).

The fruit is a hairy pod that grows in cluster of three (3) to five (5), the pod is straight or slightly curved, varies in length from three to eight (3-8 cm)long and usually contain two to four seed of about 5-11 mm in diameter (Purcell *et al.*,2014). The shape of the seed is usually oval and can vary amongst cultivars from almost spherical to elongated and flattened, seed coat colour including black, brown, blue, yellow, green and mottled. The hulls of the matured bean are hard, water resistance and protect the cotyledon from damage. If the seed coat is cracked, the seed will not germinate. The scar visible on the seed coat is called the hilum and at one end of the hilum is the micropyle; a small opening in the seed coat which can allow the absorption of water for sprouting (Acquaah, 2007).

Seeds such as soybeans contain very high level of protein, can undergo desiccation, yet survive and revive after water absorption. It was found that soybean and corn have a range of soluble carbohydrates protecting the seed viability (Blackman *et al.*, 2007).

2.4 Growth and Development of Soybeans

Growth, development and yield of soybean are all result of a given variety's genetic potential interacting with its environment. In field situation, nature provides the major portion of the environmental influence on soybeans development and yield (Lobell and Field, 2014).

Soybean varieties are classified for their morphological (form and structure) growth habit and for their day length and temperature requirement to initiate floral reproductive development. The indeterminate growth is typical of most Corn Belt soybean varieties and is characterized by continuation of vegetative growth after flowering begins. Determinate soybean varieties characteristically have finished most of their vegetative growth when flowering begins and are typically grown in the Southern United States. (Sebatha *et al.*, 2018).

The classification for maturity is based upon the adaptability of a soybean variety to effectively utilize the growing season in a given region. An early maturing variety may develop fewer leaves or progress through different stages at a faster rate especially when planted late, a late maturing variety may develop more leaves and progress more slowly (Zhang *et al.*, 2007)

The rate of plant development of a variety is directly related to temperature, so the length of time between the different stages will vary as the temperature varies both between and within

growing season. Deficiencies of nutrients, moisture or other stress condition may lengthen the time between vegetative stages but shorten the time between reproductive stages. Soybeans planted at high density tend to grow taller and produce few branches, pods and seeds per plant than those at low densities. High density soybean will also set pods higher off the ground and have a greater tendency to lodge (Suhre *et al.*, 2014)

Planted soybeans begin germination by absorbing water in amount equal to about 50% of its weight. Emergence typically occurs one to two weeks after planting depending on soil moisture, temperature and planting depth (Purcell *et al.*, 2014). Soybean root normally become infected with *Rhizobium japonicum* bacteria which causes formation of round or oval shape root growth termed nodules. Millions of these bacteria are located within each nodule and provide much of soybean plant nutrient supply through a process called Nitrogen fixation. Through nitrogen fixation, the bacteria changes non available Nitrogen gas from the air into nitrogen product that the soybeans can use. The plants in turns provide the bacteria carbohydrate supply. Such a relationship is called symbiotic relationship. Nodulated fixing nitrogen for plants appears pink or red on the inside nut white, brown or green if nitrogen fixation is not occurring (Deacon, 2015).

A soybean plant grown without competition from other plant will branch profusely and develop into a large plant increasing the number of plant in a given area (plant density), increases plant height and lodging tendency, reducing branching and pod number per plant but allow more pods and bean per unit up to an optimum plant density is different for different varieties and environment (Hanway and Thompson, 1967).

The environment in which soybean grows is extremely influential upon the plant development and yield. Environmental stress occurring at any stage of soybean development will reduce yield. Stress such as nutrient deficiencies, inadequate moisture, frost damage, hull damage, insect damage or lodging causes great yield reduction. Highest yield are obtained only where environment conditions are favorable at all stages of growth.

2.5 Chemical Composition and Nutrition of *Glycine Max*

Protein and soybean oil content account for 56 % of dry soybeans by weight, 36 % protein, 20 % fat, 30 % carbohydrate, 9 % water and 5 % ash. Soybeans comprise approximately 8 % seed coat or hull, 90 % cotyledons and 2 % hypocotyl axis or germ (Corke *et al.*, 2004).

2.6 Nutrition

Soybean is a source of complete protein (Henkel, 2009). A complete protein is one that contain significant amount of all essential amino acid that must be provided to the human body because of the body inability to synthesize them. Soy protein product can be good substitute for animal product because unlike some other beans, soy offers a complete protein profile. Soy protein product can replace animal based foods which also have complete protein but tends to contain more fat especially saturated fat without requiring major adjustment elsewhere in the diet (Henkel, 2009).

Soybeans protein is the nutritional equivalent of meat, egg and casein for human growth and health. Soybeans protein isolate have a biological value of 74, whole soybean 96, soybean milk 91 and egg 97 (Smith and Smith, 2014).

Soy protein is essentially identical to protein of other legume seeds and pulse (Derbyshire *et al.*, 2006). Moreover, soybeans can produce at least twice as much protein per acre than any other major vegetable. 5-10 times more protein per acre than land set aside for grazing animal to make milk and up to 15 times more protein per acre of land set aside for meat production. Consumption of soybean may also reduce the risk of colon cancer possibly due to the sphingo lipids (Symolon *et al.*, 2004).

2.7 Ecology of Soybeans

2.7.1 Ecological requirements

Climate conditions: Soybean, an important global crop providing oil and protein is successful in climate with hot summers and temperature of 20°C to 30°C (68°F to 86°F). Temperature of below 20°C and over 40°C (68°F, 104°F), retards growth significantly (Fargione *et al.*, 2008).

Soil: Soybean can grow in wide range of soil with optimum growth in most alluvial soil with a good organic content. It requires well drained sandy loam or sandy soil with pH of 6.0- 6.5. Soybean soil must contain the proper nitrogen fixing bacteria. Soybean will grow better than many crops on soil that is low in fertility, drought or poorly drained (Jeong- Dong *et al.*, 2018).

2.7.2 Cultural practices

Seed bed preparation: Soils should be cultivated deeply enough to ensure that no barrier to penetration of taproot (such as hard pan) exists. Soybean may be adversely affected by soil crushing under certain soil and environmental conditions

Planting: Soybean should not be planted until soil temperature is consistently above 68⁰F and soil moisture is adequate for germination and growth. Seed will decay in cool, wet soils.

Fertility and lime requirement: Soybean like all legume, forms a symbiotic relationship with a soil bacterium called *Rhizobium japonicum*. *Rhizobium japonicum* causes formation of round or oval shaped root growth termed nodules. This bacterium found on the nodules provides much of soybean plant's nitrogen supply through a process called Nitrogen fixation. Through fixation the bacteria changes N₂ gas from air into nitrogen product for soybean use and the plant in turns provide the bacteria with carbohydrate supply. Excess nitrogen promotes vegetative growth, delays maturity, may reduce seed yield and may suppress nitrogen fixation.

Weed control: Adequate weed control is necessary for good growth and high yields. There are various methods for the control of weed in soybean family namely:

Mechanical method: This involves the use of hoe and row cultivators, light and any other mechanical means of controlling weeds.

Chemical Method: Involves the use of herbicides

2.8 Pest and Diseases of Soybeans

The insects known to attack soybeans include corn earthworm, Mexican bean, beetle, caterpillar etc. Occurrence prevalence and rate of reproduction of soybean insect vary greatly from one part of the country to the other. All insects can be controlled by timely dusting or spraying with proper insecticides. Various disease caused by fungi, bacteria, virus and nematodes affect both the stem and leaves and also the root of soybean. They include:

1. **Bacterial blight:** This disease is caused by *Bacterium pseudomonas*; initial symptoms are small angular water soaked spot on leaves. Lesion centers dry out and turn brown to black with water soak margin and yellow halos. Lesion may coalesce resulting in large blighted area. Affected tissue often drops out, giving a tattered appearance to the leaves.

Control: Avoid cultivation when foliage is wet and crop rotation practices should be carried out.

2. **Brown spot:** This disease is caused by fungus *Septoriaglycines*. Symptoms are irregular light brown lesions, ranging in size from small speck to a few mm in diameter. Lesion eventually darkens to brownish black. Lesions are primarily found on leaves but can also occur on stem, petioles and pods.

Control: The use of foliar fungicide, plow under crop residue and crop rotation should be carried out.

3. **Soybean cyst nematodes:** This disease is caused by *Heterodera glycine*. It is associated with slight to severe stunting with slight to severe chlorosis. Gradual yield decline over several years. Decreased nodulation and canopy slow to close.

Control: By crop rotation for two or more years with susceptible crop, good weed control. Avoid moving infested soil with equipment or seed.

4. **Charcoal root rot:** Caused by *Macrophominaphaseolina*. It is associated with loss of vigor in mature plant; leaves turn yellow and wilt but remain attached, light, and gray or sliver discoloration in tap root and lower after flowering.

Control: By crop rotation, adequate fertilization, irrigate to keep soil moisture high.

5. **Soybean mosaic virus:** Soybean mosaic virus is accompanied by stunting, mottling, curling of leaves. Leaves may be puckered and misshapen, resembling 2, 4-D injury. Diseased pods may be stunted and curved. Seed from diseased pods may be discolored and causes significant yield losses.

Control: Planting virus free soybean seed, remove symptomatic plant from seed production field.

2.9 Harvesting

Nearly 95 million hectares of soybean were harvested worldwide in 2007 with 19 million in Asia, 8.5 million In USA and 1.2 million in Africa. All seeds on soybean plant mature at essentially the same time which is accompanied by a rapid dropping of leaves and drying of stem. Harvesting loss can amount to 10 – 20 % of the crop during combining. Depending on the variety soybean can be harvested between 100- 150 days after planting. Labour requirement in Africa are highly stressful since harvesting are done manually. Soybean require clear, dry bin for storage

2.10 Uses of Soybeans

Approximately 85% of the world's soybean crop meal and vegetable oil (Lusas *et al.*, 2012). Soybean can be broadly classified as vegetable or field types. The bulk of soybean crop is grown for oil production with high protein defatted and toasted soymeal used as livestock feed. A smaller percentage of soybeans were used directly for human consumption. Soybean oil both liquid and partially hydrogenated is exported abroad, sold as vegetable oil or end up

in a wide variety of processed food (Edmund, 1995). Soybean meal is an essential element of the America production method of growing farm animals such as poultry and swine.

Soy flour has 50 % protein, 5 % fiber and do not contain gluten (Lim, 2012). It thickens sauces, prevents staling in baked food and reduces oil absorption during frying. Baked food with soy flour gives it tenderness, moistness, a rich colour and fine texture. Soybean are primary ingredient in many diary product substitute e.g. soy milk, soy ice cream, soy yoghurt, soy cheese etc. and meat substitute e.g. veggie burgers. Soybeans are used in industrial product such as oil, soap, cosmetics, resins, plastics, ink, crayon, solvent and clothing. Soybean oil is primary source of biodiesel in the United States. Consumption of soybean reduces prostate cancer risk in men, breast cancer in women, decreased risk of death and also act as oxidant and chelating agent (Mahumoud *et al.*, 2004).

Soy protein is increasingly found in fish food both for home aquarium and for fish growing for eating. Most marine species were feed fish meal at one time but the scarcity and increasing cost of fish meal has lead producers to switch to high protein soymeal for a variety of marine species. Soy oil provides an environmentally friendly solvent; safely and rapidly remove oil from creeks, streams and shorelines without harming people, animals and the environment (Kissinger *et al.*, 2016)

2.11 Genetic Variability in Crop Breeding

Variability is the occurrence of differences among individuals due to differences in their genetic composition and/or the environment in which they are raised (Falconer and Trudy, 1996; Allard, 1999). Genetic variability is a measure of the tendency of individual

genotypes in a population to vary from one another. Variations are simply differences in genetic sequence. The variability of a trait describes how much that trait tends to vary in response to environmental and genetic influences (Anonymous, 2011).

Crop breeding programme is highly dependent on the extent of variability present in the available accession, choice of the parents and the selection procedure. The genetic diversity and relationship can be assessed by the difference in morphological and agronomic traits, pedigree information, geographic origin, isozymes and DNA markers. However, some factors affect these methods such as the influence of environmental factors on morphological and agronomic traits, uncertain or incomplete data, possible errors in pedigree information and origin of accessions and limitation of data provided by isozymes. Morphological traits or characters reflect not only on the genetic composition of a cultivar, but also the interaction of the genotype with the environment in which it is expressed (Smith and Smith, 2014). Shadakshari *et al.* (2011) carried out genetic variability study on 12 morphological characters of 50 soybeans genotypes and observed that the trait, seed per plant and seed yield per plant contributed maximum genetic diversity. The clustering pattern revealed that there was no correlation between the geographical diversity and genetic diversity. Pusha and Ketoswara, (2013) worked on Forty five genotypes of soybean (*Glycine max* (L.) Merrill.) of diverse origin, they were evaluated for variability. Analysis of variance revealed highly significant differences among the genotypes for all the characters.

According to Suliystyo and Majeya (2018), the value of GCV and PCV is divided into 3 groups that is if GCV and PCV is < 10 % it is considered low, if between 10- 20 % is considered medium and if < 30 % is considered high, Abady *et al.* (2013), Dilanesaw *et al.*

(2013), Ghodrati *et al.* (2013) and Barh *et al.* (2014) all reported high GCV for plant height, Number of pod per plant and seed yield per plant. Ali *et al.* (2016), also reported high GCV for plant height, number of branches, seed yield per plant, likewise, Suliystyo and Mejeja (2018) recorded from their findings low GCV for plant height, number of branches, number of nodes, number of pods per plant and seed weight per plant,

Heritability and genetic advance is low if it is < 20 %, it is said to be moderate if it is between 20 %-50 % and is high if it is >50 % (Suliystyo and Majeya, 2018). Malik *et al.* (2007), Aditya *et al.* (2013) and Barh *et al.* (2014), all from their individual research also discovered high heritability for plant height, number of branches, number of internode, number of node, number of pod per plant and pod length all has moderate heritability. Ali *et al.* (2016) also from their findings reported high heritability for plant height and seed yield per plant but moderate heritability for number of pods per plant. Bakale *et al.* (2012) reported moderate GAM for days to flowering, low for days to pod production, high for plant height and moderate for seed yield per plant. Balla and Seifelden (2010) observed moderate GAM for days to flowering, low GAM for days to pod production and moderate GAM for seed yield per plant. In conclusion, these findings revealed that soybean genotypes vary from one another in terms of original genetic makeups; these have influenced the variation in virtually all the traits that have been studied so far.

2.14 Pollen Viability in Plant Breeding

Pollen viability refers to the ability of the pollen to perform its function of delivering male gamete to the embryo sac (Lankinen *et al.*, 2018). This functional property of the pollen after their release from the anther varies greatly from species to species and its quality is

assessed on the basis of its viability. According to research by Lankinen *et al.* (2018) on pollen traits of Cherry Laurel using an invitro medium containing 0 %, 5 %, 10 %, 15 % and 20 % Sucrose to determine the best sucrose concentration for germination. It was observed that the viability rates were different according to genotypes; iodine potassium iodide (IKI) and TriphenylTetrazolium chloride (TTC) staining Test were used. Pollen viability estimated with TTC staining Test was better than that estimated with IKI staining test.

2.15 Molecular Markers for Breeding

Traditionally, genetic diversity of cultivars of *Glycine max* is determined by a combination of morphological or agronomic traits and biochemical tests/assays (Chowdhury *et al.*, 2001; Dayaman *et al.*, 2009). Most commercial and released soybean cultivars arose from hybridization between members of an elite group of genotypes; hence the amount of genetic variability among those cultivars is small (Chowdhury *et al.*, 2001). Such cultivars are often indistinguishable based on agro morphological traits or biochemical tests which are often subjected to environmental influence interplaying with a number of genes and thus may not represent genetic divergence in the entire genome (Diwan and Cregan, 1997; Brown-Guidira *et al.*, 2000). A large number of polymorphic markers are required to measure genetic relationships and genetic diversity; as a result, it is now widely accepted that information generated from DNA-based analyses using Restricted Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPDs), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) alone, or with morphological analyses provide the best estimate of genetic diversity (Chowdhury *et al.*,

2001).

2.16 Simple Sequence Repeats (SSR) as a Molecular Marker

Microsatellites also known as simple sequence repeats (SSR) consist of tandemly repeated, short DNA sequence motifs (Maughan *et al.*, 1995). The popularity of microsatellites stems from a unique combination of several important advantages; they are codominant markers, have high genomic abundance in a population, and random distribution throughout the genome (Morgante *et al.*, 2002). They exhibit allelic diversity. Their reproducibility is much higher than RAPDs (Demeke *et al.*, 1997; Karp *et al.*, 1997). The flanking sequences of microsatellites are usually highly conserved, making it possible to design universal primers for their study across genomes (Akkaya *et al.*, 1992; Diwan and Cregan, 1997). Although microsatellites are very useful in general, they also have certain disadvantages; including relatively high cost of marker development, occasional occurrence of artifacts, such as stutter bands (Walsh *et al.*, 1996). In general, microsatellites show a high level of polymorphism, so they are very informative markers. They can be used for population genetic studies and gene mapping, ranging from the individual level (e.g. clone and strain identification) to that of closely related species (Jarne and Lagoda, 1996).

2.17 Using SSR Markers to Assessing Genetic Diversity in Soybean.

Molecular markers are frequently used in the analysis of soybean germplasm. Simple sequence repeats markers have been shown to be highly polymorphic in soybean (Akkaya *et al.*, 1992; Diwan and Cregan, 1997). The analysis of the polymorphism in DNA sequences allow for a more accurate genetic characterization. Abe *et al.* (2003) used 20 SSR loci in 131 accessions of soybeans introduced from 14 Asian countries to detect

genetic diversity among them. Morgante and Olivieri (1994) detected similar levels of polymorphism in seven SSR loci in a group of soybean genotypes. Akkaya *et al.* (1992) used several types of SSRs to analyze the diversity of 43 soybean genotypes including ancestral and domestic cultivars representing the northern and southern U.S. germplasm. Doldi *et al.* (1997) found two to six alleles per 14 loci in a group of 18 soybean cultivars using 12 microsatellite loci. Diwan and Cregan (1997) observed an average of 10.1 alleles per locus in a total of 20 loci studied in soybean genotypes that represented 95% of all alleles of the germplasm cultivated in the north of the United States. In a study on 186 Brazilian soybean cultivars, Priolli *et al.* (2002) found four to eight alleles per loci using 12 SSR loci studied. They determined that SSR with (AT) and (ATT) repeat motifs were highly polymorphic in soybean and identified up to eight alleles at each locus. Rongwen *et al.* (1995) identified 11 to 26 alleles at each of seven SSR loci in a diverse sample of soybean genotypes that included U.S. cultivars, *G. max* and *G. soja* plant introductions and Chinese landraces. Maughan *et al.* (1995) detected 79 alleles across five SSR loci in a sample of 94 soybean accessions of *G. max* and *G. soja* genotypes. Tantasawat *et al.* (2011) used 11 SSR primers to analyse genetic relationships among 25 soybean genotypes. They reported that genetic similarity between genotypes and that the 25 genotypes formed four major clusters. Singh *et al.* (2010) also reported a cluster analysis based on coefficient of similarity classified 44 soybean genotypes into four major clusters derived from 120 SSR makers. Dayamann *et al.* (2009) used 11 SSR markers to analyze genetic diversity of 45 soybean genotypes and these accessions were grouped into 14 different clusters.

CHAPTER THREE

3.0

MATERIALS AND METHODS

3.1 Seed Collection

The accessions of soybean used, were collected from the gene bank of International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State.

3.2 Experimental Design and Planting

Evaluation of the collected twenty accessions of soybean for agronomic trait was carried out at the experimental garden Department of Plant Biology, Federal University of Technology, Minna Nigeria. The seeds of each accession were sown in the field at the depth of 1-2 cm in a complete randomized block design (CRBD) with 3 replicates each. The seeds were sown at inter and intra - row spacing of 15 cm × 15 cm, plant to plant with five seeds per stand. At two (2) weeks after germination, the seedlings were thinned to two plants per stand.

3.3 Morphological Parameter

Morphological parameters evaluation of the accessions for seed characters such as coat colour, coat pattern, helium colour, coat surface lustre and cotyledon colour was done by physical examination of 30 randomly selected seeds from each accession. Characterization of the seedlings for number of leaflet, leaflet shape, pubescence, pubescence density, pubescence colour, pubescence type, corolla colour, mature pod colour, and leaf length size was done using standard methods as described in soybean descriptor guide by (IBPGR, 1992). Other agro- morphological parameters determined were according to Varnica *et al.*

(2018) and include:

3.3.1 Plant height: The plant height was measured using meter rule from the base to the tip of the plant in centimeters until maturity at two weeks interval

3.3.2 Number of leaves: The number of leaves of the tagged plant were counted and recorded at 2 weeks interval until maturity.

3.3.3 Number of branches: The numbers of branches per plant arising from the main stem were counted at maturity.

3.3.4 Collar diameter. The collar diameter per plant was measured at two weeks interval using stem meter gauge.

3.3.5 Days to 50 % flowering: The number of days taken from sowing to 50% of the plant to flower was recorded as days to flowering per plant.

3.3.6 Number of pods per plant: The number of pods per plant was counted at harvest.

3.3.7 Number of seed per plant. The numbers of seeds per plant were counted after harvesting.

3.3.8 100 seed weight. One hundred randomly selected seeds were taken from the bulk of the seed yield and the weight measured in grams.

3.3.9 Seed yield per plant. The number of seeds per accession was counted after harvesting

3.4 Pollen grain viability test:

Pollen grain viability test was carried out by collecting ten matured flowers whose anthers have not dehisced from each accession. The pollen grains of each flower were dusted on clean glass slide one after another and stained with 2 % aceto-carmin solution. Each slide was carefully covered with cover slip and observed under light microscope. Properly stained pollen grains containing nuclei were regarded as being viable and those that appeared empty and/ or slightly stained were considered as non-viable (Daudu *et al.*, 2017). Percentage of pollen grain

viability was calculated as the proportion of the grains that absorbed the stained to the total count using the equation below:

$$\text{Pollen viability (\%)} = \frac{\text{Number of stained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

$$\text{Pollen sterility (\%)} = \frac{\text{Number of unstained pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.5 Genetic Parameter Estimates

Broad Sense Heritability (h^2) was estimated according to Falconer (1989) using

$$h^2 = \frac{\sigma^2_g}{\sigma^2_{ph}} \quad (\text{equation 1})$$

Where σ^2_g is the genotypic variance;

σ^2_{ph} is the phenotypic variance.

Phenotypic and genotypic variances were obtained from the analysis of variance table using equation 2 and 3 as follows

$$\sigma^2_g = \frac{MS1 - MS2}{r \times s} \quad (\text{equation 2})$$

$$\sigma^2_{ph} = \frac{MS1}{r \times s} \quad (\text{equation 3})$$

Where: r: replication, s: season, MS1: Mean Square for cultivar, MS2: mean square for cultivar X season.

The mean value was used for genetic analysis to determine Genotypic Coefficient of Variation (GVC) and Phenotypic Coefficient of Variation (PVC), using equation 4 and 5 as follows:

$$\text{GCV (\%)} = \frac{\sqrt{\text{Genotypic Variance}}}{\text{Grand mean}} \times 100 \quad (\text{equation 4})$$

$$\text{PVC (\%)} = \frac{\sqrt{\text{Phenotypic Variance}}}{\text{Grand mean}} \times 100 \quad (\text{equation 5})$$

Genetic advance (GA) was calculated with the method suggested by Singh and Chaundry (1985) using equation 6

$$\text{GA} = K \cdot \sigma_p \cdot h^2 \quad 6$$

Where K: constant =2.06 at 5% selection intensity. σ_p : square root of phenotypic variance
. h^2 : heritability.

$$\text{GA as percentage of mean (GAM)} = (\text{GA}/\text{Grand Mean}) \times 100$$

3.6 Molecular Characterization Using Simple Sequence Repeat Molecular Markers

Molecular analysis of the selected samples of the soybean based on superiority in morphological traits was done at the Genetic Engineering Laboratory, International Institute of Tropical Agriculture (IITA) Ibadan. The accessions of soybean were subjected to DNA extraction using a modified procedure of Edwards (1991). Approximately 100 mg of young leaf from each sample was grinded in 1000 μ l of Dellaporta extraction buffer separately. The extracted mix was collected in sterile eppendorf tube and 40 μ l of 20 % SDS was then added. The samples were vortex and incubated at 65 $^{\circ}$ C for 10 minutes. At room temperature, 160 μ l of 5 M potassium acetate was added vortexed and centrifuged at 10000 g for 10 minutes.

The supernatant were collected in another eppendorf tube and 400 µl of cold iso propanol was added gently and kept at -20 °C for 60 minutes. Centrifugation was done at 13000 g for 10 minutes to precipitate the DNA after which the supernatant was gently decanted and ensured that the pellet was not disturbed. DNA was then washed with 500 µl of 70 % ethanol by centrifuging at 10000 g for 10 minutes. Ethanol was decanted and DNA air-dried at room temperature until no trace of ethanol was seen in the tube. Pellet was then re-suspended in 50 µl of Tris EDTA buffer to preserve and suspend the DNA.

3.6.1 SSR PCR Protocol and Bands Separation

Six (6) polymorphic SSR markers were used for genotyping of the selected soybeans genotypes. The PCR reaction was optimized at 15µl. Touch-down PCR protocol was followed, which consist of an initial denaturation at 94°C for 5 minutes followed by 9 cycles of denaturation of 94°C for 15seconds, Annealing of 65°C for 20seconds and extension of 72°C for 30seconds followed by a 25 cycles denaturation of 94°C for 15second, Annealing of 55°C for 20second and extension of 72°C 30seconds then a final extension of 72°C for 7 minutes.

Primers	Primer sequence (forward)	Primer sequence (Reverse)
Satt569	GCGCAAATTGCTTCACGCATCCAAAT	GCGGCCTACTATAGTGAAGGGTAT A
Satt572	GCG GAG CAT GTA AAT CCA GCC TAT TGA	GCG GGC TAA CTT ATG TTA CTA AAC AAT
Sath294	GCG GGT CAA ATG CAA ATT ATT TTT	GCG CTC AGT GTG AAA GTT GTT TCT AT

Satt288	GCG GGG TGA TTT AGT GTT TGA CAC CT	GCG CTT ATA ATT AAG AGC AAA AGA AG
Satt222	GCGTGT TTTGTG AAA ATA ATA ATT AAA GAT G	GCG CCA CAA GTA ACT AAT GTA ATA GGT GTT
Satt194	GGG CCC AAC TGA TAT TTA ATT GTA A	GCG CTT TGT GTT CCG ATT TTG AT

3.6.2 Band separation

The separation of bands as produced by each primer was done in a 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. Molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. Prepared 1XTAE buffer was poured into the gel tank to barely submerge the gel. Seven µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

3.7 Data Analysis

Data obtained was pooled for analysis, Analysis of Variance (ANOVA) was carried out to test for significant difference among the accessions mean for all the quantitative parameters, and Duncan Multiple Range (DMRT) was used to separate the mean where there were difference. Simple percentage was used to compare the estimate of some of the parameters like pollen sterility; pollen fertility etc. All value was considered significant at

$P \leq 0.05$.

Binary data was generated for each primer sets using 1 and 0 for presence and absence of positive amplification at a particular band size, respectively. The generated binary data was used to create a data matrix which was analyzed using the Power marker V2.35 software for genetic diversity parameters such as major allele frequency, gene diversity and polymorphic information content. The genetic relationship among treated samples was also estimated by constructing a dendrogram through unweighed pair group method with arithmetic means [UPGMA] using the mega6 software and genetic distance where computed also using the mega6 software.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Seedling morphology

Morphological observations of the leaves as presented in Table 4.1 showed that, all the accessions have broad leaf shape. In terms of size the leaflet varied from medium in accessions TGX -1485 – 1D, TGX-2019 – 1E, TGX 2008 – 4F, TGX – 2009 – 16F, and TGX – 2002 – 4E to small leaflet size in other accessions. However, pubescence is present in all the accessions, with accessions TGX- 2023-4E, TGX- 1988- 5F, TGX 1485 – 1D, TGX -2017- 6E, TGX- 2025 -8E, TGX -2027-1E, TGX 1989 -19F and TGX – 1987-62F having normal pubescence density while other accessions have dense pubescence density.

For pubescence colour, majority of the accessions are light brown except for accessions TGX – 1485- 1D, TGX – 2017-6E, TGX 1448- 2E, TGX – 1989 –19F and TGX – 2022-4E that are semi-appressed.

Colour of the cotyledon examination revealed that most accessions are yellow while accessions TGX – 2019 – 1E, TGX – 2016 – 4E, TGX 2009- 16F, TGX 1989- 19F and TGX 2011 – 6F are green. For the corolla colour, accession TGX – 2018 – 5E is white while all other accessions were purple throat colour. Accessions TGX- 1485-1D, TGX – 2016- 4E, TGX – 2007 – 3F and TGX - 2004- 9F have high shattering ability, while accessions TGX – 1988- 5F, TGX -2019 – 1E, TGX 2025-8E,, TGX – 2008 -4F, TGX 2027 – 1E, TGX 2009 – 16F and TGX- 1987- 62F have medium shattering ability and the rest accessions have slight shattering ability. For the mature Pod colour, two major colours were observed; brown (TGX-2019-1E, TGX – 2008-4F, TGX-2016-4E, TGX-2010-11F, TGX-2009-16F, TGX-1987-62F, TGX-2007-3E, TGX-2004-9F, TGX-2022-4E, TGX2011-6F and TGX-1835-10E) while others have tan colour (Table 4.1).

Table 4.1: Phenotypic Parameters of Soybean Accessions

ACCESSION NUMBER	LEAFLET SHAPE	LEAFLET SIZE	PUBESCENCE	PUBESCENCE DENSITY	PUBESCENCE COLOUR	PUBESCENCE TYPE	COTYLEDON COLOUR	CPROLLA COLOUR	SEED SHATTERING	MATURE POD COLOUR
TGX- 2018-5E	Broad	Small	Present	Dense	Light Brown	Erect	Yellow	White	Slight	Tan
TGX- 2023 4E	Broad	Small	Present	Normal	Light Brown	Semi-Appressed	Yellow	Purple Throat	Slight	Tan
TGX- 1988 - 5F	Broad	Small	Present	Normal	Light Brown	Semi-Appressed	Yellow	Purple Throat	Medium	Tan
TGX - 1485 - 1D	Broad	Medium	Present	Normal	Grey	Erect	Yellow	Purple Throat	Shattering	Tan
TGX -2017- 6E	Broad	Small	Present	Normal	Grey	Erect	Yellow	Purple Throat	Slight	Tan
TGX- 2019- 1E	Broad	Medium	Present	Dense	Light Brown	Semi-Appressed	Green	Purple Throat	Medium	Brown
TGX - 2025 8E	Broad	Small	Present	Normal	Light Brown	Erect	Yellow	Purple Throat	Medium	Tan
TGX - 1448 - 2E	Broad	Small	Present	Dense	Grey	Erect	Yellow	Purple Throat	Slight	Tan
TGX - 2008 - 4F	Broad	Medium	Present	Dense	Light Brown	Erect	Yellow	Purple Throat	Medium	Brown
TGX -2016- 4E	Broad	Small	Present	Dense	Light Brown	Erect	Green	Purple Throat	Shattering	Brown
TGX - 2010 - 11F	Broad	Small	Present	Dense	Light Brown	Semi-Appressed	Yellow	Purple Throat	Slight	Brown
TGX - 2027 - 1E	Broad	Small	Present	Normal	Light Brown	Erect	Yellow	Purple Throat	Medium	Tan
TGX -2009 - 16F	Broad	Medium	Present	Dense	Light Brown	Erect	Green	Purple Throat	Medium	Brown
TGX -1989 19F	Broad	Small	Present	Normal	Grey	Erect	Green	Purple Throat	Medium	Tan
TGX - 1987 62F	Broad	Small	Present	Normal	Light Brown	Erect	Yellow	Purple Throat	Slight	Brown
TGX - 2007 3F	Broad	Small	Present	Dense	Light Brown	Semi-Appressed	Yellow	Purple Throat	Shattering	Brown
TGX - 2004 - 9F	Broad	Small	Present	Dense	Light Brown	Semi-Appressed	Yellow	Purple Throat	Shattering	Brown
TGX -2022 - 4E	Broad	Medium	Present	Dense	Grey	Erect	Yellow	Purple Throat	Slight	Brown

TGX - 2011 - 6F TGX -183510E	Broad Broad	Small Small	Present Present	Dense Dense	Light Brown Grey	Erect Erect	Green Yellow	Purple Throat Purple Throat	Slight Slight	Brown Brown
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4.1.2 Plant height

The result of plant height of the soybean accessions are presented in Table 4.2. The analysis of variance (ANOVA) showed that, there were significant differences among all the accessions of soybean in plant height. At two (2) weeks after planting, the highest plant height was due to accession TGX- 1488-2E with the value of 9.33cm, while TGX-2023-4E has the least with the value of 6.23cm. Similarly, at week 4 after germination, TGX-1488-2E have significant highest plant height with value of 18.37cm and the least was recorded in accession TGX- 2007-3F with the value of 12.28cm. However, there is no significant difference between the least height (12.28 cm) in TGX -2007-3F and the value of TGX -1988 - 5F (12.33 cm). At sixth week, statistical analysis revealed that there was significant difference among accessions with accession TGX- 1448- 2E having the highest height of 31.97 cm and TGX – 2010 – 11F has the least with value of 20.08cm. These values were significantly different from one another and from the values of all other accession except for TGX -2016 -4E (30.50 cm) and TGX -2007- 3F (20.47 cm) which were not significant to the highest and the least, respectively. At the eighth week, there were significant differences among accessions. The highest plant height was recorded in accession TGX – 2016- 4E with a value of 46.17 and the least value was due to TGX -2008- 4F with a value of 27.38. This highest value (46.71 cm) was not significantly different from the value (44.79 cm) of TGX - 1448 -2E but significantly different to the values of all other accessions studied (Table 4.2).

Table 4.2 Mean Height of the Soybean Accessions at different Weeks

Parameter	Two	Four	Six	Eight
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TGX - 2018 - 5E	7.33 ± 0.42 ^{abcd}	14.33 ± 1.72 ^{abc}	24.67 ± 2.83 ^c	34.08 ± 2.68 ^{abc}
TGX - 2023 - 4E	6.23 ± 0.89 ^a	14.5 ± 0.29 ^{abc}	25.83 ± 2.24 ^{cd}	38.0 ± 5.29 ^{abc}
TGX -1988 - 5F	7.3 ± 0.30 ^{abcd}	12.33 ± 0.33 ^a	21.0 ± 0.5 ^{ab}	31.33 ± 0.17 ^{ab}
TGX 1485 -1D	9.27 ± 0.18 ^d	17.38 ± 0.76 ^{bc}	31.75 ± 1.19 ^g	39.55 ± 2.24 ^{abc}
TGX-2017 -6E	8.73 ± 0.32 ^{cd}	17.37 ± 1.64 ^{bc}	28.47 ± 2.82 ^f	39.63 ± 3.62 ^{abc}
TGX -2019 -1E	8.38 ± 1.45 ^{abcd}	13.33 ± 2.95 ^{ab}	21.85 ± 5.50 ^{ab}	32.83 ± 8.91 ^{abc}
TGX - 2025 - 8E	9.00 ± 0.69 ^d	16.30 ± 2.30 ^{abc}	25.50 ± 2.95 ^{cd}	37.66 ± 4.17 ^{abc}
TGX - 1448 -2E	9.33 ± 0.36 ^d	18.37 ± 0.74 ^c	31.97 ± 1.35 ^g	44.79 ± 3.11 ^{bc}
TGX - 2008 -4F	8.07 ± 0.58 ^{abcd}	13.33 ± 1.37 ^{ab}	20.67 ± 2.90 ^{ab}	27.38 ± 4.53 ^a
TGX -2016 -4E	8.33 ± 0.41 ^{abcd}	17.33 ± 1.22 ^{bc}	31.50 ± 1.53 ^g	46.17 ± 2.25 ^c
TGX -2010 -11F	6.50 ± 0.37 ^{ab}	12.22 ± 0.85 ^a	20.08 ± 1.95 ^a	30.95 ± 2.77 ^a
TGX -2027 - 1E	6.75 ± 0.38 ^{abc}	13.68 ± 0.85 ^{abc}	26.17 ± 2.44 ^{cd}	37.83 ± 3.33 ^{abc}
TGX -2009 -16F	7.55 ± 0.94 ^{abcd}	15.78 ± 1.63 ^{abc}	25.25 ± 2.88 ^{cd}	37.83 ± 3.52 ^{abc}
TGX -1989 -19F	8.44 ± 0.78 ^{abcd}	15.90 ± 1.90 ^{abc}	25.70 ± 4.41 ^{cd}	37.30 ± 7.83 ^{abc}
TGX - 1987 -62F	7.2 ± 0.67 ^{abcd}	12.54 ± 0.94 ^{ab}	21.30 ± 2.08 ^{ab}	31.62 ± 3.45 ^{ab}
TGX -2007- 3F	7.53 ± 0.34 ^{abcd}	12.28 ± 0.40 ^a	20.47 ± 1.20 ^a	30.83 ± 1.82 ^a
TGX - 2004 -9F	7.63 ± 0.46 ^{abcd}	15.87 ± 0.82 ^{abc}	28.87 ± 1.17 ^{ab}	45.30 ± 2.40 ^{bc}
TGX- 2022 -4E	7.67 ± 0.75 ^{abcd}	14.47 ± 1.85 ^{abc}	26.17 ± 3.25 ^f	37.83 ± 3.83 ^b
TGX -2011 -6F	7.52 ± 0.69 ^{abcd}	14.38 ± 1.28 ^{abc}	24.67 ± 3.08 ^d	37.25 ± 4.39 ^{abc}

Values are means ± Standard Error, Values followed by the same alphabet(s) on the column are significantly different at P < 0.05 tested by Duncan Multiple Range Test.

4.1.3 Number of Leaves

The number of leaves as shown in Table 4.3 showed significant difference among the accessions analyzed. At week 2, accession TGX – 1448-2E recorded the highest number of leaves with an average value of 8.17 and TGX- 2023-4E has the least number with a value of 4.67. Similarly, in week 4, the accession with the highest number of leaves per plant (19.67) was TGX -1485-1D while the least (13.00) was due to TGX -1998-5F. However, there is no significant difference between accession (TGX-2009-16F) and accession TGX-2023-4E (13.67). At week 6, the highest number of leaves (48.55) was due to accession TGX- 1485-1D while the least was due to accession TGX-1988- 5F with a value of 18.33. These values were significantly different from one another and from the values of the other accessions. In week 8, the highest number of leaves 117.17 was recorded in accession TGX – 1485-1D while the least value of 36.83 was obtained in TGX -2010- 11F. However, these values were significantly different from one another and from the values of all other accessions studied (Table 4.3).

Table 4.3 Mean Number of Leaves of the Soybean Accessions at different Weeks

Parameter	TWO	FOUR	SIX	EIGHT
TGX - 2018 - 5E	6.50±0.81 ^{abcd}	16.33±1.28 ^{abcde}	28.83±2.82 ^{ab}	52.33±6.47 ^{abcde}
TGX - 2023 - 4E	4.67±0.33 ^a	13.67±0.33 ^a	24.33±1.45 ^{ab}	48.33±2.91 ^{abcd}
TGX -1988 - 5F	5.00±1.00 ^{ab}	13.0±0.00 ^a	18.33±3.17 ^a	49.00±0.00 ^{abcd}
TGX 1485 -1D	7.17±0.48 ^{bcd}	19.67±1.54 ^{de}	48.55±5.55 ^{cd}	117.17±13.89 ^g
TGX-2017 -6E	7.67±0.33 ^{abcd}	17.50±1.20 ^{abcde}	32.00±4.02 ^{ab}	59.50±7.83 ^{abcde}
TGX -2019 -1E	6.75±1.25 ^{abcd}	15.50±1.50 ^{abcde}	26.00±4.53 ^{ab}	43.75±11.04 ^{abc}
TGX - 2025 - 8E	6.40±0.93 ^{abcd}	15.00±0.63 ^{abcd}	24.20±1.66 ^{ab}	45.60±3.31 ^{abcd}
TGX - 1448 -2E	8.17±0.17 ^d	20.00±0.58 ^e	53.50±2.09 ^d	101.17±14.67 ^{fg}

TGX - 2008 -4F	7.00±0.52 ^{abcd}	15.17±1.66 ^{abcd}	23.17±4.76 ^{ab}	40.67±10.90 ^{ab}
TGX -2016 -4E	6.00±0.68 ^{abcd}	19.17±2.10 ^{cde}	47.33±6.29 ^{cd}	86.67±7.78 ^{efg}
TGX -2010 -11F	5.17±0.17 ^d	14.50±0.85 ^{abc}	20.50±2.08 ^a	36.83±3.21 ^a
TGX -2027 - 1E	5.33±0.21 ^{abc}	16.67±1.52 ^{abcde}	36.00±7.93 ^{bc}	80.83±12.81 ^{def}
TGX -2009 -16F	6.00±0.93 ^{abcd}	13.67±1.52 ^a	24.67±3.17 ^{ab}	46.00±7.86 ^{abcd}
TGX -1989 -19F	5.80±0.58 ^{abcd}	15.00±1.18 ^{abcd}	29.20±5.38 ^{ab}	79.02±0.18 ^{cdef}
TGX - 1987 -62F	7.00±1.00 ^{abcd}	15.20±1.83 ^{abcd}	24.40±1.86 ^{ab}	51.40±7.94 ^{abcd}
TGX -2007- 3F	7.00±0.63 ^{abcd}	14.67±1.02 ^{abc}	24.00±1.77 ^{ab}	49.83±6.01 ^{abcd}
TGX - 2004 -9F	6.67±0.84 ^{abcd}	16.00±0.50 ^{abcde}	28.83±2.14 ^{ab}	62.17±7.78 ^{abcde}
TGX- 2022 -4E	7.83±0.65 ^d	18.83±1.94 ^{bcde}	36.00±4.89 ^{bc}	76.01±1.38 ^{bcdef}
TGX -2011 -6F	6.17±0.79 ^{abcd}	14.17±1.19 ^{ab}	25.17±2.44 ^{ab}	54.83±7.97 ^{abcde}

Values are means ± Standard Error, Values followed by the same alphabet(s) on the column do not statistically differ at $P < 0.05$ tested by Duncan Multiple Range Test.

4.1.4 Number of nodes per stem

Significant differences in the number of nodes per stem in Table 4.4 were obtained in all the weeks studied. At week 2, accession TGX – 1488 -2E have the highest number of node with a value of 8.17 and TGX – 2019-1E has the least number of node with a value of 0.00. At week 4, accession TGX – 1485 -1D have significantly highest number (7.50) of node per stem and accession TGX- 2011-6F has the least number of node (5.67) per stem. However, there are no significant difference between accession TGX- 2023- 4E (6.33) and accession TGX-1988-5F (6.33) also between accession TGX- 2017-6E (7.00) and TGX- 1448-2E (7.00). At week 6, there were significant differences among accessions. Accession TGX – 1485- 1D have the highest value of 15.51 while accession TGX -1988 -5F has the least value of 7.00. At week 8, there were

significant differences between accessions. Accession TGX – 1488-2E have the highest number of node with a value of 16.33 while accession TGX – 1988-5F has the least number of node with a value of 8.67 (Table 4.4).

Table 4.4 Mean Number of Nodes of the Soybean Accessions at different Weeks

Parameter	TWO	FOUR	SIX	EIGHTH
TGX - 2018 - 5E	6.17±0.40 ^{bcdef}	6.17±0.40 ^{abc}	10.67±0.84 ^{abc}	12.67±0.76 ^{bcdef}
TGX - 2023 - 4E	4.67±0.33 ^b	6.33±0.33 ^{abc}	8.67±0.33 ^{ab}	10.67±0.33 ^{abcd}
TGX -1988 - 5F	5.00±1.00 ^{bc}	6.33±0.33 ^{abc}	7.00±0.00 ^a	8.67±0.33 ^a
TGX 1485 -1D	7.17±0.48 ^{def}	7.50±0.22 ^c	15.50±1.89 ^d	14.83±0.48 ^{efg}
TGX-2017 -6E	7.67±0.33 ^{ef}	7.00±0.45 ^{bc}	14.17±1.60 ^{cd}	13.83±0.79 ^{defg}
TGX -2019 -1E	0.00±0.00 ^a	5.75±0.95 ^a	8.50±1.19 ^{ab}	10.00±1.22 ^{ab}
TGX - 2025 - 8E	6.40±0.93 ^{bcdef}	6.00±0.32 ^{ab}	8.80±0.58 ^{ab}	12.20±0.20 ^{bcdef}
TGX - 1448 -2E	8.17±0.17 ^f	7.00±0.45 ^{bc}	12.17±1.17 ^{bcd}	16.33±0.92 ^g

TGX - 2008 -4F	7.00±0.52 ^{cdef}	6.17±0.40 ^{abc}	9.17±1.68 ^{ab}	11.17±1.80 ^{abcde}
TGX -2016 -4E	6.00±0.68 ^{dcd}	7.33±0.49 ^{bc}	14.17±1.78 ^{cd}	13.83±0.31 ^{defg}
TGX -2010 -11F	5.17±0.17 ^{bcd}	6.00±0.26 ^{ab}	8.17±0.91 ^{ab}	10.50±1.12 ^{abc}
TGX -2027 - 1E	5.33±0.21 ^{bcd}	6.83±0.40 ^{abc}	12.67±1.93 ^{ab}	12.67±0.71 ^{bcdef}
TGX -2009 -16F	6.00±0.93 ^{bcde}	6.17±0.40 ^{abc}	9.00±0.93 ^{ab}	11.67±0.88 ^{abcdef}
TGX -1989 -19F	5.80±0.58 ^{bcde}	6.60±0.40 ^{abc}	10.20±1.50 ^{abc}	12.20±1.93 ^{bcdef}
TGX - 1987 -62F	7.00±1.00 ^{cdef}	6.40±0.24 ^{abc}	9.00±0.77 ^{ab}	12.20±1.07 ^{bcdef}
TGX -2007- 3F	7.00±0.63 ^{cdef}	6.00±0.26 ^{ab}	8.67±0.71 ^{ab}	12.33±0.33 ^{bcdef}
TGX - 2004 -9F	6.67±0.84 ^{bcdef}	6.83±0.17 ^{abc}	9.50±0.2 ^{ab}	13.67±0.56 ^{cdefg}
TGX- 2022 -4E	7.83±0.65 ^{ef}	6.83±0.40 ^{abc}	11.50±1.41 ^{abcd}	14.17±0.60 ^{efg}
TGX -2011 -6F	5.33±3.33 ^{bcd}	5.67±0.33 ^a	10.17±0.87 ^{abc}	12.50±0.62 ^{bcdef}

Values are means ± Standard Error, Values followed by the same alphabet(s) on the column do not statistically differ at P < 0.05 tested by Duncan Multiple Range Test.

4.1.5 Number of branches

Number of branches shows significant differences (p<0.05) among accessions as presented in Table 4.5 with accession TGX -1485 -1D having the highest number of branches at maturity with the value of 7.80 per plant and accession TGX -1988 -5F has the least with a value of 0.33 per plant. This result also revealed that there was no significant difference between accessions TGX -1987 -62F (2.67 per plant) and TGX- 2004- 9F (2.67 per plant) (Table 4.5).

4.1.6 Number of internode

In term of number of internodes per plant, statistical analysis shows that there were significant differences ($p < 0.05$) among the accessions at maturity as presented in Table 4.5. Accession TGX – 2004- 9F recorded the highest number of internode per plant with the value of 13.17 while accession TGX – 1988- 5F had the least number of internode with a value of 1.50 per plant. However, accessions TGX – 1485- 1D (12.00 internodes per plant) and TGX- 2016-4E (12.00 internodes per plant); TGX – 2010-11F (9.83 internodes per plant) and TGX – 2009-16F (9.83 internodes per plant); TGX – 2011 – 6F (11.33 internodes per plant) and TGX- 2018-5E (11.33 internodes per plant) were not significant different from one another (Table 4.5).

4.1.7 Colar diameter

The statistical analysis of colar diameter at maturity as presented in Table 4.5 revealed significant differences among the accessions with TGX -2027- 1E having the highest value of 14.80cm and TGX – 1988- 5F has the least value of 1.32cm. Accession TGX – 1485 – 1D, and TGX – 2017- 6E have no significant difference from each other. Also, accessions TGX – 1488 – 2E and TGX – 2004 – 9F were not significantly difference from each other (Table 4.5).

4.1.8 Number of peduncle

Significant highest number of Peduncle was obtained in TGX – 1488-2E with the value of 31.33 while TGX – 1988 – 5F has the least with a value of 1.50 as presented in Table 4.5. These values are significantly different from one another and with the values of all other accessions (Table 4.5).

4.1.9 Peduncle length

Accession TGX- 2027-1E recorded the highest peduncle length with the value of 1.85cm while TGX -1988 – 5F has the least with a value of 0.13 cm as presented in Table 4.5. However, there were no significant difference between the peduncle length of accessions TGX- 2018- 5E, TGX – 2017- 1E, TGX – 2008- 4F, TGX- 2010 – 11F and TGX – 2004- 9F. Also accessions TGX – 2017- 6E, TGX – 2009- 16F, TGX – 1989 -19f and TGX – 2022 – 4E; and accessions TGX- 2023-4E, TGX- 1988 -5F, TGX – 1485- 1D, TGX- 1488-2E, TGX – 2016 – 4E and TGX – 2027- 1E were significantly different from one another (Table 4.5).

4.1.10 Days to flowering

Significant differences were observed among accessions in days to flowering as presented in Table 4.5. Accession TGX – 2022- 4E have the highest days to flowering 66.00 days while accession TGX – 1988- 5F has the least of 8.17 days to flowering (Table 4.5).

4.1.11 Days to pod production

The result of statistical analysis shows that there were significant differences in days to pod production among the accessions as presented in Table 4.5. Accession TGX – 2022-4E have the highest days to pod production with the average of 86.67 days while accession TGX – 1988 – 5F has the least with average days of 10.33 (Table 4.5).

Table 4.5: Mean Numbers of some Morphological Parameters at Maturity

Parameter	NBR	NIN	COD	CODM	NPE	PEL	DF	DPP
TGX - 2018 - 5E	3.67±0.67 ^{bcd}	11.33±0.56 ^{dc}	6.41±0.68 ^{cdef}	8.29±0.40 ^{cde}	12.00±2.22 ^{abcde}	1.05±0.20 ^{bcd}	46.00±1.39 ^{cd}	59.67±2.33 ^{cde}
TGX - 2023 - 4E	1.50±0.81 ^{ab}	5.17±2.32 ^{ab}	2.49±1.16 ^{ab}	3.77±1.16 ^{ab}	5.67±2.72 ^{ab}	0.55±0.30 ^{ab}	25.33±11.39 ^b	32.67±14.65 ^b
TGX -1988 - 5F	0.33±0.33 ^a	1.50±1.50 ^a	0.67±0.67 ^a	1.32±1.32 ^a	1.50±1.50 ^a	0.133±0.13 ^a	8.17±8.17 ^a	10.63 ± 0.33 ^a
TGX 1485 -1D	7.83±0.70 ^f	12.00±0.73 ^e	7.50±0.52 ^{def}	8.94±0.71 ^{cdef}	27.50±6.60 ^{fg}	1.83±0.26 ^e	43.00±1.15 ^{cd}	61.5 ± 1.61 ^{cde}
TGX-2017 -6E	2.83±0.48 ^{abcd}	13.00±1.03 ^e	6.86±0.48 ^{def}	9.16±1.05 ^{cdef}	18.00±3.06 ^{bcd}	1.17±0.15 ^{bcd}	45.83±0.40 ^{cd}	65.00 ± 1.67 ^{def}
TGX -2019 -1E	1.50±0.81 ^{ab}	6.33±2.17 ^{bc}	3.88±1.62 ^{bc}	4.16±1.48 ^{ab}	7.33±3.00 ^{ab}	0.97±0.37 ^{bcd}	33.17±10.57 ^{bc}	43.00 ± 12.73 ^{bc}
TGX - 2025 - 8E	2.33±0.56 ^{abc}	9.33±2.03 ^{bcd}	4.86±1.03 ^{bcd}	6.94±1.44 ^{bcd}	8.83±2.09 ^{abcd}	0.833±0.32 ^{abc}	41.67±8.58 ^{cd}	54.30 ± 11.17 ^{bc}
TGX - 1448 -2E	6.83±1.11 ^{ef}	12.83±0.54 ^e	7.37±0.54 ^{def}	10.40±0.53 ^{def}	31.33±8.11 ^g	1.78±0.40 ^{de}	49.17±2.27 ^{cd}	68.00 ± 2.78 ^{def}
TGX - 2008 -4F	2.17±1.28 ^{abc}	7.17±1.47 ^{bcd}	4.71±0.57 ^{bcd}	6.91±1.10 ^{bcd}	17.50±6.25 ^{bcd}	1.25±0.23 ^{bcd}	47.83±1.54 ^{cd}	64.50 ± 2.78 ^{de}
TGX -2016 -4E	6.67±0.80 ^{ef}	12.00±0.82 ^e	7.58±0.63 ^{def}	11.06±0.62 ^{ef}	21.33±2.87 ^{defg}	1.58±0.10 ^{cde}	54.00±0.37 ^{de}	72.67 ± 0.95 ^{def}
TGX -2010 -11F	1.50±0.34 ^{ab}	9.83±1.05 ^{cde}	4.90±0.29 ^{bcd}	6.30±1.35 ^{bc}	8.00±0.68 ^{abc}	1.07±0.10 ^{bcd}	54.67±1.48 ^{de}	75.00 ± 1.83 ^{def}
TGX -2027 - 1E	5.33±0.88 ^{def}	10.83±0.98 ^{cde}	8.30±1.00 ^f	14.83±0.96 ^h	21.00±3.98 ^{cdefg}	1.85±0.03 ^e	47.50±0.50 ^{cd}	65.50 ± 1.88 ^{def}
TGX -2009 -16F	2.33±1.05 ^{abc}	9.83±0.91 ^{cde}	5.66±0.75 ^{cdef}	7.98±1.24 ^{cde}	11.33±3.29 ^{abcde}	0.98±0.22 ^{bcd}	50.83±0.91 ^{de}	70.00 ± 1.77 ^{def}
TGX -1989 -19F	3.17±1.45 ^{abcd}	9.17±2.46 ^{bcd}	5.20±1.27 ^{bcd}	8.61±1.96 ^{cde}	23.50±5.96 ^{efg}	0.98±0.43 ^{bcd}	42.50±8.61 ^{cd}	54.00 ± 10.85 ^{bc}
TGX - 1987 -62F	2.67±0.88 ^{abcd}	8.50±2.03 ^{bcd}	4.75±1.01 ^{bcd}	6.35±1.40 ^{bc}	7.50±2.28 ^{ab}	0.63±0.20 ^{ab}	55.33±1.94 ^{de}	73.17 ± 3.36 ^{def}
TGX -2007- 3F	3.83±0.83 ^{bcd}	9.67±0.42 ^{cde}	5.56±0.48 ^{cdef}	8.17±0.65 ^{cde}	18.67±3.86 ^{bcd}	1.73±0.33 ^{de}	49.17±1.11 ^{cd}	66.50 ± 0.84 ^{def}
TGX - 2004 -9F	2.67±0.49 ^{abcd}	13.17±0.60 ^e	7.93±0.89 ^{ef}	10.40±0.36 ^{def}	14.17±1.92 ^{abcde}	1.10±0.17 ^{bcd}	49.83±0.54 ^{cd}	67.67 ± 0.71 ^{def}
TGX- 2022 -4E	5.00±1.06 ^{cde}	12.8±30.48 ^e	7.26±0.81 ^{def}	12.49±1.39 ^{fg}	16.83±2.77 ^{bcd}	1.00±0.23 ^{bcd}	66.00±0.89 ^e	86.67 ± 0.95 ^f
TGX -2011 -6F	4.83±1.01 ^{cde}	11.33±0.84 ^{de}	5.62±0.48 ^{cdef}	10.89±0.82 ^{ef}	11.00±1.91 ^{abcde}	0.87±0.15 ^{abc}	57.50±1.09 ^{de}	77.5 ± 1.38 ^{ef}
Total	3.53±0.26	9.78±0.40	5.66±0.25	8.26±0.38	14.89±1.09	1.12±0.07	45.66±1.57	61.46±2.01

Values are means ± Standard Error, Values followed by the same alphabet(s) on the column do not statistically differ at P < 0.05 tested by Duncan Multiple Range Test.

NBR= Number of branches,
NPE= Number of peduncle,

NIN= Number of internode,
PEL= Peduncle length,

COD = Colar diameter,
DF= Days to flowering,

CODM = Colar diameter at maturity,
DPP= Days to pod production

4.1.12 Number of pod per plant

The statistical analysis shows that there was significant differences ($P < 0.05$) among the accessions for number of pod per plant as presented in Table 4.6. The highest number of pod per plant was recorded in accession TGX – 2027- 1E with the value 158.30 while the least was recorded in accession TGX- 1988- 5F with a value of 10.83 (Table 4.6).

4.1.13 Pod length

Significant highest pod length was recorded in accession TGX – 2018-5E with a value of 4.67 cm, while the least value was obtained in TGX – 1988- 5F with a value of 0.70 cm. The results as presented in Table 4.6 further shows that there was no significant difference between the pod length of accessions TGX – 1485 – 1D (4.00 cm) and TGX- 2019-6E (4.00 cm); similarly between accession TGX – 2016- 4E (4.13 cm) and TGX – 2010 – 11F (4.13 cm) (Table 4.6).

4.1.14 50% flowering and & 75 % maturity

Table 4.6 revealed that there were no significant difference among all accessions in 50% flowering and 75 % maturity (Table 4.6).

4.1.15 Pod production

The result of statistical analysis as presented in Table 4.6 shows that there were significant differences among accessions in terms of pod production per plant. The highest number of pod production was recorded in accession TGX – 1485-1D with a value of 216.17 while the least was recorded in accession TGX – 1988-5F with a value of 19.17. These values were significantly different from one another and from the number of pod produced in all other accessions.

Accession TGX – 1988-5F, TGX – 2023- 4E, TGX – 1485- 1D, TGX – 2027 -1E and TGX – 2022 -4E shows significant difference from the other accessions (Table 4.6).

Table 4.6 Mean Number of some Yield Parameters of the Soybean Accessions

Parameter	NPD	PDL	50%	75%	PDP
TGX - 2018 - 5E	73.17±7.81 ^{abcd}	4.67±0.28 ^d	28.75±9.56 ^a	54.83±20.51 ^a	102.67±41.51 ^{abc}
TGX - 2023 - 4E	27.16±0.16 ^a	1.70±0.78 ^{ab}	27.51±1.75 ^a	54.69±73.43 ^a	29.33±15.53 ^{ab}
TGX -1988 - 5F	10.83±10.83 ^a	0.70±0.70 ^a	9.56±78.72 ^a	18.98±37.45 ^a	19.17±17.43 ^a
TGX 1485 -1D	145.33±25.70 ^{def}	4.00±0.12 ^d	27.03±8.95 ^a	54.94±21.06 ^a	216.17±87.35 ^c
TGX-2017 -6E	75.17±18.64 ^{abcd}	4.00±0.22 ^d	28.66±9.72 ^a	57.58±21.81 ^a	68.33±27.49 ^{abc}
TGX -2019 -1E	48.83±21.62 ^{ab}	2.65±0.85 ^{bc}	29.68±11.44 ^a	57.94±23.97 ^a	55.00±21.47 ^{abc}
TGX - 2025 - 8E	44.83±12.32 ^{ab}	4.08±0.23 ^d	29.24±11.56 ^a	62.13±25.52 ^a	50.50±21.30 ^{abc}
TGX - 1448 -2E	145.17±15.86 ^{def}	3.52±0.09 ^{cd}	28.67±9.86 ^a	60.07±22.64 ^a	173.00±73.45 ^{abc}
TGX - 2008 -4F	58.67±23.36 ^{ab}	3.62±0.25 ^{cd}	28.72±10.81 ^a	56.88±22.42 ^a	68.50±40.10 ^{abc}
TGX -2016 -4E	132.12±16.29 ^{cdef}	4.13±0.18 ^d	30.21±10.55 ^a	57.66±21.03 ^a	117.67±44.22 ^{abc}
TGX -2010 -11F	42.00±9.77 ^{ab}	4.13±0.19 ^d	30.82±11.72 ^a	57.48±23.37 ^a	41.67±15.72 ^{abc}
TGX -2027 - 1E	158.50±33. 70 ^f	4.38±0.09 ^d	30.09±10.15 ^a	61.91±21.57 ^a	199.67±98.10 ^{bc}
TGX -2009 -16F	111.33±28.88 ^{bedef}	3.62±0.11 ^{cd}	30.62±11.53 ^a	59.68±23.56 ^a	116.17±58.85 ^{abc}

TGX -1989 -19F	66.17±28.05 ^{abc}	3.78±0.20 ^{cd}	27.72±10.76 ^a	57.59±22.91 ^a	50.00±28.67 ^{abc}
TGX - 1987 -62F	52.67±15.51 ^{ab}	2.67±0.54 ^{bc}	31.14±12.53 ^a	54.05±22.36 ^a	57.50±26.14 ^{abc}
TGX -2007- 3F	68.83±9.94 ^{abc}	4.43±0.13 ^d	24.12±8.62 ^a	57.06±22.34 ^a	92.17±39.86 ^{abc}
TGX - 2004 -9F	110.83±20.16 ^{bcdef}	3.95±0.23 ^d	30.38±9.98 ^a	58.75±21.73 ^a	119.50±52.99 ^{abc}
TGX- 2022 -4E	148.50±47.92 ^{ef}	3.72±0.11 ^{cd}	42.21±16.19 ^a	64.74±23.98 ^a	214.67±12.70 ^c
TGX -2011 -6F	77.33±18.13 ^{abcde}	4.07±0.18 ^d	34.06±12.52 ^a	64.08±23.14 ^a	96.00±37.96 ^{abc}

Values are means ± Standard Error, Values followed by the same alphabet(s) on the column do not statistically differ at $P < 0.05$ tested by Duncan Multiple Range Test.

NPD= number of pod, PDL = pod length, 50% =50% flowering, 75% = 75% maturity, PDP = pod production

4.1.16 100 pod weight

Statistical analysis from Table 4.7 shows that there was significant difference among accession, with accession TGX – 2027- 1E having the highest 100 pod weight of 62.26 g while accession TGX- 1987 – 62F has the least value of 36.31 g. These values were significantly different from one another and from the value of all other accessions. However, there were no significant difference among accessions TGX 2022 – 4E, TGX -2011-6F, TGX – 2007- 3F, TGX – 1989- 19F and TGX – 2010- 11F; TGX – 2023- 4E, TGX -1988 -5E, TGX -2019 -1E, TGX -1488-2E, TGX- 2016- 4E and TGX- 2004- 9F (Table 4.7).

4.1.17 100 seed weight

TGX -2025 – 8E have the highest value for 100 seed weight of 16.99 g, followed by TGX- 200911F (16.61 g) and accession TGX – 1987- 62F has the least value of 10.37 g. These values highest and lowest seed weight were significantly different from one another and from the value

of all other accessions as presented in Table 4.7. However, there are no significant difference between TGX – 2004- 9F (15.19 g) and TGX – 2007 -3F (15.19 g) (Table 4.7).

4.1.18 Weight per seed

Statistical analysis revealed that there were significant differences among the accessions at 5% level of significance as presented in Table 4.7. Accession TGX – 2025 – 8E have the highest value of 0.24g and accession TGX – 1987 – 62F has the least value of 0.11 g. However, there were no significant difference among the following accessions TGX – 2004- 9F(0.15 g), TGX – 2007 -3F (0.15 g), TGX – 1989- 19F (0.15 g), TGX- 1448- 2E (0.15 g) and TGX - 1485 – 1D (0.15 g); TGX - 2018– 5E(0.14 g), TGX- 2023-4E(0.14 g) and TGX -2019 – 1E(0.14 g);TGX – 2017– 6E(0.16 g), TGX -2016- 4E(0.16 g), TGX – 2010 -11F(0.16 g); and TGX 2027 -1E(0.16 g), TGX – 2022 – 4E (0.18 g) and TGX -2011-6F(0.18 g) (Table 4.7).

4.1.19 Number of seed per pod

Table 4.7 revealed that Accession TGX – 1485 – 1D have the highest number of seed per pod with the value of 3.00 seed per pod and accession TGX – 1987- 62F has the least value of 1.80 seed per pod. However, there were no significant difference among the following accessions TGX 2018 – 5E (2.60 seed per pod), TGX – 2023- 4E (2.60 seed per pod), TGX – 1448- 2E (2.60 seed per pod), TGX-2017-6E (2.60 seed per pod),TGX – 2016 -4E(2.60 seed per pod) and TGX – TGX- 1988 – 5F (2.20 seed per pod), TGX – 2008 -4F (2.20 seed per pod), TGX – 2027- 1E (2.20seed per pod), TGX – 2007- 3F (2.20 seed per pod); TGX – 2022 – 4E(2.00 seed per pod), TGX -1989 – 19F(2.00 seed per pod) and TGX – 2009- 11F (2.00 seed per pod); TGX – 2019- 1E (2.40 seed per pod) and TGX -2010 -11F(2.40 seed per pod) (Table 4.7)

4.1.20 Seed yield per plant

In term of seed yield per plant, there were significant differences among accessions at 5% level of significance as presented in Table 4.7, with accession TGX -2027- 1E, having the highest value of 1167.67 and accession TGX 1988 – 5F has the least value of 77.33. These values highest and lowest seed yield per plant were significantly different from one another and from the value of all other accessions (Table 4.7).

Table 4.7 Mean Value of some Yield Parameters of the Soybean Accessions

Parameter	100 Pod Weight	100 seed weight	weight per seed	Number of Seeds	seed yield per	
TGX-2018-5E	59.06 ±4.62 ^{cd}	15.87±0.23 ⁱ	0.14±0.28 ^{ab}	2.60±0.24 ^{bcd}	294.67±2.03 ^{def}	
TGX-2023-4E	47.0±4.20 ^{abc}	13.73±0.08 ^d	0.14± 0.01 ^{ab}	2.60±0.25 ^{bcd}	107.67 ±5.24 ^{ab}	
Values means	TGX-1988-5F 44.95 ±4.99 ^{abc}	13.88±0.06 ^d	0.22±0.026 ^{de}	2.20±0.20 ^{abc}	77.33±6.94 ^a	are ±
TGX-1485-ID	38.83±3.73 ^{ab}	14.12±0.51 ^d	0.15±0.01 ^{ab}	3.00±0.00 ^d	641.67±31.54 ^h	
TGX-2017-6E	59.23±5.77 ^{cd}	16.15±0.20 ^{ij}	0.16±0.02 ^{abc}	2.60±0.25 ^{bcd}	307.33±23.10 ^{efg}	
TGX-2019-IE	46.22±4.34 ^{abc}	11.45±0.16 ^b	0.14±0.01 ^{ab}	2.40 ±0.25 ^{abcd}	176.00±27.73 ^{abc}	
TGX-2025-8E	63.88±5.40 ^d	16.99±0.15 ^k	0.24±0.03 ^e	2.80±0.20 ^{cd}	116.00±26.56 ^{ab}	
TGX-1448-2E	47.82±4.53 ^{abc}	13.83±0.18 ^d	0.15±0.02 ^{ab}	2.60.±0.25 ^{bcd}	790.00±16.17 ⁱ	
TGX-2008-4F	54.68±4.68 ^{bcd}	15.60±0.18 ^{ghi}	0.17±0.02 ^{abcd}	2.20±0.20 ^{abc}	263.00±6.93 ^{cde}	
TGX-2016-4E	45.33±3.99 ^{abc}	12.67±0.26 ^c	0.16±0.01 ^{abc}	2.60±0.25 ^{bcd}	762.33±40.70 ⁱ	
TGX-2010-11F	50.78±4.59 ^{abcd}	15.67±0.17 ^{hi}	0.16±0.01 ^{ab}	2.40±0.25 ^{abcd}	203.33±13.59 ^{bcd}	
TGX-2027-IE	64.26±7.96 ^d	15.01±0.10 ^{fgh}	0.16±0.02 ^{abcd}	2.20±0.20 ^{abc}	1167.67±105.33 ^j	
TGX-2009-11F	56.12±5.37 ^{cd}	16.61±0.36 ^{jk}	0.23±0.02 ^{cde}	2.00±0.00 ^{ab}	405.33±7.84 ^g	
TGX-1989-19F	48.84±4.52 ^{abcd}	14.81±0.16 ^{ef}	0.15±0.02 ^{ab}	2.00±0.32 ^{ab}	190.00±24.25 ^{bc}	
TGX-1987-62F	36.31±3.06 ^a	10.39±0.07 ^a	0.11±0.01 ^a	1.80±0.20 ^a	257.00±23.09 ^{cde}	
TGX-2007-3F	48.78±4.78 ^{abcd}	14.94±0.25 ^{fg}	0.15±0.01 ^{ab}	2.20±0.20 ^{abc}	154.67±13.62 ^{ab}	
TGX-2004-9F	47.25±4.66 ^{abc}	15.19±0.16 ^{fgh}	0.15±0.01 ^{ab}	1.80.±0.20 ^a	386.00±15.01 ^{fg}	
TGX-2022-4E	48.54±2.13 ^{abcd}	14.30.±0.09 ^{de}	0.18±0.02 ^{bcd}	2.00±0.32 ^{ab}	736.67±21.65 ⁱ	
TGX-2011-6F	51.99±4.92 ^{abcd}	15.19±0.09 ^{fgh}	0.18±0.02 ^{bcd}	2.60±0.25 ^{bcd}	356.67±28.58 ^{efg}	
Stand Total	50.52±1.24	14.55±0.17	0.17±0.01	2.35±0.06	389.12±38.84	

ard Error, Values followed by the same alphabet(s) on the column do not statistically differ at $P < 0.05$ tested by Duncan Multiple Range Test.

4.1.21 Cluster analysis based on agro -morphological parameters

At a genetic distance of 80, The Euclidean hierarchical diagram on the bases of 31 quantitative and qualitative characters, clustered the accessions into 4 different groups as seen in figure 4.1; accession (TGX -2008-4F, TGX- 2023-4E, TGX-2009-16F and TGX-1448-2E) and into 3 different groups at a genetic distance of 100 (TGX- 1448-2E, TGX- 2016- 4E and TGX- 20185E been clustered as a distinct genotype.)The similarity index at a genetic distance of 80 revealed that accessions TGX -1485-1D, TGX – 2027- 1E, TGX1448- 2E and TGX- 2022-4E, likewise TGX -2004- 9F and TGX- 2009-16F and TGX-2016-4E. Also TGX-2023-4F and TGX -1988-5F were grouped together as a distinct unit. In terms of dissimilarity, at a genetic distance of 100, accessions TGX- 1448-2E, TGX- 2016- 4E and TGX- 2018-5E were clustered as a distinct genotype (Figure 4.1).

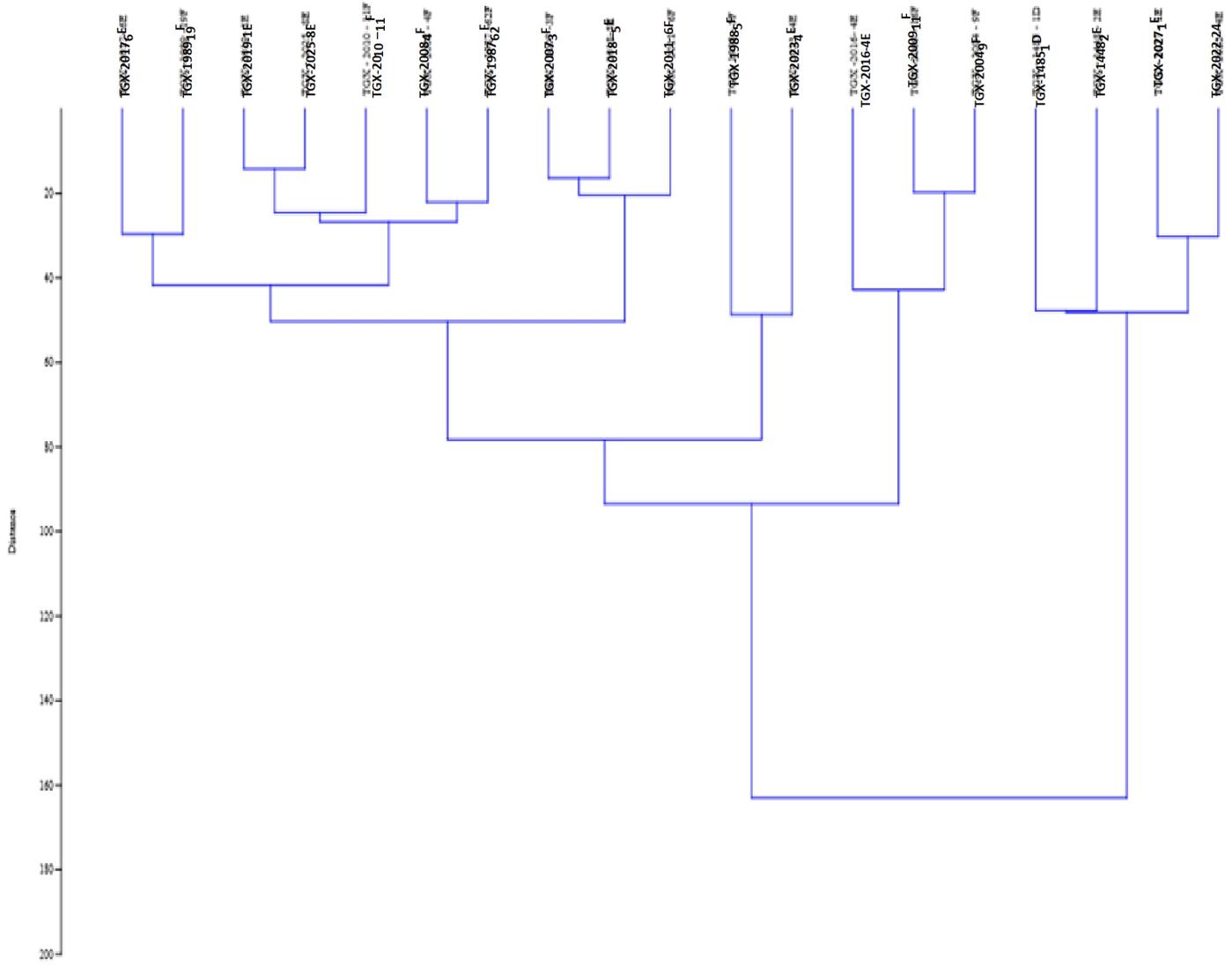


Figure 4.1: UPGMA Dendrogram on morphological traits of 19 soybean accessions

4.1.22 Estimation of genetic parameters

Genetic and phenotypic coefficient of variation gives information about the nature and magnitude of variation, if it is due to genetic or environmental causes. From table 4.8 the phenotypic variance is greater than the genotypic variance; also the phenotypic coefficient of variation is greater than the genotypic coefficient of variation.

The highest genetic variance value (86020.74) was due to seed yield per plant, followed by number of pod per plant (1711.38), days to pod production (70.90) while the least (0.00) was due to weight per seed. For phenotypic variance, the highest (89053.53) was obtained in seed yield per plant, followed by number of pod per plant (4806.96) and the least (0.00) was recorded in weight per seed. Genetic coefficient of variation have highest value (75.37) for seed yield per plant, followed by number of pod production (44.03), number of branches (42.18) and the least value (10.55) was due to pod length. The highest phenotypic coefficient of variation value (76.26) was due seed yield per plant while the least value (14.09) was recorded in days to pod production. Broad sense heritability was highest (95.37) for 100 seed weight with an expected genetic advance of 29.65 %, followed by days to flowering (80.91) with expected genetic advance of 24.91%, days to pod production (75.66) with expected genetic advance of 21.96 % and colar diameter (59.33) have high heritability. Moderate heritability were observed in pod length (46.48) with a genetic advance of 14.81 %, number of branches (45.93) with a genetic advance of 58.88 % while number of seed per plant (0.30) with expected genetic advance of 0.16 % have the lowest heritability. This indicates that some of the agro morphological traits could be selected for the crop improvement (Table 4.8).

Table 4.8 Estimation of some Component of Genetic Parameters for some Agro – metrical characters among the 20 Soybean Accessions

Traits	Means	Genotypic variance	Phenotypic variance	Environmental variance	Broad sense heritability (h ²)	Genotypic Coefficient of Variation	Phenotypic coefficient of variation	GA	GAM (%)
PH	37.09	21.25	106.26	85.00	20.00	12.43	27.79	1144.99	11.44
NB	4.37	3.40	7.40	4.00	45.93	42.18	62.24	5888.79	58.89
NI	11.04	2.64	7.99	5.36	32.99	14.71	25.61	1740.04	17.40
CD	9.42	6.61	11.15	4.53	59.33	27.30	35.45	4332.64	43.33
NN	12.72	3.02	7.85	4.83	38.45	13.66	22.02	1744.56	17.45
DF	51.03	47.09	58.20	11.11	80.91	13.45	14.95	2491.83	24.92
DPP	68.69	70.90	93.70	22.81	75.66	12.26	14.09	2196.62	21.97
NPP	93.96	1711.38	4806.96	3095.58	35.60	44.03	73.79	5411.68	54.12
PL	3.95	0.17	0.37	0.20	46.48	10.55	15.47	1481.46	14.81
100PW	50.52	56.54	170.76	114.22	0.33	14.88	25.86	17.64	0.18
100SW	14.55	4.60	4.82	0.22	95.37	14.74	15.09	2965.34	29.65
WPS	0.17	0.00	0.00	0.00	0.33	19.17	33.20	22.79	0.23
NSPP	2.35	0.11	0.36	0.25	0.30	13.93	25.56	15.65	0.16
SYPP	389.12	86020.74	89053.53	3032.79	0.97	75.37	76.69	152.60	1.53

PH= plant height, NB= number of branches, NI= number of internode, CD= colar diameter, NN= number of node, DF= days to flowering, DPP= days to pod production, NPP= number of pod per plant, PL= pod length, 100PW= 100 pod weight, 100SW= 100 seed weight, WPS= weight per seed, NSPP= number of seed per plant, SYPP= seed yield per plant

4.1.23 Pollen parameters estimates

The Table 4.9 below shows that TGX- 2018 -5E have the highest pollen viability value of 84.6 % and the least viability of 26.7 % as seen in accession TGX -2010 -11F. Most of these accessions were seen to be highly fertile. The highest sterility value was recorded in TGX- 2010- 11F with a value of 73.3 % and the least value of 15.4 % was recorded in TGX- 2018-5E. For pollen diameter, the result shows significant differences among accessions at 5 % level of significance with accession TGX- 2007- 3F having the highest value of 0.27 and accession TGX -1989- 19F having the least value of 0.21. However, there were no significant difference in accessions TGX-2018-5E, TGX-1988-5F, TGX-1485- 1D, TGX 2025-8E, TGX-2010 -11F and TGX- 2027- 1E; TGX- 2023- 4E, TGX -2017- 6E, TGX- 1987-62F and TGX-2004-9F; TGX 2011-6F, TGX -2016-4E, TGX -2008- 4F, TGX -1488-2E and TGX – 2019- 1E (Table 4.9).

Table 4.9 Pollen Viability and Diameter of the Soybean Accessions

Trait	Total pollen Count	Fertile (Stained)	Sterile (unstained)	Viability (%)	Sterility (%)	Pollen diameter
TGX-2018-5E	13.00	11.00	2.00	84.60	15.40	0.24±0.00 ^{bc}
TGX-2023-4E	19.00	11.00	8.00	57.90	42.10	0.24±0.00 ^{bc}
TGX-1988-5F	20.00	15.00	5.00	75.00	25.00	0.24±0.00 ^{bc}
TGX-1485-ID	23.00	19.00	4.00	82.60	17.40	0.25±0.00 ^d
TGX-2017-6E	9.00	7.00	2.00	77.80	22.30	0.24 ±0.01 ^{bc}
TGX-2019-IE	11.00	8.00	3.00	72.70	27.30	0.24±0.00 ^{bc}
TGX-2025-8E	17.00	12.00	5.00	70.60	26.30	0.24±0.00 ^{bc}
TGX-1448-2E	21.00	16.00	5.00	76.20	23.80	0.25±0.00 ^d
TGX-2008-4F	5.00	3.00	2.00	60.00	40.00	0.25±0.00 ^d
TGX-2016-4E	17.00	14.00	3.00	82.40	18.80	0.25±0.00 ^d
TGX-2010-11F	30.00	8.00	22.00	26.70	73.3	0.24±0.00 ^{bc}
TGX-2027-IE	22.00	16.00	6.00	72.70	27.3	0.24±0.00 ^{bc}
TGX-2009-11F	13.00	10.00	3.00	76.90	23.10	0.23±0.01 ^b
TGX-1989-19F	11.00	5.00	6.00	45.50	54.54	0.21±0.00 ^a
TGX-1987-62F	8.00	5.00	3.00	62.50	37.5	0.24±0.01 ^{bc}
TGX-2007-3F	15.00	10.00	5.00	66.70	33.30	0.27±0.01 ^e
TGX-2004-9F	10.00	7.00	3.00	70.00	30.0	0.24±0.00 ^{bc}
TGX-2022-4E	12.00	8.00	4.00	66.70	33.40	0.25±0.01 ^d
TGX-2011-6F	21.00	15.00	6.00	71.40	28.6	0.25±0.00 ^d

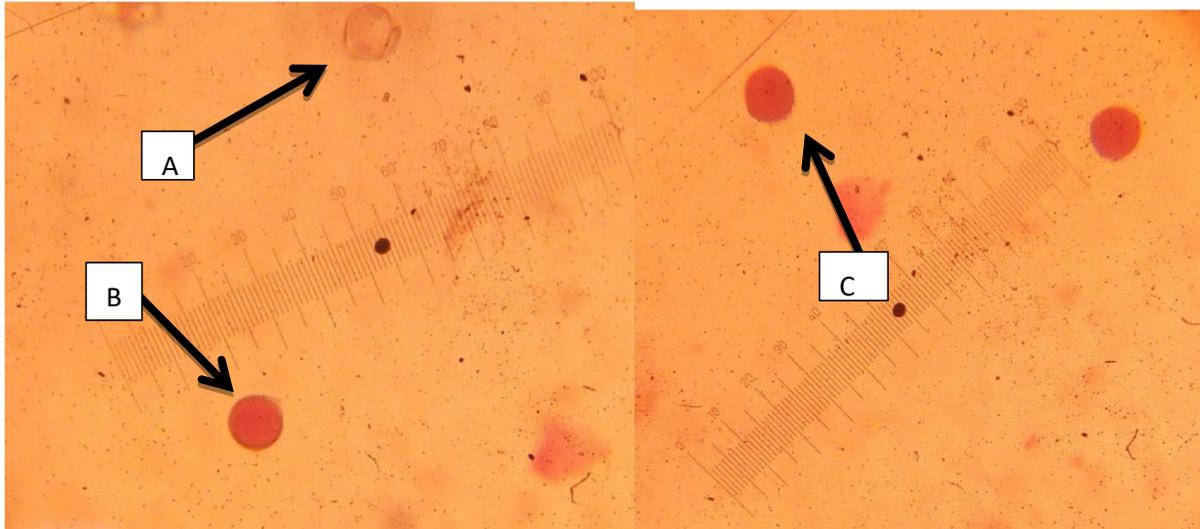


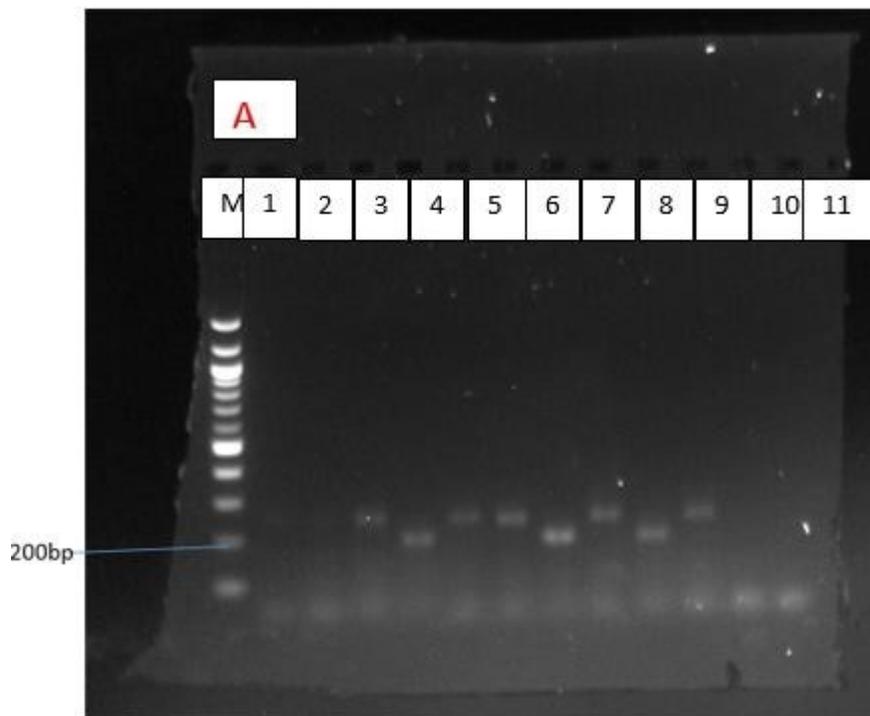
Plate 4.1: Fertile and non fertile pollens of soybean accessions B & C: Fertile pollen A: Unfertile pollen

4.1.24 Molecular characterization of selected soybean genotypes

In this study, Table 4.10 shows that all the 6 pairs of SSR markers produced a total number of 12 repeatable and scoreable polymorphic bands. DNA fragments ranged from 150 to 320 bp. The number of alleles per locus was within 1 to 3 with an average of 2.0. Although, Satt 596, Satt 572 and Satt 194 did not produce any polymorphic band, Satt 294, Satt 288 and Satt 222 were highly polymorphic, each producing three different alleles. Out of the six markers, Satt 294, Satt 288 and Satt 222 were considered suitable markers for detecting genetic diversity among individuals each having high gene diversity values. The highest and lowest gene diversity values were determined as 0.6446 and 0.4298 for Satt 288 and Satt 294, respectively with an average of

0.2782. Polymorphic Information Content (PIC) values were within average ranging from 0.3855 to 0.5721 with an average of 0.2473 (Table 4.10).

The evolutionary history inferred using the Neighbor-Joining method shows optimal tree with the sum of branch length of 1.63685646. Based on the phylogenetic dendrogram as presented in figure 4.2, the tree generated consisted of 2 major clades with each Claude consisting of branches. The major clade has 3 branches with TGX-2016-4E clustering with TGX-2017-6E and TGX-2019-1E clustering with TGX-1987-62F. The major branch on the other hand consists of a close cluster consisting of TGX-2007-3F and TGX-2010-11F and both related to TGX1448-2E. The second clade consists of a close cluster between TGX-2023-4E and TGX-2018-5E which are both closely related to TGX-2011-6F and TGX-2022-4E (Figure 4.2).



Primer satt 288

Plate 4.2: SSR Amplification of two DNA Primers

M= ladder and number 1 to 11 on the first row represent the soybean genotypes

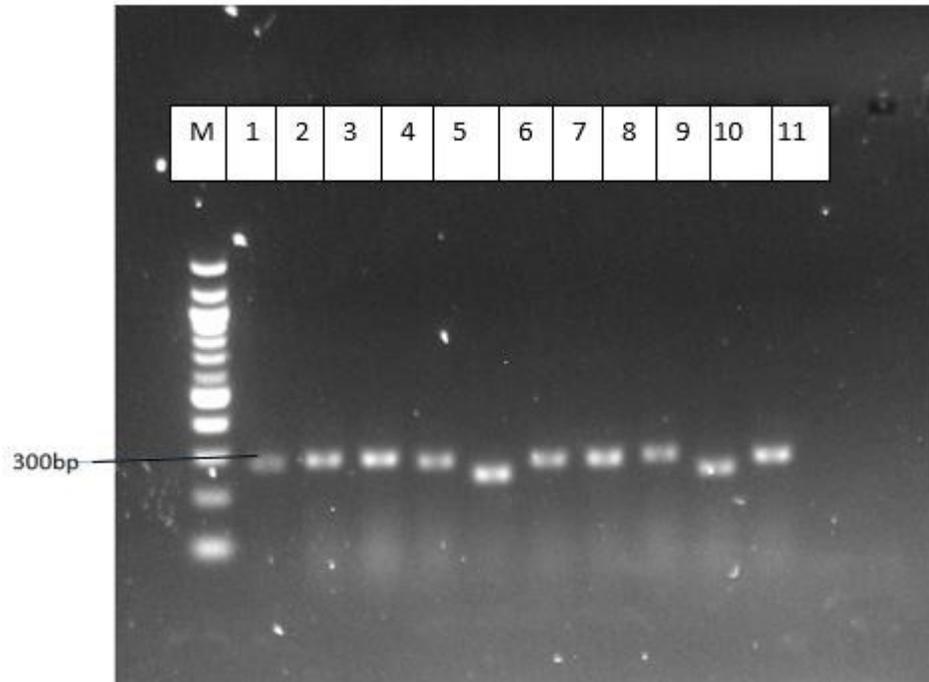
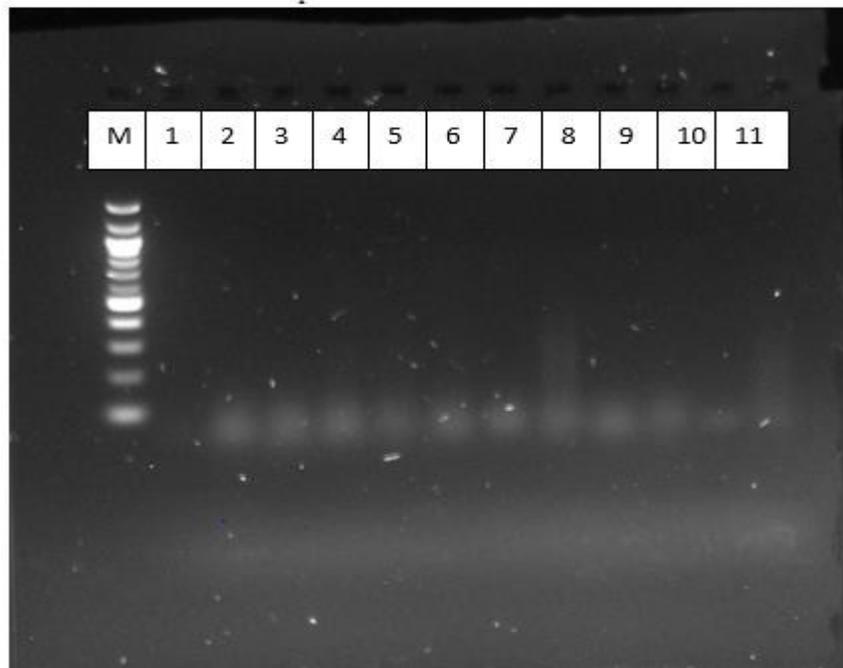


Plate 4.3: SSR Amplification of two DNA Primers



Primer 596

Plate 4.4: SSR Amplification of two DNA Primers

Table 4.10 SSR Molecular Parameters Detected by Different DNA Primers

Marker	Major.Allele.Frequency	SampleSize	AlleleNo	Availability	GeneDiversity	PIC
596	1.0000	11.0000	1.0000	1.0000	0.0000	0.0000
572	1.0000	11.0000	1.0000	1.0000	0.0000	0.0000
294	0.7273	11.0000	3.0000	1.0000	0.4298	0.3855
288	0.4545	11.0000	3.0000	1.0000	0.6446	0.5721
194	1.0000	11.0000	1.0000	1.0000	0.0000	0.0000
222	0.5455	11.0000	3.0000	1.0000	0.5950	0.5262
Mean	0.7879	11.0000	2.0000	1.0000	0.2782	0.2473

Table 4.11 Data Matrix of SSR-PCR Primers for the Selected Soybean genotypes

#	596	572	294	288	194	222
1	0	1	1/0	0/0	0	1/0
2	0	1	1/0	0/0	0	0/1
3	0	1	1/0	1/0	0	1/1
4	0	1	1/0	0/1	0	1/0
5	0	1	0/1	1/0	0	1/1
6	0	1	1/0	1/0	0	1/0
7	0	1	1/0	0/1	0	0/1
8	0	1	1/0	1/0	0	1/0
9	0	1	0/0	0/1	0	1/0
10	0	1	1/0	1/0	0	1/0
11	0	1	0/0	0/0	0	0/1

Table 4.12 Dice Dissimilarity Index among the Selected Soybean Genotypes

	1	2	3	4	5	6	7	8	9	10	11
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1	TGX-2019-1E										
2	TGX-1987-62F	0.00									
3	TGX-2022-4E	0.17	0.17								
4	TGX-2007-3F	0.17	0.17	0.44							
5	TGX-2017-6E	1.21	1.21	0.44	4.09						
6	TGX-2011-6F	0.17	0.17	0.00	0.44	0.44					
7	TGX-2010-11F	0.17	0.17	0.44	0.00	4.09	0.44				
8	TGX-2018-5E	0.17	0.17	0.00	0.44	0.44	0.00	0.44			
9	TGX-1448-2E	0.44	0.44	1.21	0.17	1.21	1.21	0.17	1.21		
10	TGX-2023-4E	0.17	0.17	0.00	0.44	0.44	0.00	0.44	0.00	1.21	
11	TGX-2016-4E	0.17	0.17	0.44	0.44	0.44	0.44	0.44	0.44	0.17	0.44

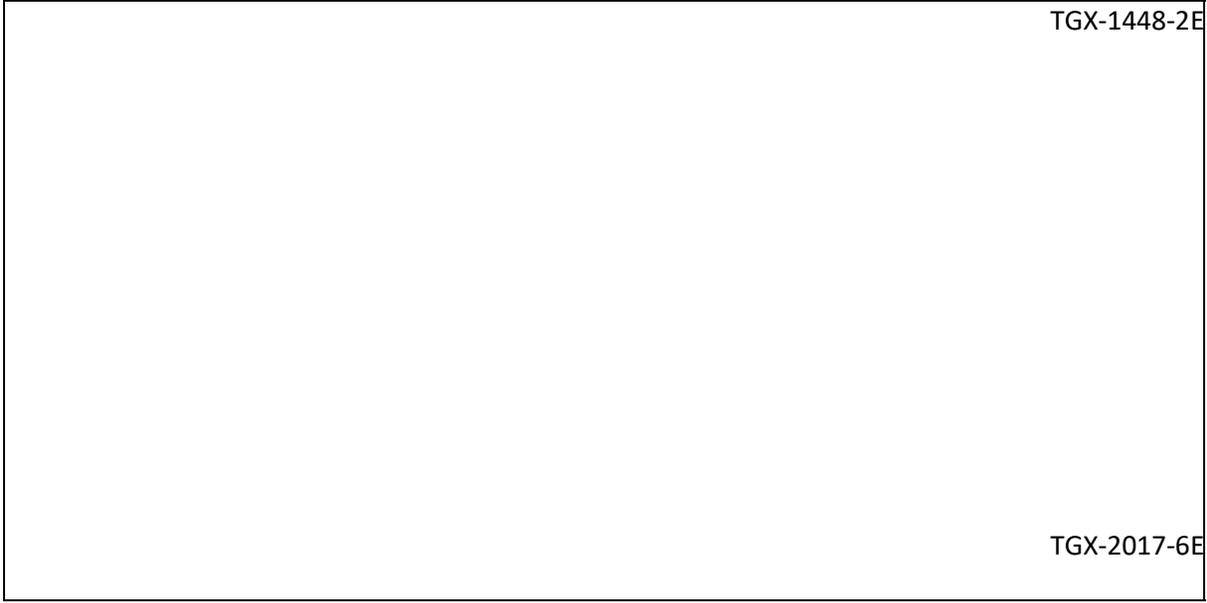
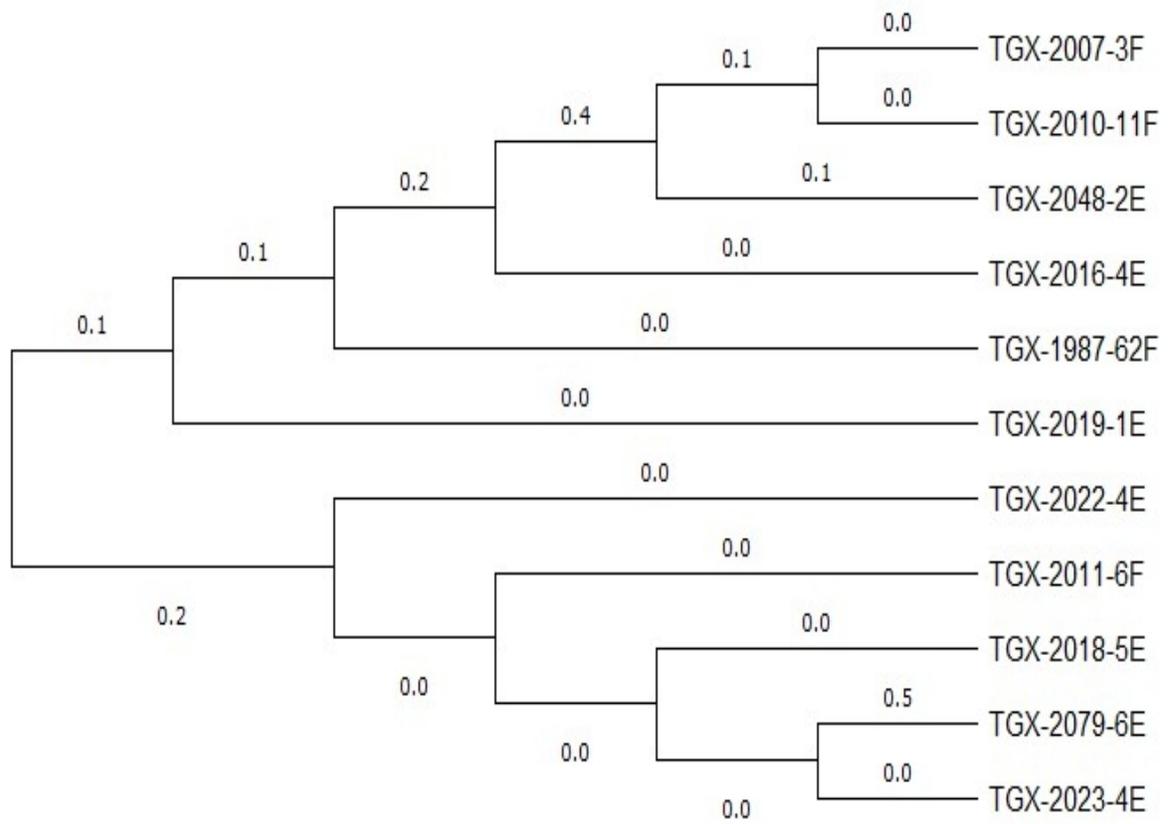


Figure 4.2 Dendrogram Based on UPGMA Analysis of Genetic Dissimilarity of Soybean Genotypes , showing relationship among them

4.2 Discussion

Characterisations of soybean accession based on variations in qualitative characters are useful for identification and avoid duplication of genotypes (Ramteke and Murlidharan, 2012). Qualitative characters are considered as marker character in the identification of soybean accession which are influenced by genes and less influenced by environmental fluctuations, as such, visually observed traits as seed shape and colour can be used to classify accessions into few broad groups (Arun- Kumar *et al.*, 2018) The dominance in terms of yellow cotyledon colour and purple throat collar colour observed in the study could be attributed to its abundance in the collection site. However, this predominant purple collar colour and yellow seed colour was earlier observed by (Ramteke and Murlidharan, 2012). The work of Gupta *et al.* (2010) substantiate that flower colour, seed colour, presence or absence of pod hair are the most stable characters across agro climatic zone.

The range of plant height at maturity (27.38 cm- 46.17 cm) with an average of 36.98 cm obtained in this study falls within the range reported by Pusha and Ketoswara (2013), 30cm-53cm with mean value of 38.35 cm. Also in agreement with these findings, Padameshwar (2006) reported the range of 21.12 cm – 78.12 cm with a mean value of 45.12 cm and Rajkumar *et al.* (2010) reported 19.93- 67.00 cm with a mean value of 45.12 cm. The close agreement of these results could be attributed to sources, ecology of seed collection and genetic diversity. However, contrary to these results Acquah (2007) opined that soybean plant varies from less than 0.2- 2.0

m in height. This might also be due to variation in genetic makeup of some of the soybean as well as variation in agro-ecological zones where experiment were performed.

Number of leaves per plant is an essential trait for photosynthesis and enhances/increases production in crop. In concordance with the findings of this study, the range number of leaves (37.47-43.49) earlier reported by Afolayan and Eguavon (2017) falls within the range value of 36.83-117.17 obtained. The variation in the value could be attributed to difference in cultural management, farm input such as fertilizer, insecticide, environmental and ecological. Variation was also reported by Bhagasara *et al.* (2017). Variation in genetic makeup could also results in the gross variations in these genotypes.

The range of number of branches 0.33-7.8 with a mean value of 3.53 obtained in this study similar that reported by Chandrawat *et al.* (2017) with range of 2.90-8.50 with a mean of 4.88; Pusha and Ketoswara (2013) 1.00- 4.96 with a mean value of 2.51. These variations among these results could be attributed to the differences in climatic condition where they were raised and other soil amendments.

The range of value for number of nodes 8.67- 14.83 is in agreement with the earlier report of Parameshwar (2006) (8.47-14.97). Number of Peduncle ranged from 1.50-27.50, this is in contrast to range of 11.00- 18.00 earlier reported by (Ajayi *et al.*, 2014) for cowpea. Peduncle length of 13.58-39.52 cm reported by Chandrawat *et al.* (2017) is also in contrast with the present study with a range of 0.13-1.85 cm. This variation could be attributed to difference in the genetic makeup of the crops.

Variation in days to anthesis (8.17-66.00) with a mean 45.66, this range falls within the range of 27.12-56.34 with a mean value of 38.15 reported by Parameshwar (2006); 30.67-57.33 reported by Rajkumar *et al.* (2010), and 30.00- 46.00 with a mean 38.35 for Pusha and Ketoswara (2013). This variation could be attributed to different in anthesis time, to photoperiod, sensitivity of the plant, its response to the environmental condition and genetic nature of the seed.

The range of Days to pod production is between 10.63-86.67 with a mean value of 61.46. Contrary to the result obtained in this study Parameshwar (2006), 74.65-109.12 with a mean value of 93.13; Chandrawat *et al.* (2017) 95.00-111.50 with a mean of 102.60. This difference could be attributed to variation in environmental factors of the environmental site and genetics constitution of the crop.

The differences observed among the accession of soybean in yield parameter are indication of variability in their genetic makeup. The range of number of pod per plant was 10.83-1.58 with an average mean of 84.07, this result is totally different from the report of Parameshwar (2006) with range 22.78-48.75; Pusha and Ketoswara (2013) with range 12.35- 49.56 and Chandrawat *et al.* (2017) with range 13.50 -50.88. This variation could be attributed to pollen fertility of the plant and absorption of pollen of other works.

The range of value 1.80- 3.00 for number of seed per pod is in agreement with the report of Pusha and Ketoswara (2013) with a range of 1.00- 3.00. The range value of 10.39-16.99 for 100 seed weight is slightly similar to the report of Pusha and Ketoswara (2013) with range 7.2615.38 but different from the work of Chandrawat *et al.* (2017). The range of 77.55-1167.67 for seed yield per plant is higher than the range of 3.34-14.97 reported by Pusha and Ketoswara (2013) and 2.71- 8.46 reported by Chandrawat *et al.* (2017). These variations might be attributed to

different ecological regions, planting seasons as well as pod shattering that might had occur at the experimental site

The coefficient of variation; Genotypic coefficient of variation GCV and phenotypic coefficient variation (PCV) gives information about the nature and magnitude of variation while heritability gives information about the inheritance of the character, Ali *et al.* (2016). The genotypic coefficient of variation is higher than environmental coefficient of variation for colar diameter, days to flowering, days to pod production and 100 seed weight. This shows that these traits were influenced by genetic factors more than the environmental factors, which further buttress the fact that high variability exist in the crop for morphological parameters. These observed variations could be attributed to both environmental and genetic factors since the PCV was high for all the traits studied. Ali *et al.* (2016) and Suliystyo and Mejaya (2018) also reported similar results and attributed it to the influence of environmental factors on the expression of the character.

The value of heritability is used to predict the progress of the selection of characters whether controlled by genetic factor or environmental factors, Ali *et al.* (2016). The high broad sense heritability in conjunction with moderate to high genetic advanced obtained for some of the studied traits indicate the importance and reliability of this characters for selection. High heritability coupled with moderate genetic advance has been attributed to equal importance of additive and non- additive gene action (Singh *et al.*, 2018). Therefore, the high heritability obtained number of branches, colar diameter, days to flowering; days to pod production, pod length and 100 seed weight in the study could be attributed to additive gene action of the characters and could be selected for crop improvement. Similar findings were reported by Malik *et al.* (2007); Aditya *et al.* (2013); Dilaneswa *et al.*(2013); Barh *et al.*(2014); Ali *et al.* (2016);

and Suliystyo and Majaya (2018). Salihu *et al.* (2017) reported that selection based on these traits could assist in successful isolation of desirable genotype for crop improvement.

High fertility and viability observed in this study buttress the fact that they are good source of hybridization for crop improvement. Olaoye *et al.* (2014) also reported similar findings in tomatoes. The pollen diameter from this study ranges from 0.21-0.27. These result shows that the characters are fairly uniform within the accessions. This result is in contrast to the work of Olaoye *et al.*, (2014) who reported a range of 1.93- 2.57. These variations could be attributed to the difference in sample. According to Animasaun *et al.* (2014) utilization of the knowledge of pollen viability as selection criterion for high yield could provide vital information for effective breeding programme

SSR markers are efficient for measuring genetic diversity and relatedness as well as identifying varieties of soybean (Tantasawat *et al.*,2011).The low mean polymorphic information content (PIC) of 0.25 obtained for all the SSR markers used indicated the ineffectiveness of the markers used on the selected Soybean accessions. Ahmad *et al.* (2015) reported that marker is effective if the PIC value is higher than 0.5. The ranged of polymorphic information content 0.38-0.57 recorded in the present study is slightly similar to the work of Sudeshina *et al.* (2018), who reported PIC range from 0.4 -0.9 on 40 genotype using 34 primers, similarly Ghosh *et al.*(2014) also reported arrange of 0.1-0.4 for 32 genotypes using 10 primers. The slight variations could be attributed to the number of genotypes and primers used.

High number of alleles and high polymorphism are very important for correct estimation of genetic diversity and effectiveness of marker development (Pfieifer *et al.*, 2011). The allele number in this study ranges from 1-3 with an average of 2.0 indicate the high specific and reproducible nature of the SSR markers used and also shows that genetic variability exist among

the studied soybean samples . Koutu *et al.* (2019) reported an allele range of 2-6 with an average of 4.0. Similarly, Kumar *et al.* (2009); Singh *et al.* (2010) and Bisen *et al.* (2015) also had range of 2.0- 6.0 with an average of 4.0.

The gene diversity obtained in this present study (0.0-0.6) signifies high genetic diversity among the chosen genotypes. This result is in accordance to the findings of Ghosh *et al.* (2014) 0.0- 0.63 and Chauhan *et al.*(2015). The Clustering of the accessions into two major clade indicates that most of the accessions are similar in their genetic constitution and geographical origin.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This present study has provided information on the vast variability that exists among soybean selection. Accessions TGX- 2027-1E, TGX- 1448-2E, TGX – 1485-1D, TGX- 2022-4E, and TGX-2016-4E showed excellent agro- morphological parameters and can be utilized for effective breeding programs of soybean in Nigeria.

High genotypic variance and high broad sense heritability as well as genetic advance as percentage of mean (GAM) for 100 seed weight, Days to Pod production, colar diameter and days to flowering indicates that these characters can be selected for further breeding programme

High pollen viability observed in accessions TGX- 2018-5E, TGX-1485-1D and TGX- 2016- 4E results in high seed yield; they can serve as source of gene for hybridization for higher yield.

The high polymorphic information content (0.53and 0.57) produced by primers 222 and 288 respectively indicates their reliability and effectiveness in determination of diversity among soybean accessions. This further buttressed the variation detected by agro morphological parameters.

5.2 Recommendations

- I. Accessions TGX- 2027-1E, TGX- 1448-2E, TGX – 1485-1D, TGX- 2022-4E,and TGX- 2016-4E is recommended for commercial cultivation for its high yield traits.
- II. Recurrent selection of the high yielding accessions should be done until a superior trait is attained for its improvement and utilization

- III. Further research should be carried out on the amino acid composition, oil content and fatty acid composition on the soybean accessions.

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Appendix

PLANT HEIGHT

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
WEEK2	Between Groups	73.651	18	4.092	2.065	0.014
	Within Groups	164.488	83	1.982		
	Total	238.139	101			
WEEK4	Between Groups	363.323	18	20.185	1.83	0.035

	Within Groups	904.578	82	11.031		
	Total	1267.9	100			
WEEK6	Between Groups	1457.052	18	80.947	2.094	0.013
	Within Groups	3170.04	82	38.659		
	Total	4627.092	100			
WEEK8	Between Groups	2677.656	18	148.759	1.75	0.047
	Within Groups	6970.338	82	85.004		
	Total	9647.994	100			

NO OF LEAVES

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
WEEK2	Between Groups	85.828	18	4.768	1.853	0.032
	Within Groups	213.583	83	2.573		
	Total	299.412	101			
WEEK4	Between Groups	416.816	18	23.156	2.17	0.01
	Within Groups	874.967	82	10.67		
	Total	1291.782	100			
WEEK6	Between Groups	9387.796	18	521.544	5.311	0
	Within Groups	8051.967	82	98.195		
	Total	17439.762	100			
WEEK8	Between Groups	49404.335	18	2744.685	4.632	0
	Within Groups	48589.15	82	592.551		

Total	97993.485	100
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NO NODES

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
WEEK2	Between Groups	253.412	18	14.078	7.183	0
	Within Groups	162.667	83	1.96		
	Total	416.078	101			
WEEK4	Between Groups	28.264	18	1.57	1.814	0.037
	Within Groups	70.983	82	0.866		
	Total	99.248	100			
WEEK6	Between Groups	506.718	18	28.151	3.003	0
	Within Groups	768.767	82	9.375		
	Total	1275.485	100			
WEEK8	Between Groups	250.004	18	13.889	2.874	0.001
	Within Groups	396.233	82	4.832		
	Total	646.238	100			

ANOVA AT WEEK 8

		Sum of Squares	Df	Mean Square	F	Sig.
BRRANCHES AT WK8	Between Groups	455.421	18	25.301	5.577	0
	Within Groups	431	95	4.537		
	Total	886.421	113			
INTERNODES AT WK8	Between Groups	1002.351	18	55.686	4.902	0

	Within Groups	1079.167	95	11.36		
	Total	2081.518	113			
COLAR DIAMETER WK 8	Between Groups	407.853	18	22.659	5.265	0
	Within Groups	408.871	95	4.304		
	Total	816.724	113			
COLAR DIAMETER AT MATURITY	Between Groups	1096.114	18	60.895	7.238	0
	Within Groups	799.305	95	8.414		
	Total	1895.419	113			
NJUMBER OF PENDUCLE WK8	Between Groups	6682.737	18	371.263	4.03	0
	Within Groups	8752	95	92.126		
	Total	15434.737	113			
PEDUNCLE LENGTH WK8	Between Groups	20.934	18	1.163	2.879	0
	Within Groups	38.38	95	0.404		
	Total	59.314	113			

YEILD PARAMETERS

ANOVA

		Sum of Squares	Df	Mean square	F	Sig.
DTF	Between Groups	17299.158	18	961.064	6.374	0
	Within Groups	14324.5	95	150.784		
	Total	31623.658	113			
DTPP	Between Groups	32698.614	18	1816.59	7.164	0
	Within Groups	24087.667	95	253.554		
	Total	56786.281	113			

DTFF	Between Groups	6578.035	18	365.446	6.15	0
	Within Groups	2258	38	59.421		
	Total	8836.035	56			
MATURITY75	Between Groups	16711.228	18	928.402	4.303	0
	Within Groups	8199.333	38	215.772		
	Total	24910.561	56			
NPPP	Between Groups	771218.877	18	42845.493	5.036	0
	Within Groups	323299.333	38	8507.877		
	Total	1094518.211	56			
HPW	Between Groups	5109.251	18	283.847	2.485	0.003
	Within Groups	8680.587	76	114.218		
	Total	13789.838	94			
HSW	Between Groups	252.294	18	14.016	62.715	0
	Within Groups	16.986	76	0.223		
	Total	269.279	94			
WPS	Between Groups	0.088	18	0.005	2.969	0
	Within Groups	0.125	76	0.002		
	Total	0.213	94			
NSPP	Between Groups	10.337	18	0.574	2.273	0.007
	Within Groups	19.2	76	0.253		
	Total	29.537	94			
