GENETIC STUDIES ON DATE PALM (*Phoenix dactylifera* L.*)* GERMPLASM GENE POOLS FROM JIGAWA, NIGERIA

BY

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ABSTRACT

Date palm is cultivated in arid and semiarid regions worldwide. In Nigeria, date palm is one of the most important tree crops of great socioeconomic importance in the Sahel, Sudan and Guinea Savannah ecologies. In order to assess the genetic diversity of the crop, survey and exploration were undertaken to collect the fruits from the populations of the female tree germplasm across the gene pools of the Nigerian Institute for Oil Palm Research (NIFOR), date palm research substation located in Dutse, Jigawa state, Nigeria. A total of 21 accessions were randomly collected across the gene pools. The fruits collected were analysed for their proximate and phytochemical compositions. The accessions were also evaluated for morphological characteristics using completely randomised block design (CRBD), Quantitative and qualitative characters were taken for the fruits following standard procedures. Random amplified polymorphic DNA (RAPD) technique was used to determine genetic diversity and sex identification. The cytological analysis was carried out using standard procedures. Results of the proximate composition showed that the accession R13P5 had the highest moisture content of 7.65 %. The highest carbohydrate content was observed in R4P12 with 82.32 %. The result of the mineral analysis showed that R24P9 had the highest calcium, magnesium, sodium and potassium with 119.39, 80.55, 13.44 and 423 mg/100 g respectively. The sugar content analysis showed that R5P8 had the highest glucose (320 mg/g) content while R15P6 recorded highest in fructose and sucrose (102 and 92 mg/g) content respectively. At late seedling stage, the highest plant height and girth size were observed in accessions R13P5 (39.00 cm) and R5P8 (10.00 cm). The results of the fruit characteristics showed that R7P1 had the highest fruit weight, fruit diameter and fruit thickness of 11.85 g, 2.10 mm, and 4.00 mm, respectively. The molecular diversity results revealed a total of 125 amplified fragments with 6 primers, of which 106 (84.3 %) were polymorphic and 19 (15.2 %) were monomorphic. Out of the total of 21 accessions screened for sex determination, 12 accessions (R13P1, R4P12, R5P8, R3P22, R1P18, R7P1, R13P5, R13P9, R14P21, R4P29, R16P31 and zariya showed male pattern and 9 accessions (R24P9, R5P20, R2P4, R9P2, R6P20, R9P21, R5P24, R5P6, R1P10) were observed to be female. The morphological variations in the male accessions revealed a larger girth size and higher number of lower leaf spines while the female accessions showed a smaller girth size with little to absence of lower leaf spines. Cytological examination of some of the accessions confirmed 2n=36 number of chromosomes in accessions (R5P8 and R16P31) and 2n = 28 chromosomes in accession R1P18 with metacentric and submetacentric chromosomes respectively. The high genetic variability observed among the date palm germplasm for proximate, morphological and molecular characterisation has provided baseline information among the date palm germplasm in the gene pools. The developed sex determinant qualitative marker could be used for gender identification at the seedling stage of date palms in order to save time. Plant breeders and growers may adopt this marker as a potential tool for gender identification in date palm seedlings before they are transplanted in the field.

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ABBREVIATIONS, GLOSSARIES AND SYMBOLS

ANOVA	Analysis of Variance
DMRT	Duncan Multiple Range Test
IITA	International Institute of Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute
RAPD	Random Amplified Polymorphic Dna
AFLP	Amplified Fragment Length Polymorphism
SSR	Simple Sequence Repeats
ISSR	Inter- Simple Sequence Repeats

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Date fruits are the products of date palm tree (*Phoenix dactylifera* L.), belonging to the family Arecaceae. It is one of the oldest cultivated plants in the world and is the most important subsistence crop in Northern Africa and the Middle East, although it is also cultivated in other parts of the world (Al-Shahib and Marshall, 2003). In Nigeria, date palm is one of the most important tree crops in Sahel, Sudan and Guinea Savannah ecologies where it has remained restricted within compounds, homesteads and orchards in the Northern part of the country i.e. above latitude 10^{o}N (Okolo *et al.*, 2005). It is generally believed that the date palm was introduced into Nigeria in the early 17th century by traders and Muslim pilgrims on pilgrimage to the holy cities of Mecca and Madinah through the Trans-Saharan trade route from North Africa and Middle East (Omoti and Okolo, 2000).

Although the date palm is not indigenous to Nigeria, but with over 400 years of its existence, the crop has been cultivated for sufficiently long time to have acquired agro – climatic adaptation and so evolved as landraces. Most national collections of date palm germplasm rely primarily on these land races. In Nigeria, several collection missions have been undertaken to capture these land races by the Nigerian Institute For Oil Palm Research (NIFOR) for date palm germplasm conservation and crop improvement (Ataga *et al.*, 2012). The States where date palm is grown in Northern Nigeria include Kaduna, Katsina, Kano, Sokoto, Kebbi, Jigawa, Yobe, Borno, Gombe, Bauchi and Adamawa. These are generally referred to as the Nigerian main date palm growing belt of the country. Other States include Plateau, Taraba, Nasarawa, Kaduna and Niger. These states are classified as

marginal areas for date palm cultivation in the country (Abdulqadir *et al.*, 2011). Date production in Nigeria has two fruiting seasons (dry season, that is from February to June; and wet season, from July to August), but only the dry season fruit is economically useful (Abdulqadir *et al.*, 2011). Little or no research is carried out on wet season date fruit because it is harvested and consumed during the season in Nigeria.

The date palm is dioecious, which means there are separate male and female plants, therefore pollination is required for fruit bearing (Maria *et al.,* 2014). The flowers are yellowish, small and attached directly to the spikelets; male flowers are sweet-scented and have six stamens while the female flowers consist of three carpels with ovules, of which normally only one will develop into a fruit.

For fruit setting, fertilisation of the female flowers by male pollen is required. In date palm, pollination is not left to the wind or insects but is done traditionally by man where they insert a piece of spikelet of male flower at the moment when the female flowers are getting open. Besides traditional method, pollination can be done using machines, which has made the process quicker, easier, and efficient (Huntrods, 2011).

Date trees typically reach about 21–23 metres (69–75 ft) in height, growing singly or forming a clump with several stems from a single root system. This tree typically has feathery leaves at the top which may be anywhere from 0.9 to 1.5 feet (3 to 5 m) in length. The trunk is usually very long and narrow with rough-textured bark over most of its surface. There are no branches on a date palm tree. The leaves normally cascade downward from the crown of the plant to form a sort of canopy. They might spread anywhere from 19.7 to 32.8 feet (6 to 10 m) in diameter. This can often provide some shade for a person to

sit in or for other small plants to grow in. This tree is believed to have originated in Africa or Asia (Wisegeek, 2011). Date fruits (dates) are oval-cylindrical, 3–7 cm long, and about an inch (2.5 cm) in diameter, ranging from bright red to bright yellow in colour, depending on variety. They are very sweet, containing about 75 percent of sugar when dry (Divya and Bichu, 2015).

Germplasm is a term used to describe living genetic resources such as seeds or tissue, maintained for the purpose of breeding, preservation and research. These resources may take the form of seed collections stored in seed banks, trees growing in nurseries, animal breeding lines maintained in animal breeding programmes or gene banks, etc. It provides potential diversity base in genetic resources of cultivated plants (Mahmut, 2012). Germplasm collections can range from collections of wild species to elite, domesticated breeding lines that have undergone extensive human selection.

Genetic diversity is the sum total of genetic characteristics within any species or genus (Rao and Hodgkins, 2002). Genetic diversity is required by breeders for the development of new, superior crop varieties with desirable qualities that can ensure a stable, abundant supply of food, feed and fibre (Jain *et al.*, 2004). Therefore, genetic studies cannot be conducted if species do not show any variation. It is clear that gene erosion is a very dangerous and alarming feature of present-day exploitation of genetic resources (Bennett, 1965). This prospect alarms both geneticists and breeders, since lack of diversity severely impairs the future improvement of crops and/or limits the possibilities for addressing new production constraints (Vetelainen *et al.*, 2008). The progress of any genetic preservation is dependent on understanding the amount and distribution of the genetic variation present in the gene pool (Jubrael *et al*., 2005).

Date fruits have great importance in human nutrition owing to their rich content of essential nutrients which include carbohydrate sugar, ranging from 65% to 80% on dry weight basis, and mostly of inverted form i.e glucose and fructose (Aldjain *et al.,* 2011). In addition, date fruit has been reported to have other important components like proteins, fat, vitamins, dietary fiber, fatty acids, polyphenols, antioxidant and amino acids (Chandrasekaran *et al.*, 2013). Date palm fruits have been reported to contain natural constituents like phytochemicals, sterols, carotenes and flavonoids, and have been screened for various medicinal activities to reduce the side-effects of artificial drugs that bring harm to human body systems. The pulp is rich in iron, calcium,cobalt, copper, fluorine, manganese, sodium, copper and zinc.

Morphological characters of the tree are taken into consideration for cultivar identification. For the male trees, identification is a cumbersome process because they are mostly are seed-borne and are hardly identical to any female cultivar. Date palm varieties are very similar; however, studies have shown that there are clear differences based on the vegetative characteristics and spathe (Djerouni *et al*., 2015). Since the palm leaf components look very different, the measurements which were taken from leaf palm like thorn length, pinna number and leaf palm length have shown the similarities and the differences between the palms (Saker *et al*., 2006, Haider *et al*., 2015). In addition, several cultivars of date palm have been reported to be nearly morphologically similar and are of similar features (Ali *et al.,* 2008).

The use of DNA markers provides a powerful tool to certify the identity of date palm cultivars at the seedling stage through the variety of fingerprinting. Random Amplified Polymorphic DNA (RAPD) markers can be used for fast screening of nuclear genome

variations (Williams *et al.*, 1990). RAPD markers have been used for germplasm characterisation in date palm (Tirfi *et al.*, 2000; Askari *et al.*, 2003; Soliman *et al.*, 2003).

Moreover, Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Restriction Fragment Length Polymorphism (RFLPs), have been used to characterise date palm genotypes (Sedra *et al*., 1998; Ben Abdallah *et al*., 2000; Tirfi *et al*., 2000). Random amplified polymorphic DNAs (RAPD) are DNA fragments amplified by the polymerase chain reaction using short (usually 10 bp) synthetic primers of random sequence. RAPD markers have been used for identification and DNA fingerprinting of date palm varieties, (El-Tarras, 2007).

In Nigeria, sex determination in date palm at seedling stage has been very difficult to accomplish by date palm growers. Thus, many efforts have been reported to ascertain the sexes of date palm plants. Al-Mahmoud *et al.* (2012) reported that the regions involved in sex determination in date palm employ a XX/XY (2n = 36) system with the male being heterogametic. Furthermore, they observed that the critical regions showed significant polymorphism between the male and female alleles. This polymorphism can be used in the development of assays to distinguish sexes at an early stage. Noppharat and Peerasak (2018) also used sex-specific DNA markers and concluded that such markers produced a reliable technique for sex determination in date palm, which could be used to predict the sex of date palm at the seedling stage. They also reported that this technique was easy to apply and accurate when tested repeatedly. It has shown to be successful when used to screen a large number of seedlings with results matching the theory of cross-breeding in dioecious plants where the ratio of male to female is 1:1.

1.2 Statement of the Research Problem

Despite the nutritional and economic potentials of date palm trees, the genetic resources have not been well utilised in enhancing the nutritional qualities of the crop. There is no significant information regarding the phytochemical study (e.g phytochemical screening) of the date fruits available in the germplasm gene pool in Jigawa, Nigeria. Morphological parameters are not sufficient to distinguish the closely related cultivars before fruiting. However, a large number of phenotypic characters need to be scored in date palms which are very laborious and time-consuming. Despite the fact that the germplasm gene pools at Jigawa have the largest diversity of date palm in Nigeria, the genetic diversity of the crop is yet to be ascertained, most especially using molecular markers.

The dioecy in plants represents the major challenge in development of breeding programmes especially in crops like date palm. In Nigeria, although there is an increasing research efforts on a number of different plant species, very limited information is available on the molecular basis of gender determination of date palm at seedling stage so that growers can cultivate in their orchards a sufficiently large number of productive female trees with only a minimal number of male trees.

In Nigeria, inspite of many uses of date palm as an economically important crop, its cytogenetic study is rarely published because of the lack of understanding of the chromosomal behaviour in order to facilitate breeding for its improvement. Thus, Tahira *et al.* (2019) earlier reported that the limited number of roots, small and numerous numbers of chromosomes and the slow growth nature of palm trees have rendered the cytogenetic studies in date palm difficult.

1.3 Aim and Objectives

The aim of this study was to evaluate genetic characteristics of the date palm (*Phoenix dactytlifera* L.) germplasm gene pools in Jigawa State.

The objectives of this study were to:

(i) quantify the proximate and phytochemical compositions of the different date palm fruits in the gene pool of Jigawa state.

(ii) determine the morphological parameters and fruit characteristics of date palm samples.

(iii) characterise the germplasm of date palm gene pool from Jigawa State into different genotypes using random amplified polymorphic DNA (RAPD) marker.

(iv) determine the sex of date palm germplasm at seedling stage using sex specific RAPD marker

(v) determine the cytological relationships among the accessions.

1.4 Justification for the Study

Date fruit is marketed all over the world as a high-value confectionery and remains an extremely important subsistence crop in most of the desert regions (Nadeem *et al.,* 2011). Understanding the nutritional composition of various genotypes of the date palm within the gene pool would enhance and facilitate the consumption of date fruits; this will undoubtedly play an important role in human health since they may be a rich source of minerals and anti-oxidants which can serve as a great source of energy. Due to the high sugar content in the pulp, they can be used as a substitute for sugar in the food industry (Ricardo *et al.,* 2019). The scientific clarification of the phytochemical screening in date

fruits will provide information on dates as an alternative source of natural anti-oxidants that could improve health status.

Morphological parameters will be informative for description, cultivar characterisation, phenotypic diversity analysis and phylogenetic relationship exploration among date-palm ecotypes. Moreover, phenotypic diversity evaluation constitutes an available basic step for the elaboration of a programme to improve germplasm management and utilisation of the crop (Eissa *et al.,* 2009). The use of data based on molecular markers such as Random Amplified Polymorphic DNA (RAPD) in the characterisation of date palm genotypes and for sex determination would accelerate genetic improvement and phylogenetic relationships in closely related groups of fruit trees through marker-assisted selection. Characterisation into main known genotypes is therefore, necessary to know some existing ones. This would be most important for speeding up breeding and thereby saving time, cost and other resources. Similarly, the use of data based on molecular markers such as Random Amplified Polymorphic DNA (RAPD) in the characterisation of date palm genotypes and for sex determination would accelerate genetic improvement and phylogenetic relationships in closely related groups of fruit trees through marker-assisted selection.

Cytology is still considered an important technique for the characterisation of plant species. The chromosomal data of date palm will serve as an important tool in understanding the similarities and differences on the basis of chromosome number, shape and size during chromosomal evolution and genetic diversity of the crop.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Biology of Date Palm

The date palm fruit (*Phoenix dactylifera* L., 2n = 36) is a perennial monocotyledonous fruit plant, belonging to the family of Arecaceae (Barrow, 1998). Date palm tree is an excellent crop for cultivation in arid and semi-arid regions of the world due to its high tolerance to environmental stresses (Krueger, 1995).The date palm has been cultivated and subjected to selection by man since ancient times and the distinction between wild and cultivated is blurred (Krueger, 2001). Within the genus *Phoenix*, it is generally accepted that there are 12 – 13 species (Barrow, 1998). Wild *Phoenix* species are found in the tropics and sub-tropics of Africa and Asia while *Phoenix dactylifera* originated in the Middle East, Western India and Iraq (Barrow, 1998).

The date palm develops a cylindrical, unbranched and large stem (10-20 m tall) marked with leaf scars, and generally produces basal suckers. The adult date palm has a crown of up to 100-125 green leaves with 150 pinnae, and having acanthophylls on the petiole. Leaves, pinnae and acanthophylls vary in length depending on cultivars (Chao and Krueger, 2007). Only female trees yield fruits and fifty female trees can be pollinated manually by one male tree. *Phoenix* spp are dioecious, with the inflorescences arising from the leaves. The small, pale yellowish flowers are borne singly, with the sepals being united into a cupule. There are 3 petals. Female flowers have 3 carpels, only one of which matures; male flowers generally have 6 stamens. The fruits of *Phoenix* spp are drupes of variable size, depending on the species, with a single grooved seed (Krueger, 1995).

In Nigeria, date palm is one of the most important tree crops of Sahel, Sudan and Guinea Savannah ecologies, where it has remained restricted within compounds, homesteads and orchards in the northern part of the country i.e. above latitude 100^0 N (Okolo *et al.*, 2005). It is generally believed that the date palm was introduced into Nigeria in the early 17th century by traders and Muslim pilgrims on pilgrimage to the holy cities of Mecca and Madinah through the Trans Saharan trade route from North Africa and Middle East (Omoti and Okolo, 2000), It is essentially cultivated for its edible fruits which are very nutritious and energy-producing in Nigeria.

Although the date palm is not indigenous to Nigeria, but with over 400 years of its existence, the crop has been cultivated for sufficiently long time to have acquired agro – climatic adaptation and so evolved as landraces. Most national collections of date palm germplasm rely primarily on these landraces. Several collection missions have been undertaken to capture these landraces by the Nigerian Institute for Oil Palm Research (NIFOR) for date palm germplasm conservation and crop improvement in Nigeria.

2.2 The Genus *Phoenix*

The genus *Phoenix* is a monocotyledonous angiosperm, which is woody and diploid (2n=2x= 36). It is a dioecious species of the Arecaceae (palm family) family. There is a confusion regarding the exact number of species in the genus *Phoenix* (Abul-Soad *et al.*, 2017). *Phoenix* spp are either single-trunked or clumping. Trunks range in size from nearly trunkless to over 30 m. *Phoenix* spp may be distinguished from other palms having feather-type leaves by the modification of the basal leaflets into spines, the presence of a terminal leaflet, and a central fold or ridge on the leaflets, which cause the leaflets to remain erect at all times. *Phoenix* spp are dioecious, with the inflorescences arising from among the leaves.

The small, pale-yellowish flowers are borne singly, with the sepals being united into acupule. There are 3 petals. Female flowers have 3 carpels, only one of which matures; male flowers generally have 6 stamens. The fruits of *Phoenix* spp are drupes of variable size depending on the species, with a single grooved seed.

The taxonomy of *Phoenix* is not well established and lacks an authoritative treatment. There is disagreement between various taxonomic treatments and some confusion about species names and validity. *Phoenix* spp hybridise readily, which can lead to confusion, especially when several species are present, as may occur in *ex-situ* collections. There is some suggestion that all species should be treated as a single species (Wrigley, 1995). The genus *Phoenix* was considered as monotypic because different *Phoenix* species hybridise readily and produce fertile hybrids. The genus *Phoenix* is the only member of the tribe *Phoeniceae*. Dioecy is a rare sexual system in flowering plants, with only 4-6% of all plant species being dioecious.

Phoenix spp. may be distinguished from other palms by a number of morphological features: the leaves have feathery surfaces; the basal leaflets are modified into spines; there is a terminal leaflet for each leaf; and there is a central fold or ridge on the leaflets which causes them to remain erect at all times. The inflorescences in the *Phoenix* spp arise from among the leaves; the small, pale yellowish flowers are borne singly; the sepals of each flower are fused into a cupule (cup-shaped structure), and there are three petals per flower. In this dioecious plant, male and female flowers are borne on separate plants. Female flowers have three carpels, only one of which matures; whereas, male flowers usually have six stamens. The fruits of *Phoenix* spp. are drupes of variable sizes, depending on the species, and each fruit has a single grooved seed.

2.3 Genetic Diversity of Date Palm

The genetic improvement of a crop species depends on the ability to select promising plant material. To facilitate the selection process, molecular markers that are associated with important traits can be used as selection tools (Eissa *et al.,* 2009). According to Chaludvadi *et al.* (2014), genetic diversity in date palm may be the result of dissemination of the germplasm with human migration, human selection and clonal propagation. They reported that commercial cultivars of date palm have been disseminated by offshoots from oasis situated in the centre of origin and diversity of date palm in lower Mesopotamia and eastern Arabia. Cultivars propagated by offshoots are almost similar, whereas the less important and non-commercial cultivars are result from seed dissemination.

In addition, Soliman *et al.* (2006) suggested that molecular markers can be used to establish genetic maps, which in turn are important tools for more refined marker-assisted selection in breeding programmes as well as for in-depth genetic and systematic analyses. Soliman *et al.* (2006) reported that an integrated approach is needed in incorporating genetic studies to improve the knowledge of date palm taxonomy and diversity. They also suggested that proteins and/or DNA attributes could be used successfully for variety identification, and for studying the genetic diversity of date palm cultivars. This crop can be promoted best through better characterisation and evaluation.

Morphological traits have been used to describe the genetic variation in date palm cultivars which are mainly related to the fruit and influenced by the environment (Askari and Al-Khalifah, 2003). Furthermore, biochemical studies, such as isozyme analyses of peroxidases, have been used to characterise date palms genetically in Morocco and Tunisia (Majourhat *et al.,* 2002). This has also been reported by Elshibli and Korpelainen (2008)

on genetic diversity in Sudan germplasm representing 37 female and 23 male accessions using 16 SSR primers

2.4 Nutritional Importance of Date Palm

Proximate analyses have been reported to give the overall nutritional composition of the date fruit sample; this has briefly been complemented by anti nutrient and mineral composition (Adesuyi *et al.,* 2012). Hamad *et al.* (2015) reported that fresh varieties have a higher content of inverted sugars; the semi dried varieties contain equal amount of inverted sugars and sucrose, while dried varieties contain higher sucrose. Date fruits have great importance in human nutrition owing to the rich content of essential nutrients which include carbohydrate sugar ranging from 65% to 80% on dry weight basis and mostly of inverted form i.e glucose and fructose, (Ahmed *et al.,* 2018).

Chandrasekaran *et al.* (2013), reported that the nutritional value of dates was due to their high sugar content as well as other important micro and macro-nutrients such as potassium (2.5 times more than bananas), calcium, magnesium and iron. Other important components are proteins, fat, vitamins, dietary fiber, fatty acids, polyphenols, antioxidant and amino acids. Date fruits have been reported to contain about seven fatty acids of which three were 11.38 % saturated, three were 58.06 % mono-unsaturated and one was 30.56 % poly-unsaturated. The most abundant fatty acids were oleic acid (52.34 %), linoleic acid (30.56 %), palmitic acid (6.75 %), vaccenic acid (4.8 %) and stearic acid (3.98 %).

Dates are a good source of dietary fiber which, depending upon the variety and stage of ripening, it ranges from 6.4 % to 11.5 % in 14 different varieties (Al-Shahib and Marshall, 2003). Some of the low-quality dates, which are used for industrial purposes, have been

found to contain up to 10% of crude fiber (Barreveld, 1993). Dietary fiber content of dates can further contribute to their nutritional significance as dates can be used in the preparation of fiber-based foods and dietary supplements. Daily intake of 100g of dates can meet the 32% of recommended dietary allowance for dietary fiber (Marlett *et al.,* 2002).

Parvin *et al.* (2015) reported that date fruit has been recommended in folk remedies for the treatment of various diseases like diabetes, obesity, cancer and heart diseases. Recently, it has been found that date fruits might be of benefit in glycemic and lipid control of diabetic patients. They have also been identified as having antioxidant and anti-mutagenic properties of poly-phenolic compounds and vitamins. Higher content of the insoluble fiber induces satiety and has a laxative effect due to increased stool weight. Dietary fiber exhibits many therapeutic benefits and helps in lowering the blood cholesterol levels. It has been shown to reduce the risk of many disease conditions such as diabetes, hypertension, bowel and colon cancers, cardiovascular diseases and diverticulosis (Cummings *et al.,* 1992; Marlett *et al.*, 2002).

The date flesh and seed both contain a wide range of saturated and unsaturated fatty acids. The saturated fatty acids include capric, lauric, myristic, palmitic, stearic, margaric, arachidic, heneicosanoic, behenic and tricosanoic acids. Al-Shahib and Marshall (2003), observed significant variability in the fatty acids content in seeds of 14 date cultivars. They reported that the concentration of oleic acid varied from 41.1% to 58.8% and suggested that date seeds could be used as a source of oleic acid provided the technical problems related to its extraction are overcome.

2.5 Phytochemical Screening in Date Palm Fruits

Phytochemicals are plant-derived chemicals which may give health benefits when taken as medicine, drug or as a part of daily diet. They are classified into two main categories: primary metabolites, which occur in all cells and play an essential role in the reproduction and metabolism of those cells; for example, nucleic acids, the common amino acids and carbohydrates (sugars); secondary metabolites such as terpenes (a group of lipids), phenolics (derived from carbohydrates), alkaloids (derived from amino acids), which are characteristic of a limited range of species and have a biological effect on other organisms (Thatoi and Patra, 2011; Dias *et al.*, 2012).

Many of the biologically active constituents of medicinal, commercial and poisonous plants are classified as secondary metabolites. Date fruit is rich in phytochemicals such as carotenoids, polyphenols (e.g., phenolic acids, isoflavons, lignans and flavonoids), tannins and sterols (Martín-Sánchez *et al.*, 2014). The concentration and composition of these constituents are widely varied depending on several parameters, including date variety, stage of fruit picking, storage, postharvest processing, the geographical origin of thedates and soil conditions (Al-Laith, 2009; Amorós *et al.*, 2009; Al-Turki *et al.*, 2010).

Several researchers have reported that the chemical constituents and functional composition of date fruits are dramatically changed during date maturing period with increasing levels of reducing sugars, while fiber, mineral and vitamin levels decrease steadily (Kikuchi and Miki, 1978; Al-Farsi *et al.*, 2007; Al-Turki *et al.*, 2010).Date pulps contain easily digestible sugars (70%), mostly glucose, sucrose and fructose; dietary fibers and enclose less proteins and fats (Al-Farsi and Lee, 2008). Currently, several human health problems are related to diets. Date palm fruits constitute an important part of a balanced diet as they are natural

sources of food nutrient needed by humans and animals. Such food nutrient includes protein, carbohydrate, minerals and dietary fiber.

Date fruits are highly nutritious, being high in carbohydrates, fibre and potassium, certain vitamins and minerals, but are low in fat and virtually free from cholesterol and sodium (Mortazavi *et al*., 2007). Dates are also used as food preparations like sweets, snacks, confectionary, baking products, institutional feeding and healthy foods. The wholesome savoury taste of all natural sugar invites the most culinary creativity. As an ingredient to any recipe, dates provide the perfect natural alternative to added sugar. Wonderfully delicious, dates are one of the most popular fruits packed with an impressive list of essential nutrients, vitamins and minerals that are required for normal growth, development and overall well-being.

2.6 Morphological Parameters of Date Palm

Date palm varieties are very similar; however, studies have shown that there are clear differences based on the vegetative characteristics and spathe (Djerouni *et al*., 2015). Since the palm leaf spathe constituents look very different, the measurements which were taken from leaf palm like thorns length, pinnae number and leaf palm length have shown similarities and differences between the palms (Haider *et al*., 2015). Genotype identification of date palm is commonly based on morphological characters (Sedra *et al.*, 1998). In date palm, most of the female cultivars are recognised by their fruit characteristics such as size, shape, colour and taste as well as the morphological characters of the tree for cultivar identification. During the ripening process, the date fruits pass through four distinct stages of maturity, i.e. kimri, beser (khalal), rutaband tamar (Al-Ghamdi, 1993).

According to Al-Khalifah *et al.* (2012), some date palm cultivars have similar or narrow distinguishing morphological characters that complicate cultivar identification and require evidence to prove phylogenetic relationships at the interspecific level. Morphological characterisation needs a generous set of phenotypic records that are sometimes problematic to measure as a result of sensitivity to the environmental influences (Rao, 2004). The vegetative parameters are informative for description, phenotypic diversity and phylogenetic relationship among date palm ecotypes.

Haider *et al.* (2015) studied assessment of morphological attributes of date palm accessions from Pakistan, and concluded that quantitative and qualitative traits such as leaves, number of leaflets, length and grouping of spines, spathe, fruit and spadices possess quantitative markers mainly used for identification, description, differentiation and characterisation of date palm. Ahmed *et al.* (2016) studied the phenotypic characteristics of 75 cultivars of date palm from Algeria ,and concluded that the precise number of cultivars was still unknown since the cultivars exhibited homogenous traits and differed mainly by the fruit parameters; also they were morphologically nearly similar and of similar denomination.

Despite its great diversity, date palm is currently threatened by genetic erosion due to abiotic stresses (high seasonal temperature, drought and rainfall irregularities). Since problems of synonymy and homonymy often occur, the establishment of research strategies aiming at the evaluation of the genetic diversity of this local date palm germplasm has become imperative. Criteria related either to the vegetative or the fruit parameters are useful for cultivar characterisation, phenotypic diversity analysis and phylogenetic relationship exploration among date palm ecotypes. Moreover, phenotypic diversity

evaluation constitutes an available basic step for the elaboration of a programme to improve germplasm management and utilisation of any crop (Chang, 1992).

2.7 Fruit and Seed Characteristics in Date Palm

The date fruit is 7 cm long and has an oblong shape, but some species can reach a sphere-like shape (Al-Alawi *et al.,* 2017). The date fruit consists of exocarp (skin), mesocarp (pulp), endocarp (inner layer) and seeds (pits). Mesocarp is the bulk of the fruit (85-90 %), consists of epithelial cells and is divided into external and internal mesocarp (Dayang *et al.,* 2014). The flesh is surrounded by a thin layer of skin that is meant to protect the fruit (Dayang *et al.,* 2014).

The date seed represents 6-15 % of the fruit weight, depending on the species, and is a valuable by-product of the date processing industry. The seed is characterised by the presence of a furrow of variable depth and width along its length. Seeds of different date varieties differ in the depth of the furrow. The date palm fruit seed is characterised by a high content of dietary fiber and can be used to increase the content of dietary fiber in some products (Ghnimi *et al.,* 2017). The seed contains mainly insoluble fractions of dietary fiber, e.g. the date seed Deglet Noor contains 50 % cellulose and 20 % hemicellulose. Date seeds are mainly used in the production of animal feed (Shafiei *et al.,* 2010)

Muhammad *et al.* (2016) studied the yield parameters of major date palm cultivars planted in Pakistan, and reported significant differences among the date palm cultivars for number of bunches per plant, weight of single bunch plant(kg), number of strand bunches, number of fruit strands, single fruit weight, fruit diameter, fruit size, yield per plant. It was concluded that all the studied date palm cultivars varied among the yield parameters and fruit characters.

Ghulam *et al.* (2010) reported that the fruit weight and color of date fruits were mainly controlled by the type of pollen grain which may differ from season to season depending on the source of grains (Metaxenia) and time of pollination. Elshibli and Korpelainen (2010), evaluated 37date palm cultivars from Sudan which exhibited large variations in fruit and seed morphology for all characters. Among the 37 date palm cultivars studied, it was suggested that the cultivars were a mix of vegetative and seed-propagated material and concluded that the effect of environment and/or genetic-environment interactions might have led to the pronounced differences observed in the apparent characteristics of the fruits.

The date palm fruit seed is characterised by a high content of dietary fiber and can be used to increase the content of dietary fiber in some products (Ghimni *et al.*, 2017). The seed contains mainly insoluble fractions of dietary fiber. For example, the date seed Deglet Noor contains 50 % cellulose and 20 % hemicellulose. Date seeds are mainly used in the production of animal feed (Shafiei *et al.,* 2010).

Katarzyna *et al.* (2010) reported that date seed represents 6-15 % of the fruit weight, depending on the species, and that the seeds are a valuable by-product of the date processing industry. The seed is characterised by the presence of a furrow of variable depth and width along its length. Seeds of different date varieties differ in the depth of the furrow.

2.8 Metaxenia Effect on Date Palm

Artificial pollination of date palm spathes is one of the major practices that are necessary for successful fruiting. It has been documented and reported that there is a direct effect of the type of pollen on some fruit characteristics outside the embryo and the endosperm. This effect is known as metaxenia. Metaxenia effects have been reported on fruit size (Swingle,

1928; El-Ghayaty, 1983; Abdelal *et al*., 1983 and Shaheen *et al.,* 1989) on fruit colour and the time of ripening (Al-Delamiy and Ali, 1970) and on fruit and seed weight (El-Ghayaty, 1983; Abdelah *et al*., 1983., Al-Hamoudi *et al.,* 2006., Abd El-Zaher, 2008).

Many earlier authors have concluded that there is a direct effect of male parent used on date palm qualities including flavour or aroma of the fruit (Ben Salah and Hellali, 1998). Pollen from some male selections produced larger fruits in conjunction with increased size due to thinning. The increased size of the fruit produced from pollen from male selections did not lead to an increase in checking as did the increased size due to thinning (Nixon, 1950). These observations corroborated the fact that some males are better than others for pollinating certain varieties. This underlies the identification and selection of superior males and the development of clones thereof to produce a desirable type of pollen in quantity. Nixon (1950), suggested that growers should observe male palms for their performance in regard to the following:

i Time of blooming: The prospective male should flower at the same time as its prospective female partner. This means that the males should receive the same cultural care that the females do.

ii Number and size of flower clusters and quantity of pollen: Fewer males will be required if they have more and larger inflorescences with abundant pollen. Flowers that tend to adhere to the strands without shedding easily are preferred.

iii Compatibility: In some varieties, fruit set is better with pollen from certain males as compared to others.

Most of date palm males available for pollinating different female cultivars are mainly originated from seed propagation; resulting in many different local males that represent a source of genetic diversity. Characterisation and evaluation of the present male palm as a superior one for each female cultivar should be first step to establish an intensive programme to produce superior (highly potent) males through different procedures. Soliman and Al-Obeed (2013) observed that the Safry Male followed by Succary male significantly increased germination percentage and dimensions of pollen grains compared with other date palm males in both seasons.

The shape pores frequency and exine patterns of pollen grains are used to differentiate and identify date palm males. Therefore, it is important to conduct an evaluation of these males in terms of vegetative and flowering characteristics, determination of biodiversity and time of opening (early or delay). It is also important to monitor similarities and differences, and to analyse the causes of differences, which would contribute to information used for the identification of especially the male. It is also necessary to evaluate the physical and chemical quality of fruits resulting from females that are fertilised with pollen of these males since the source of pollen is an important factor to improve production and fruits quality in different cultivars of date palm.

2.9 The Role of Molecular Markers in Date Palm

Molecular markers have been used to evaluate genotypic and phenotypic variations in date palm in the last decade. A gene of interest can be linked from molecular markers thereby allowing identification of commercial varieties and indirect selection of desired genotypes (Diaz *et al.,* 2003). DNA markers have yielded good results in some breeding programmes (Hawken *et al.,* 1998) and in studying crop genetic relationships (Loh *et al.,* 2000).

Techniques used for DNA fingerprinting in date palm research are Restriction fragment length polymorphism (RFLP), (Bostein, 1980), RAPD (William *et al.,* 1990) microsatellite (Morgante and Oliviery, 1993) and AFLP (Vos *et al*., 2000). These markers have been used to identify different date cultivars singly or in combination with other markers for in-depth studies to see if they can complement one other (Adawy *et al.,* 2005). RFLP was used in Egypt to identify 5 cultivars (Corniquel and Mercier, 1997), while RAPD was used to identify various date cultivars in Morocco (Lashermes *et al.,* 1998), Egypt (Soliman *et al.,* 2003) and Saudi Arabia. Amplified fragment length polymorphism was used to identify date palm cultivars in California (Cao and Chao, 2002) Spain (Diaz *et al.,* 2003) and Egypt (Ashraf *et al.,* 2005). Microsatellite markers were used in Tunisia (Hamza *et al.,* 2012), Egypt (Hussein *et al.,* 2005), Qatar (Ahmed and Al-Qaradawi., 2009) and Sudan (Elshibli, 2009).

Some of these markers could not distinguish cultivars based on geographical location (Elshibli and Korpelainen, 2009), while some detected low level of genetic diversity (Adawy *et al.,* 2005). This may be why recent studies used combination of molecular and morphological markers to increase the discriminating power for date palm identification (Al-khalifah *et al*., 2012). The studies using both markers are still new but may raise a new hope for proper identification of date palm genotypes.

Corniquel and Mercier (1997) reported in their studies that all DNA markers have their merits. For instance, AFLP and RAPD markers allow identification of large amount of number of loci without sequence knowledge, and they are time-saving and provide useful information about the genetic relationship and diversity in many fruit crops. Zehdi *et al.* (2004) reported that RAPD markers were the most efficient strategy among the makers they

used because it can detect more genetic relationships. Maguire *et al.* (2002) reported that RAPD was more suited for population-based investigations and more informative for DNA finger printing. Rajora and Rahman (2003), reported that it has high level of polymorphism because of their abundance throughout eukaryotic genome.

2.9.1 Random amplified polymorphic DNA (RAPD) technique

Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence. Low cost, efficiency in developing a large number of DNA markers in a short time and requirement for less sophisticated equipment have made the RAPD technique valuable, although the reproducibility of the RAPD profile is still debatable.

Random Amplified Polymorphic DNA (RAPD) markers can be used for fast screening of nuclear genome variations (Williams *et al.,* 1990). RAPD markers have been used for germplasm characterisation in date palm (Soliman *et al.,* 2006), the construction of genetic linkage maps in many different species and the estimation of genetic diversity within and among species including tree species. Ganga *et al.* (2018), analysed the use of RAPD marker for delineating of genetic diversity among eight (8) date palm cultivars in India, and observed that the maximum polymorphism percent was found in primer OPE 2 and OPF 9

(77.78 %) and a genetic similarity to be between 0.558 to 0.835. It was and concluded that RAPD marker are an effective tool to discriminate various date palm genotypes.

RAPD technique and other DNA molecular techniques are useful to differentiate the varieties that cannot be discriminated by the morphology, but most importantly to study the genetic diversity of unnamed genotypes. In the case of the confirmation of uniqueness and specificity of some markers, RAPD and other marker techniques provide information to growers about the authenticity of the varieties delivered by private and government nurseries multiplying the most suited varieties.

Eissa *et al.* (2009) reported that RAPD-PCR primers allowed for enough distinction among the seven (7) date palm cultivars used for morphological and genetic characterisation in Egypt. The overall comparison among cultivars across the eight primers revealed the effectiveness of RAPD in distinguishing among date palm cultivars grown in the same location. It was concluded that molecular markers such as RAPD marker might be a better tool to distinguish date palm cultivars in Egypt than morphological attributes, and that there was no specific morphological criteria to distinguish the closely related date palm cultivars in Egypt.

RAPD markers have been successively used for phylogenetic studies in many plant species (Xuemei *et al.*, 2012). RAPD analysis could be used for an effective identification and DNA fingerprinting of date palm (Abdulla and Gamal, 2010). RAPD markers are reliable for identification of date palm cultivars (Eissa *et al.*, 2009). This has been supported by several authors like Xuemei *et al.* (2012), who concluded that RAPD marker system reveal high levels of polymorphism among species, indicating its effectiveness for evaluating intra

and inter-specific genetic diversity in the genus. On the basis of above observations at DNA level, it can be concluded that there is high genetic diversity and stable differences between date palm cultivars. Markhand *et al.* (2010) reported that date palm fruits differ from one cultivar to another.

2.10 Sex Determination in Date Palm Seedlings using SCAR Marker

Molecular markers based on the direct analysis of genomic DNA are used for the study of phylogenetic relationship, genetic diversity, genetic fidelity of date palm cultivars (Al-Qurainy *et al.,* 2011). These markers may be useful in the study of sex determination in dioecious plants. Despite increasing research efforts on a number of different plant species, very limited information is available on the molecular basis of gender determination, and it may be very difficult to estimate the numbers of genes involved. However, in some plant species, sex-determining genes have been discovered including *Carica papaya* and *Asparagus officinalis* (Murase *et al.,* 2017).

Research related to date palm is greatly restricted, owing to the lack of measures to identify its gender at the seedling stage. Date palm cultivation is more cost-effective through the cultivation of female plants than male plants. An increase in the number of female date palm plants per hectare may result in an increase in date production, thereby making the plantation more profitable. This has prompted the farmers to solely propagate date palm cultivars via offshoots which results in the reduction in genetic variations. The high genetic diversity is very important in plants for their survival in their natural habitat (Elleuch *et al*., 2008).

The seedlings produced may be either male or female, and no reproducible technique is currently available for gender determination in germinated seeds of date palm. Several efforts have been directed recently to establish a method for the early detection of seedling gender before transplanting them in the fields. However, no methodology has so far been developed for gender identification at the seedling stage (Hafiz *et al.*, 1980).

2.10.1 Sex determination of date palm seedlings using RAPD marker

Several researchers have identified molecular makers that could segregate sex in date palm and methods have been developed for identifying the sex at an early stage, including the use of isozymes (Torres and Tisserat, 1980), peroxydases (Majourhat *et al.*, 2002) and Random amplified polymorphic DNA (RAPD) (Moghaieb *et al.*, 2010). A major limitation of all of these studies has been that none of this markers has been shown to work across abroad range of date palm cultivars (Al-Mahmoud *et al.*, 2012).Some markers could be used to identify sex in one or two cultivars (Younis *et al.*, 2008). Investigation of the regions involved in sex determination has revealed that date palm employs a XX/XY (2n = 36) system with the male being heterogametic. The critical regions also showed significant polymorphism between the male and female alleles. This polymorphism can be used in the development of assays to distinguish sexes at an early stage (Al-Mahmoud *et al.*, 2012).

Shibu *et al.* (2001) used 60 RAPD primers for sex identification in Myristica; Yang *et al.* (2005) screened a total of 1041 RAPD primers to generate a 500bp male-specific DNA fragment in *Calamus simplicifolius*. A number of sex-specific DNA markers have been identified in several dioecious plants (Dhawan *et al.*, 2013). In other dioecious plants, sex identification was achieved using various DNA markers, as in *Ginkgo biloba*, *Carica papaya* and *Actinidia deliciosa*, (Shirkot *et al.*, 2002),

2.11 Cytology and Karyotype Variations among Date palm Cultivars of Sindh, Pakistan

Cytology is still considered an important technique for the characterisation of plant species. In addition, Naruhashi and Iwatsubo (1991) reported thatthe chromosomal data of plant species is more important to understand in terms of their similarities and differences based on the chromosome number, shape and size. Shan *et al.* (2003) also noted that karyotype analysis had been conducted for closely related varieties to know the changes in chromosome shape during chromosomal evolution. Al-Salih *et al.* (1987) studied chromosome number of two specific female date palm cultivars and reported 2n=32, 34, 36, and 64 chromosomes. Al-Ani *et al.* (2010) noted that the inconsistent and unpredictable chromosome number could be due to unavailability of soft roots at mitosis stage from adult palm trees. Alzahrani (2016) reported variation in chromosome number to be 2n=34, 36 in Khalas and Sheeshi date palm cultivars. A very limited research has been conducted on genetic diversity and no work has been reported regarding the chromosomal studies of this economically important plant which is cultivated in Nigeria.

2.11.1 Cytological studies on date palm tissue culture-derived plants

Date palm is a tree crop of economic importance in Egypt. It represents an income to the oases inhabitants, protects the under-crops from the effects of the climate and reduces the speed and damage from sand storms and wind erosion. It is a dioecious, perennial, monocotyledon, diploid (2n = 36) with long generation time (Almaarry, 1995). Somaclonal variations canoccur through utilisation of tissue culture technique. One of these variations is chromosomal apparitions (duplication, deletion, translocation and ploidy). Mohamed *et al.* (2010) carried out cytological studies on date palm mother plant and tissue culture-

derived plants in Egypt. They reported that there were about 36 chromosomes arranged in 18 pairs according to their lengths. Almaarry (1995) studied date palm propagation through tissue culture techniques and reported that date palm contains 36 chromosomes (2n).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Exploration and Collection of Plant Materials

Survey and exploration were undertaken to collect the fruits from the population of the Female date palm germplasm across the gene pools in the experimental field of Nigerian Institute for Oil palm Research (NIFOR) date palm research substation, Jigawa State, Nigeria. The Institute has the national mandate for the cultivation of the crop. The exploration and collection missions were undertaken at Gene pool 1 which contains germplasm originally collected from Sokoto, Kebbi and Zamfara States; Gene pool 2 (collections from Kaduna and Katsina States); Gene pool 3 (containing collections from Kano and Jigawa States), Gene pool 4 (which contains germplasm from Bauchi and Gombe State) and Gene pool 5 (containing germplasm from Borno and Yobe States) Nigeria.

The collection of fruits were done during the harvesting period between February and March, 2018. This involved a systematic random sampling from selected matured female trees in all the gene pools. The collected date fruits were checked for physical damage and injury from insects and fungal infection. They were then brought to the Department of Plant Biology, Federal University of Technology, Minna, for studies. The fruits were depulped (seeds were removed from the fruits). The depulped seeds were packed and sealed in thick polyethylene bags. Each of the seed samples was assigned an entry number, the gene pool name and palm number.

3.2 Description of Study Area

The area for this study was Nigerian Institute for Oil palm Research (NIFOR), Date Palm Research Substation Dutse, Jigawa states, where the fruit samples were collected, and Federal University of Technology, Minna, Niger state in which the field experiment and some laboratory work were carried out. The substation of NIFOR is situated on latitude 10º14ˈN and longitude 4º12ˈE.The substation ecology is within the Sudan savannah with annual rainfall of about 600mm per annum and average temperature of 32ºC. The soil type is sandy to loam. The Experimental Garden is situated at the Department of Biological Sciences, Federal University of Technology, Minna, Niger State, Nigeria. Geographically, Minna is located in the North-Central zone of Nigeria. It is located within longitude 6º34ˈE and latitude 9º36ˈ North. It covers an area of 88 Km2 with an estimated human population of 348,788 (Niger State Ministry of Agriculture, 2008). The area has a tropical climate condition with mean annual temperature, relative humidity and rainfall of 20-30 ºC, 61 % and 1334 cm, respectively. The climate presents two distinct seasons: a raining season between May and October and a dry season between November and April. The vegetation is a typical Guinea savannah type consisting majorly of grassland intersparsed with trees.

3.3 Depulping and Sprouting of Seeds

The collected date fruits from the gene pools were depulped (seeds were removed from the fruits). The seeds were then sprouted according to the protocol of the Nigerian Institute for Oil palm Research (NIFOR). Seed viability test was carried out following standard procedures by soaking the seeds for a period of three days. The emergence of the radicle after a period of soaking and thoroughly washed indicates the viability of the seeds.

3.4 Experimental Design and Raising of Nursery

The viable sprouted date seeds were transplanted in polyethylene bags arranged in a randomised complete block design (RCBD) in five replicates. Each accession was grown in a polyethylene bag, at inter and intra-block spacing of 2 feets. Three seeds of each accession were planted per hole and later thinned to one plant per stand. Twenty-one date seedling plants per row were planted and data were taken from 105 plants per block. All the cultural practices on cultivation of date palm were observed.

3.5 Percentage Emergence

Germination counts were made 7 days after sowing. Number of seeds showing germination were counted and expressed in percentage (Songsri *et al.*, 2011). The percentage emergence was calculated using the formular below:

$$(\%)\ \text{Germination} = \frac{\text{No.of germinated seeds}}{\text{Total No of seeds sown}} \times 100 \quad \ldots\ldots\ldots\ldots \quad (3.1)$$

3.6 Proximate Composition

3.6.1 Determination of moisture content

Sample containers were dried in a hot - air oven at 105 ^{0}C for 30 minutes and weighed. One gram (1.0 g) of ground samples were placed in the oven-dried containers and weighed. The fruit samples in the containers were oven-dried to a constant weigh at 105 ^{0} C in three (3) hours. After three (3) hours, the samples were allowed to cool in desiccators and weighed. The loss in weight after drying for three (3) hours was determined, (AOAC, 2005). Moisture content of the fruit samples was calculated as follows:

$$\text{Moisture}\ (\%) = \frac{\text{Loss in weight after drying}}{\text{Initial sample}} \times 100 \quad \ldots\ldots\ldots\ldots \quad (3.2)$$

3.6.2 Determination of ash content

A total of 1.0 g of each date fruit sample was weighed from the oven-dried sample after moisture content determination. Crucibles were pre-heated in a muffle furnace to 550 ^{0} C, cooled in desiccators and weighed. The oven-dried samples were then transferred into the crucibles, placed in a muffle furnace and the temperature raised to 550 ^{0}C. After two (2) hours of uninterrupted heating at 550 ^{0}C, the crucibles were removed with tong and transferred into the desiccator for cooling. The cooled crucibles were weighed and the weight of the sample left was determined, (AOAC, 2000).

The ash content was calculated as:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{1 g of oven-dried weight}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.3)$$

3.6.3 Determination of crude protein

About 2.5 g of dried and ground samples was weighed into the digestion tubes. 15 g Na_2SO_4,1 g CuSO4, one or two solemnized boiling granules and 25 ml of concentrated H_2SO_4 were added to the tube. It was digested at about 400 ^{0}C until solution was almost colourless (2 hrs for inorganic material) and then at least a further 30 minutes. After it was cooled down, 200 ml of water was added. One hundred (100) ml 0.1 N HCl was pipetted into a 500 ml conical flask, 1ml Conway's indicator was added and the flask was placed under the condenser of the distillation apparatus, ensuring that the condenser tip was immersed in the acid solution. To the Kjeldahl tube containing the digested samples, 100 ml of 50 % NaOH solution was slowly added down the side of the Kjeldahl tube so that it formed a layer underneath the digestion mixture. The digestion mixture was immediately

transferred to the distilling bulb of the distillation apparatus and corked. It was heated until all ammonia passed over into the standard acid. Approximately 150 ml of the distillate was collected. Excess standard HCl in distillate was titrated with NaOH standard solution until the colour changed from purple to green, indicating end point. (AOAC, 2005).

Percentage nitrogen was calculated (wet weight basis) as follows:

$$\% \text{ Nitrogen (wet)} = \frac{(A-B) \times 1.4007}{\text{Weight (g) of sample}} \times 100 \quad \text{..... (3.4)}$$

Where:

A = vol. (mL) std. HCl x normality of std. HCl

B = vol. (mL) std. NaOH x normality of std. NaOH

Nitrogen content on dry weight basis was calculated (when moisture content is known) as follows:

$$\% \text{ Nitrogen (dry)} = \frac{\% \text{ Nitrogen (wet)}}{(100 - \% \text{ moisture})} \times 100 \quad \text{..... (3.5)}$$

The percentage protein (wet or dry basis) was calculated as follows:

% Protein = % Nitrogen x 6.25

Where:

6.25 is the protein-nitrogen conversion factor.

3.6.4 Determination of crude lipid

About 2 g of date fruit was weighed from the oven-dried sample obtained from moisture content determination into a thimble (W_1) which had been weighed (W_0)**.** It was then dried in the oven for 5 h at 100 ^{0}C. Beakers to be used for fat determination were dried for about 1 h at 100°C and cooled in a desiccator. The weights were taken and recorded (W_2).The thimble W_2 containing the sample was placed in a Soxhlet unit connected to a condenser

and a heating flask. About 400 ml of petroleum ether was poured into the flask in the extraction unit. The heating mantle was set at 60 °C and extraction was carried out for 3 h. The solvent containing the extracted lipid was poured into the dried beaker and evaporated in a stream of air at room temperature until there was no further weight loss, (AOAC, 2000). The beaker containing the extract was weighed (W_3).the percentage crude lipid was then computed as follows:

$$\% \text{ Crude lipid} = \frac{(W3 - W2)}{\text{weight of sample}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.6)$$

3.6.5 Determination of crude fiber

One (1)g of dried sample obtained from determination of moisture content was placed in a crucible (W_1). A quantity of 1.25 % sulfuric acid was added to the 150 ml notch, after preheating with the hot plate in order to reduce the time required for boiling. About 3-5 drops of n-octanolwas added as antifoam agent, followed by boiling for 30 minutes from the onset of boiling. Sulfuric acid was then drained off. It was washed three times with 30 ml (crucible filled up to the top) of hot deionized water and drained. About 150 ml of preheated potassium hydroxide (KOH) 1.25 % and 3-5 drops of antifoam were added, followed by boiling for 30 minutes. It was filtered and washed as point 6. It was washed again with cold deionized water aimed to cool the crucibles and then washed three times with 25 ml of acetone. The crucibles were dried to constant weight in an oven at 105 °C for one hour to constant weight. It was allowed to cool in a desicator and then weighed (W_2).

The percentage crude fiber Calculated as:

$$\% \text{ Crude fibre} = \frac{(W2 - W1)}{W1} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.7)$$

Where: W_1 = weight of one gram of the sample

W_2 = weight of crude fiber and ash content

3.7 Determination of Minerals

Determination of mineral elements was done according to the method of Association of Analytical Chemists (AOAC, 2000). One (1) gram of sample was digested with nitric acid and perchloric acid at the ratio of 3:1. That is, 15mL of nitric acid and 5mL of perchloric acid were added to 1g of sample in a digestion tube, which was then heated at 150 °C using Kiedjal heating block until the mixture became clear (turned colourless). Distilled water was added after cooling. It was then filtered with Watmann No. 1 filter paper and the filtrate was made up to 50 mL with distilled water. The samples were analysed for manganese and magnesium using the Atomic Absorption Spectrophotometer (AAS).

3.8 Phytochemical Analysis

Total saponin contents were determined according to the method described by Makkar *et al*. (2007). The alkaloid contents were quantified according to the method described by Sofowora (1993). The tannin content was determined by Folin-Ciocalteu method as described by Marinova *et al*. (2005). The total flavonoid content was determined by aluminium chloride colorimetric assay as described by Zhinshen *et al*. (1999). The total phenol content was estimated and measured spectrophotometrically by Folin-Ciocalteu colorimetric method using gallic acid as the standard (Ainsworth and Gillespie, 2007).

3.9 Determination of Sugars

The Sugar content was determined using Lane and Eynon volumetric method and the non-reducing sugar (sucrose) was calculated as the difference between total sugars and reducing sugars. Fructose and glucose were determined using an enzymatic glucose analyzer as reported by Galant and Wilson (2015).

3.10 Morphological Parameters

The morphological parameters were determined following a standard descriptor to characterise date palm (IPGRI, 2005; Rizk and Sharabasy, 2007). All measurements were performed in triplicates using measuring tape. Specifically, the days to emergence (DE) were determined as the number of days between sowing of seeds and the day the first seedling emerged from the soil. Plant height was measured from the ground level to the lowest green leaf, using a measuring tape. Petiole length was measured using a meter rule. The width of leaf, girth of plant and length of leaflets were measured using a measuring tape. Length of petiole and length of internodes were measured with a measuring tape.

3.11 Morphological Parameters of Fruits and Seeds

The morphological features of fruits and seeds were determined according to the method described by Hanen *et al*. (2009) as follows:

i. Colour of the fruit

ii. Texture of fruit

iii. Length of the seed and length of the fruit were measured with vernier calipers

iv. Seed weight and weight of the fruit were determined with a digital weighing balance

v. Diameter of the seed and width of the fruit were measured using micrometer screw guage

vi. Thickness of the pulpit was determined using micrometer screw guage

vii. Diameter of the seed/width of the fruit (gram) were determined using micrometer screw guage.

3.12 Molecular Analysis

The molecular characterisation was carried out at Bioscience centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

3.12.1 Sample collection

The leaves of each of the 21 accessions were collected from young (3 weeks- 1 month old) date palm seedlings. The leaf samples were dried using silica gel and taken to IITA, Ibadan for analysis.

3.12.2 DNA extraction

The DNA extraction was carried out using a CTAB (Cetyltrimethyl ammonium bromide) based procedure with the use of DNeasy plant mini kit (Qiagen, Germany), according to the manufacturer's instructions.

3.12.3 DNA Amplification protocol and primer selection

The PCR (Polymerase Chain Reaction) programme included an initial denaturation step at 94°C for 2 min, followed by 45 cycles at 54°C for 1 min, annealing for 30 seconds, extension at 72 °C for 30 seconds and final extension at 72°C for 10 minutes. A total of six primers were tested with twenty-one accessions of date palm. The six informative primers were selected and used to evaluate the degree of polymorphism and genetic relationship between and within all accessions under study. Table 3.1 shows their names and base sequences.

3.12.4 Gel electrophoresis

The amplified DNA fragments were separated on 1.5 % agarose gel and stained with ethidium bromide. Base pair (bp) DNA ladder (Progma) was used as a marker within molecular size from 100-1500 bp and visualized under 300 nm UV light after staining with ethidium bromide.

3.13 Sex determination

3.13.1 Plant material and genomic DNA extraction

Leaf samples (3 weeks to 1 month old) of date palm seedlings were collected from the twenty-one (21) different accessions under study at the Biological Sciences experimental field in the Federal University of Technology, Minna, Nigeria and stored at −80°C.The DNA was extracted using a CTAB (Cetyltrimethyl ammonium bromide) based procedure using DNeasy plant mini kit (Qiagen, Germany), according to the manufacturer's instructions. Quality/Quantity of DNA was assessed by electrophoresis on 0.8 % agarose gel and run in 1X TBEbuffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, pH 8.0).Analysis was carried out using a SmartView Pro 1200 Imager System (Scientific Biotech, Taiwan) and band intensity was compared with a100 bp DNA Ladder RTU (Genedirex®, Taiwan). All DNA samples were diluted to a concentration of 50 ngμL−1 using an elution buffer and stored at -20 °C.

3.13.2 Primer Selection and DNA amplification

A range of combinations of six primers was used, together with PCR amplification across the young leaf samples of the twenty-one date palm accessions. DNA amplification was performed on Applied Biosystems Veriti96-well Thermal Cycler with the following programme. First denaturation at 94 °C for 5 min., followed by 40 cycles of denaturation for 1 min at 94 °C, annealing at 36 °C for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. Amplification products were analysed by gel electrophoresis on 0.80 % agarose gel in 1x TBE buffer (Tris/borate/ethylenediaminetetra acetic acid). Gels were stained with ethidium bromide and visualised under UV light. Each amplification reaction was performed using a single primer and repeated thrice to verify the reproducibility of the results.

Table 3.1: The Names and Base Sequences of the Primers used for this Study

S/N	PRIMER NAME	PRIMER BASE SEQUENCE
1	OPT –10	5^1 – CCT TCG GAA G –3^1
2	OPT – 05	5^1 – GGG TTT GGC A –3^1
3	OPT – 01	5^1– GGG CCA CTC A –3^1
4	OPH – 04	5^1 – GGA AGT CGC C –3^1
5	OPH – 06	5^1 – ACG CAT CGC A–3^1
6	OPH –10	5^1 – CCT ACG TCA G –3^1

3.13.3 Development of sex determinant primers

In the development of the specific markers for identifying male and female date palms, two sets of primers were employed. One set of two primers (the dominant marker) was self-designed using KU323795.1 as a reference male sex determination sequence, which was

used to amplify the male sex-specific DNA target band. The second primer set was used as a positive control in the reaction. Table below documents primer details.

Table 3.2: Names and Base Sequences of the Sex Determinant Primers used on the Twenty-One Date Palm Accessions.

Primer Target		Primer sequence	Primer Name
Male determinant	Forward	CTCTTCCAATGTTTCTTTCTTGTG	Reference. Sequence
	Reverse	CTACCACTGGCTTCTGCTAAC	KU323795.1
Male/female	Forward	GCATTAGCACCATAGTAAATTGT	Positive Control
Determinant	Reverse	GTCCCAATCAGAGTGCACTCAA	

3.13.4 Identification of male specific band from RAPD profile

A total of six (6) decamer oligonucleotides (RAPD primers) were used with the bulk DNA of male and female plants for selection of male-specific band. The designed male specific primer was further used with individual DNA samples of male and female plants for reproducibility testing and selection of male-specific band.

3.13.5 Validation of male specific band from RAPD profile.

The designed primers were further used for the amplification of the genomic DNA of eight (four each) known commercial varieties namely Deglet noor and Tirgal and two accessions each of the previously determined sexes were repeated in order to authenticate the efficacy of the developed primers.

3.14 Cytological Studies

3.14.1 Mitotic studies

Mitotic study was carried out using the method of Abubakar *et al.* (2015) with slight modifications. Germinated root tips of 1mm long were collected between (6:00 - 6:30) am and were pretreated with ice-chilled water for two hours. The pretreated root tips were then fixed in freshly prepared solution of absolute ethanol and glacial acetic acid in a ratio of 3:1 (fixative solution) for 24 hours to remove water. The root tips were hydrolysed in 1 NHCl for 2 minutes, squashed and stained using aceto-carmine reagent. Photomicrograph of the observed cells on the slide was then taken under high power objective (40 x).

3.15 Data Analysis

The data collected for morphological and yield parameters, proximate analysis, mineral composition and phytochemical screening in all the accessions were subjected to analysis of variance (ANOVA) using SPSS to compare the means. Duncan's Multiple Range Test (DMRT) was used to separate the means where significant differences were observed. For molecular analysis, binary data were generated for each primer sets using 1 (presence of positive amplification at a particular band size) and 0 (absence of positive amplification at a particular band size). The generated binary data were then used to create a data matrix which was analysed using the Power marker V2.35 software.

Cluster analysis, a method for displaying differences or similarities among cultivars was employed for grouping together accessions that showed dissimilarity in several traits (Legendre and Legendre, 1998). Clustering was carried out using the unweighted pair-group method of arithmetic average (UPGMA) method. All statistical analyses were performed using Statistical Analysis System software V6.07 (SAS, 1990). In the sex

determination studies, the number and sizes of DNA bands on the resultant gel were compared at the 200 base pair (bp) ladder (for male target band). Samples were identified that had a banding DNA pattern for male and absence of DNA pattern for female plants. Male and female seedlings were labeled for subsequent evaluation for morphological variations.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Germplasm collection

The exploration and germplasm collection cut across the four experimental gene pools in the field. A total of twenty-one accessions were randomly collected (Table 4.1) in all the gene pools. The highest number of accessions was obtained from gene pool II (collections from Kaduna and Katsina states) with a total of seven accessions. This gene pool contained the highest number of female plants among all the gene pools in the field. This was closely followed by gene pool III with (five) 5 accessions. A total of (four) 4 accessions were collected from gene pool four (IV). A total of 3 accessions were collected from gene pool 1. These gene pools had the greatest genetic diversity of date palm genetic resource in Nigeria as they were all collected from the growing states in Nigeria.

The result showed that gene pool II which had the highest number of accessions also had the highest number of accessions in colour variations, ranging from brown, light-brown to dark brown (Plate 1); the lowest number of colour variations was observed in gene pool 1.

Table 4.1: Sources and Description of Date Palm Germplasm in Dutse, Nigeria

S/N	Accessions	Gene pools	Place of Collection	State	Fruit Shape	Fruit Colour
1	R1P10	Genepl II	NIFOR/Dutse	Jigawa	Obovate	Light brown
2	R1P18	Genepl II	NIFOR/Dutse	Jigawa	Cylindrical	Light brown
3	R2P4	Genepl IV	NIFOR/Dutse	Jigawa	Obovate	Brown
4	R3P22	Genepl II	NIFOR/Dutse	Jigawa	Cylindrical	Dark brown
5	R4P12	Genepl III	NIFOR/Dutse	Jigawa	Obovate	Light brown
6	R4P29	Genepl III	NIFOR/Dutse	Jigawa	Obovate	Light brown
7	R5P8	Genepl III	NIFOR/Dutse	Jigawa	Ovate	Light brown
8	R5P20	Genepl I	NIFOR/Dutse	Jigawa	Obovate	Light brown
9	R5P24	Genepl II	NIFOR/Dutse	Jigawa	Cylindrical	Dark brown
10	R6P20	Genepl II	NIFOR/Dutse	Jigawa	Ovate	Dark brown
11	R7P1	NCRP	NIFOR/Dutse	Jigawa	Obovate	Light brown
12	R9P2	Genepl I	NIFOR/Dutse	Jigawa	Cylindrical	Brown
13	R9P12	Genepl II	NIFOR/Dutse	Jigawa	Cylindrical	Dark brown
14	R13P9	Genepl1	NIFOR/Dutse	Jigawa	Ovate	Brown
15	R13P1	Genepl IV	NIFOR/Dutse	Jigawa	Spherical	Light brown
16	R13P5	Genepl IV	NIFOR/Dutse	Jigawa	Cylindrical	Brown
17	R14P21	Genepl II	NIFOR/Dutse	Jigawa	Cylindrical	Brown
18	R15P6	Genepl III	NIFOR/Dutse	Jigawa	Spherical	Light brown
19	R16P31	Genepl I	NIFOR/Dutse	Jigawa	Ovate	Dark brown
20	R24P9	Genepl III	NIFOR/Dutse	Jigawa	Cylindrical	Light/dark brown
21	ZARIYA	Zaria Field	NIFOR/Dutse	Jigawa	Cylindrical	Black

4.1.2 Phenotypic characters of the fruit

The phenotypic observation revealed colour variations among all the collected accessions irrespective of the gene pool. Four different shapes, namely ovate, obovate, spherical and cylindrical, were observed (Table 4.1). The cylindrical type of shape was the most abundant with nine (9) of the accessions being recorded, while the spherical shapes was the least observed among the accessions (Table 4.1).

The colours observed among the accessions were brown, black, light-brown and dark-brown (Plate I). A total of six accessions were recorded to have brown colour representing 28 % of the total accessions, while 23 % of the accessions were dark-brown. The highest number of colour observed among the accessions was light-brown (38 %).The least colour observed was the black, which was recorded for the accession Zaria.

The texture of the fruit among the accessions was observed to be rough or smooth. Six of the accessions were recorded to have smooth epicarp, representing 29 % of the total number of accessions studied, while 15 accessions were observed to have rough epicarp, representing 71 % of the total number of accessions studied (Plat II and Plate III).

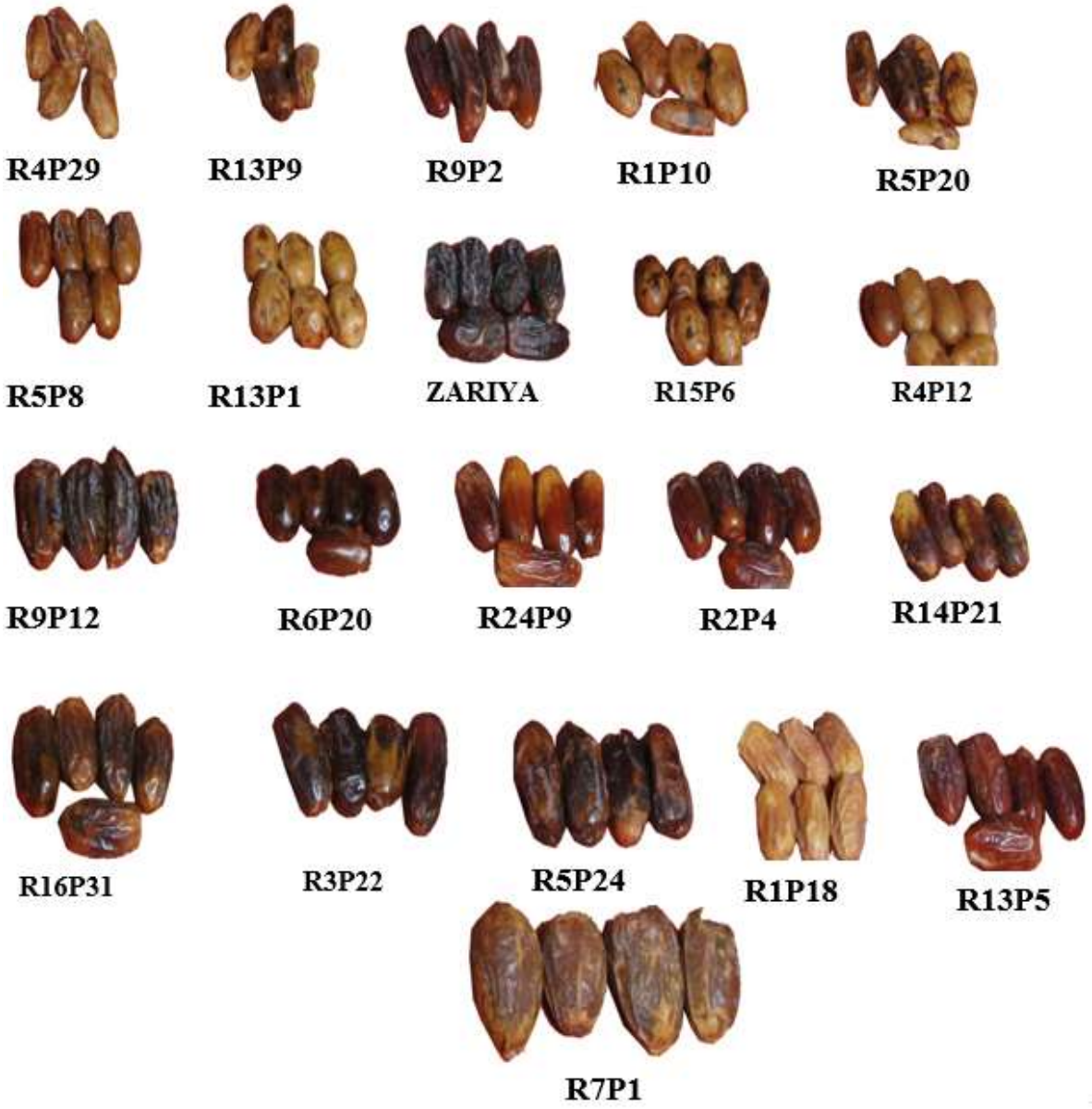

Plate I: Shape and colour variation in the date fruits

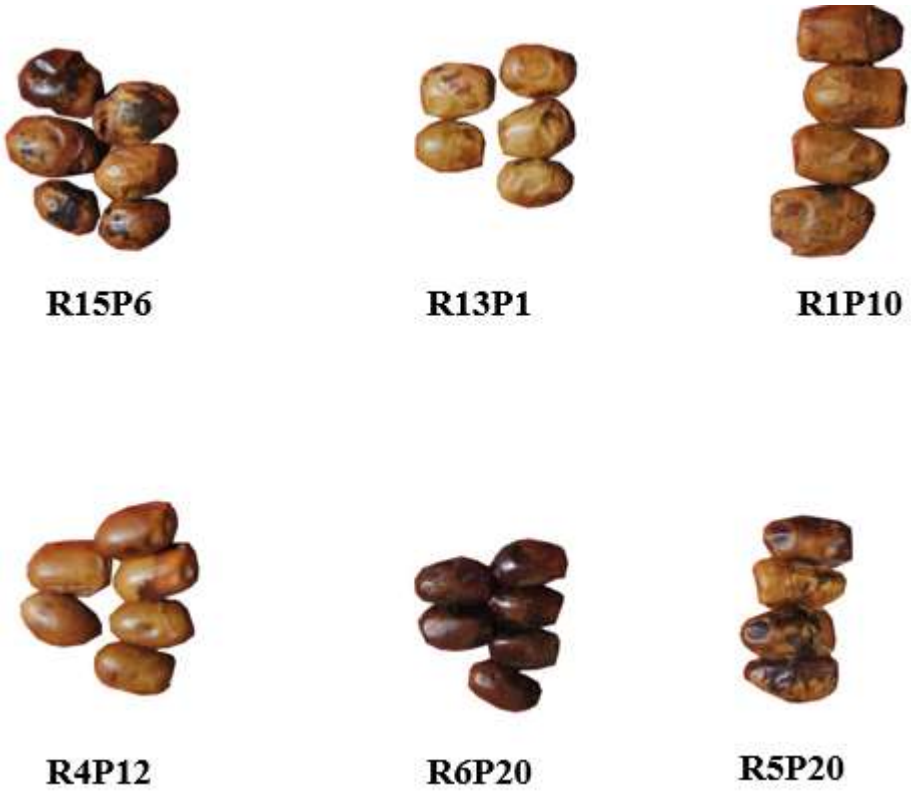

Plate II: Accessions with smooth epicarp

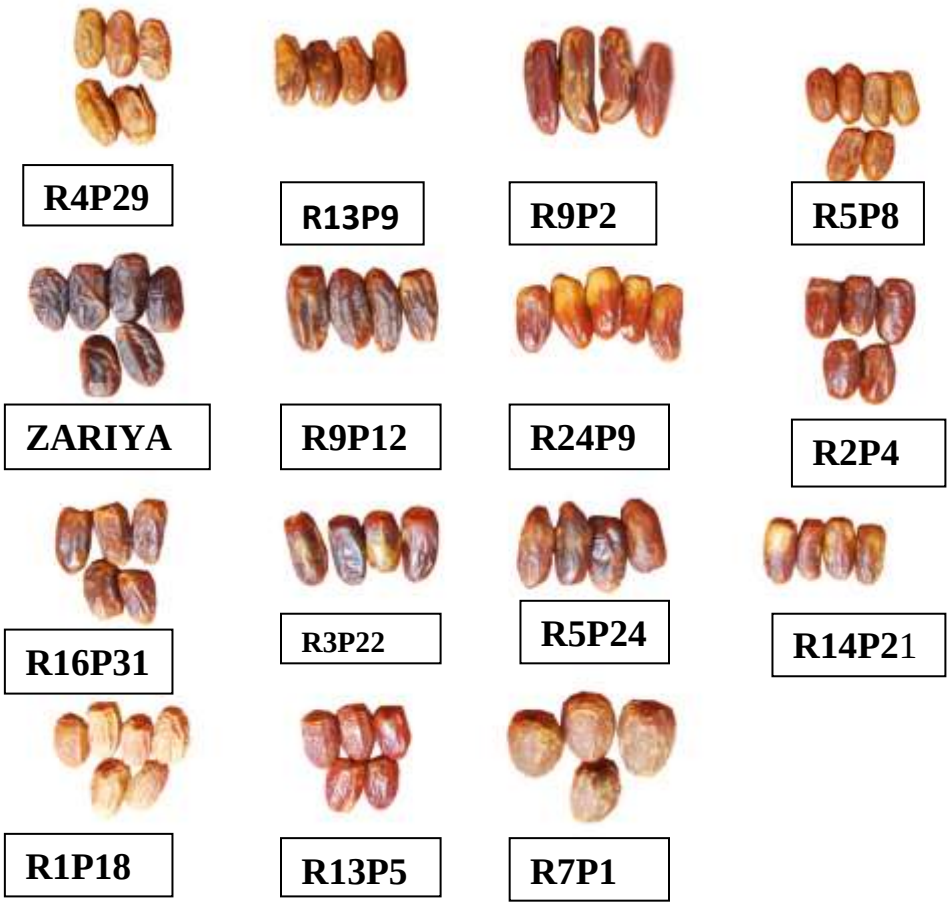

Plate III: Accessions with rough epicarp

4.1.2.1 Phenotypic characters of the seed

There were variations in the colour of seed in all the accessions studied, ranging from light-brown, and reddish-brown to a combination of both colors. The result showed that 47.60 % of the accessions had light-brown seeds while 28.50 % had reddish-brown; 33.30 % had reddish- brown seeds. Most accessions with reddish-brown seed colour were observed to have smooth seed-coat while accessions with light-brown colour and those with a combination of both colours (light-brown and reddish-brown) were observed to have rough seed coat (Plate IV)

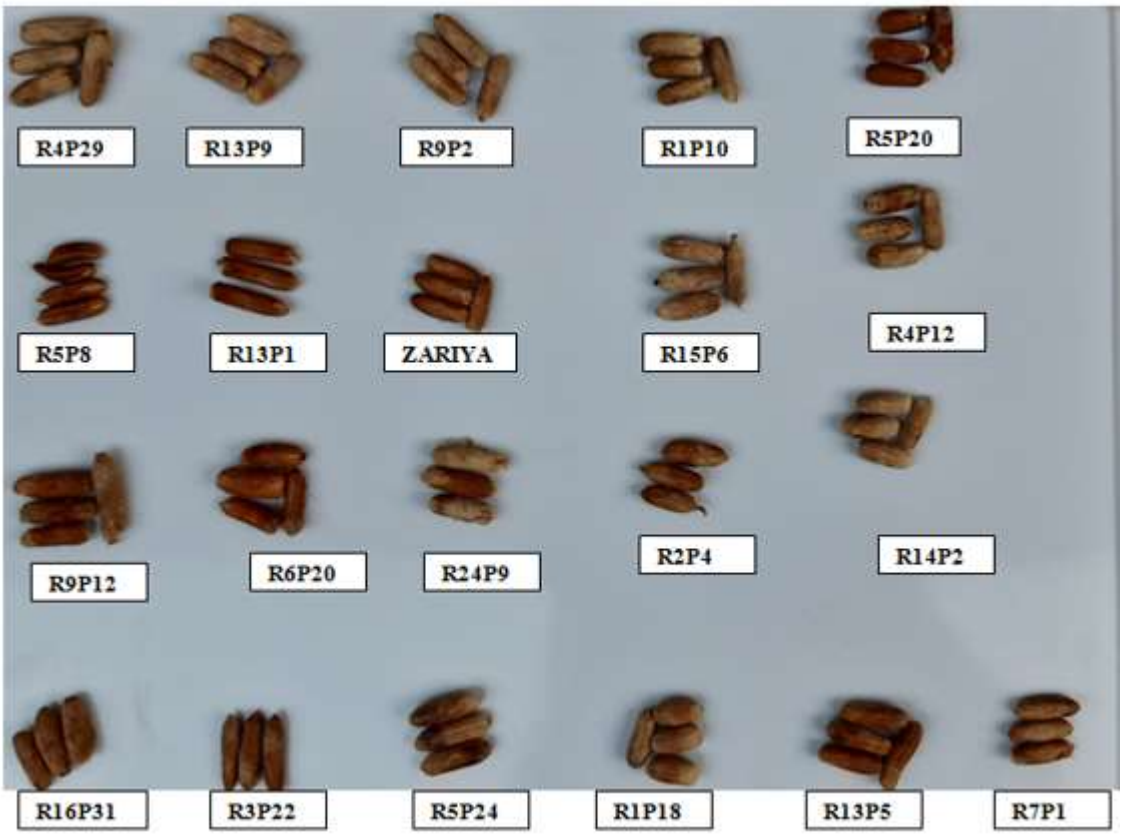

Plate IV: Colour variation of seeds of the date accessions

4.1.3 Proximate composition of date fruit accessions

The proximate analysis of date palm fruits showed the presence carbohydrates, protein, ash, fiber, lipid, calcium, magnesium, sodium, phosphorus and potassium. The accession R13P5 had the highest moisture content (7.65 %), which was significantly different ($P>0.05$) from all the other accessions. The lowest moisture content was observed in accession R16P31 with a mean of 2.25 %. No significant differences were observed in the moisture content of accessions R6P20, R5P8, R5P24, R1P18, R7P1, R24P9 and R9P12 (Table 4.2).

The lowest carbohydrate content was observed in accession R5P24 with the mean value of 64.55 %; the highest was observed in accession R9P12 with the value of 82.85 %, which was similar to the values observed in R2P4 (82.70 %) and R4P12 but significantly different from the values observed in all other accessions. The highest ash content (2.70 %) was observed in the accessions R5P8 while the lowest (1.56 %) was observed in (R13P9), and the differences ($P<0.05$) were significant. Significant differences were observed among accessions R6P20, R3P22, R5P24, R1P10, R15P6 and R13P1 (Table 4.2)

The highest protein content was observed in accession R4P29 with the mean value of 7.13 %, which was significantly different from all the other accessions; the least was observed in accession R5P8 with a mean value of 3.36 %.The result of the fibre content showed that accession R14P21 had the highest fibre with a mean value of 12.20 %, which was significantly different from all the other accessions at $p <0.05$; the least fiber content was observed in accession R5P8 with the mean value of 2.1 %, which was not significantly different from accessions R13P1 and R5P20 with the fibre content of 2.20 % and 2.13 %, respectively. The lipid content was highest in accession R5P24 with the mean value of 5.46 % followed by accession R16P31 (4.93 %). The lowest value of 2.69 % was observed in

accession R9P2, which was similar to accessions R13P9, R6P20, R5P8,R1P8,R1P18, R7P1 and R4P29 (Table 4.2).

Table 4.2: Proximate Composition in Fruits of the Twenty-One Accessions of Date Palm

Accession	Moisture (%)	Ash (%)	Protein (%)	Fibre (%)	Lipid (%)	Carbohydrates(%)
R1P10	2.29±0.01^{ij}	2.42±0.05^{abcd}	3.82±0.15^{fgh}	6.80±0.17^{de}	4.83±0.57^{abc}	75.24±2.67^{abcd}
R1P18	2.62±0.05^{h}	1.83±0.05^{ef}	5.25±0.22^{c}	9.70±0.17^{b}	3.04±0.57^{bc}	72.38±2.14^{abcd}
R2P4	3.13±0.09^{g}	2.04±0.09^{cde}	4.31±0.18^{efg}	2.35±0.47^{fg}	4.18±0.59^{abc}	82.7±1.25^{a}
R3P22	4.60±0.36^{e}	2.40±0.06^{abcd}	6.26±0.26^{b}	2.90±0.12^{fg}	4.19±1.19^{abc}	67.80±5.79^{cd}
R4P12	5.04±0.03^{d}	2.04±0.16^{cde}	3.78±0.13^{fgh}	3.61±0.5^{f}	3.50±0.61^{abc}	82.32±0.92^{a}
R4P29	7.50±0.06^{a}	2.02±0.13^{de}	7.13±0.19^{a}	7.16±0.53^{d}	2.89±0.6^{bc}	75.37±0.96^{abcd}
R5P8	2.54±0.01^{hij}	2.70±0.06^{a}	3.36±0.19^{h}	2.10±0.12^{g}	2.93±0.6^{bc}	75.14±5.8^{abcd}
R5P20	2.54±0.03^{hij}	2.49±0.08^{abc}	4.30±0.23^{efg}	2.20±0.57^{g}	4.62±0.59^{abc}	78.59±0.96^{abc}
R5P24	2.47±0.02^{hij}	2.42±0.06^{abcd}	5.29±0.19^{c}	6.40±0.17^{de}	5.46±0.44^{a}	64.55±5.73^{d}
R6P20	2.53±0.01^{hij}	2.44±0.03^{abcd}	4.37±0.24^{ef}	2.30±0.06^{fg}	2.97±0.57^{bc}	74.60±5.88^{abcd}
R7P1	2.70±0.06^{h}	1.83±0.03^{ef}	4.60±0.32^{de}	5.50±0.26^{e}	3.39±0.55^{bc}	80.39±0.17^{ab}
R9P2	5.23±0.15^{d}	2.65±0.04^{a}	6.08±0.09^{b}	5.43±0.55^{e}	2.69±0.6^{bc}	76.53±1.18^{abc}
R9P12	2.57±0.06^{hi}	2.57±0.04^{ab}	3.76±0.26^{fgh}	2.89±0.58^{e}	4.11±0.57^{abc}	82.85±1.1^{a}
R13P1	4.95±0.03^{d}	2.38±0.06^{abcd}	5.13±0.19^{cd}	2.13±0.55^{g}	3.50±0.59^{abc}	79.49±1.04^{ab}
R13P5	7.65±0.03^{a}	1.81±0.1^{ef}	4.35±0.21^{ef}	5.63±0.53^{e}	4.49±0.57^{abc}	75.08±1.09^{abcd}
R13P9	3.75±0.04^{f}	1.56±0.04^{f}	3.81±0.16^{f}	8.47±0.41^{c}	3.39±0.31^{bc}	68.05±5.81^{cd}
R14P21	3.68±0.02^{f}	2.55±0.16^{ab}	4.16±0.09^{efg}	12.20±0.55^{a}	4.40±0.59^{abc}	70.41±0.4^{bcd}
R15P6	6.55±0.03^{c}	2.46±0.04^{abcd}	6.03±0.05^{b}	2.82±0.52^{fg}	3.84±0.6^{abc}	77.19±1.33^{abc}
R16P31	2.25±0.01^{j}	2.16±0.1^{bcde}	5.40±0.32^{c}	5.56±0.3^{e}	4.93±0.33^{ab}	69.98±5.78^{bcd}
R24P9	2.59±0.02^{hi}	2.52±0.51^{ab}	5.22±0.13^{cd}	11.67±0.61^{a}	3.97±0.31^{abc}	73.41±0.9^{abcd}
ZARIYA	7.18±0.1^{b}	2.11±0.06^{bcde}	3.64±0.33^{gh}	2.36±0.36^{fg}	3.48±0.57^{abc}	80.36±1^{ab}

Values are mean±standard errors. Mean values with different letter(s) in the same column are significantly different at $p<0.05$

4.1.4 Mineral contents of date fruit accessions

The results of the mineral content of the fruit flesh of *Phoenix dactylifera* are shown in Table 4.2. The calcium content was highest in accession R24P9 (119.39 mg), followed by accession R5P24 (102.17 mg) and the lowest value of 68.83 g was observed in the accession R13P9.The highest and lowest values were significantly different from the values obtained in all other accessions ($p<0.05$). Accession R24P9 had the highest magnesium and sodium contents with the mean values of 80.55 mg and 13.44 mg, while the least values were observed in R13P5 with the value of 34.37 mg and 6.29 mg, for magnesium and sodium, respectively. Differences in sodium and magnesium were significantly different.

The highest potassium content observed in accession R5P8 (424.00 mg), followed by R24P9 (423.00 mg); the lowest value was observed in accession R2P4 (275.00 mg), which differed statistically from the other accessions. The phosphorus content was least in accession R3P22 with the mean value of 24.82 mg while the highest was observed in accession R5P8 with the mean value of 48.3 mg followed by accession R24P9 (46.8 mg). Accession R16P31 and R2P4 with the mean values of 29.40 mg and 3.13 mg respectively, did not differ significantly.

Table 4.3: Mineral Composition in Fruits of the Twenty-One Date Palm Accessions

ACCNS	Ca (mg/100 g)	Mg (mg/100 g)	Na (mg/100 g)	K (mg/100 g)	Ph (mg/100 g)
R1P10	85.38±5.93^{bcdef}	55.49±0.70^{bc}	8.80±0.06^{f}	324.00±0.58^{j}	36.26±0.05^{g}
R1P18	69.77±5.78^{ef}	40.48±0.63^{d}	8.65±0.05^{f}	354.00±0.58^{g}	35.85±0.06^{h}
R2P4	72.23±5.89^{def}	39.26±0.50^{d}	6.74±0.03^{i}	275.00±0.58^{s}	29.5±0.06^{o}
R3P22	88.88±5.74^{bcde}	60.32±0.37^{b}	7.54±0.10^{h}	295.00±0.58^{q}	24.82±0.06^{p}
R4P12	76.38±5.81^{cdef}	35.26±0.55^{d}	6.55±0.06ij	297.00±0.58^{p}	30.45±0.06^{n}
R4P29	76.82±5.80^{cdef}	38.74±0.52^{d}	6.25±0.06^{j}	334.00±0.58^{i}	32.63±0.05^{k}
R5P8	94.45±5.75^{bc}	57.3±0.54^{bc}	10.75±0.04^{d}	424.00±0.58^{a}	48.3±0.06^{a}
R5P20	91.16±5.74^{bcd}	54.35±54.35^{bc}	12.25±0.05^{b}	417.00±0.58^{b}	42.65±0.06^{d}
R5P24	102.47±5.75^{b}	78.41±0.52^{a}	11.77±0.07^{c}	404.00±0.58^{c}	46.21± 0.06^{c}
R6P20	85.33±5.72^{bcdef}	60.34±0.39^{b}	7.70±0.06^{h}	311.00±0.58^{n}	32.72±0.06^{k}
R7P1	70.49±5.75^{ef}	35.16±0.52^{d}	6.86±0.06^{i}	342.00±0.58^{h}	32.35±0.04^{l}
R9P2	85.13±5.77^{bcdef}	59.53±0.63^{b}	10.22±0.05^{e}	366.00±0.58^{f}	36.84±0.06^{f}
R9P12	92.70±5.76^{bc}	60.28±0.41^{b}	11.40±0.06^{c}	383.00±0.58^{e}	38.22±0.05^{e}
R13P1	82.22±5.74^{cdef}	52.1±0.52^{c}	6.73±0.01^{i}	365.00±0.58^{f}	34.55±0.05^{i}
R13P5	72.35±5.83^{def}	34.37±0.45^{d}	6.20±0.06^{k}	316.00±0.58^{k}	30.4±0.06^{n}
R13P9	68.83±5.95^{f}	35.38±5.93^{d}	6.60±0.57^{i}	307.67±0.88	30.41±0.06^{n}
R14P21	84.52±5.56^{bcdef}	56.12±0.53^{bc}	10.14±0.08^{e}	385.00±0.58^{d}	35.75±0.06^{h}
R15P6	88.29±5.79^{bcde}	56.61±0.59^{bc}	7.43±0.05^{h}	312.00±0.58^{mn}	34.26±0.05^{j}
R16P31	78.52±5.73^{cdef}	39.58±6.06^{d}	8.12±0.50^{g}	313.00±0.58^{l}	29.4±0.06^{o}
R24P9	119.39±0.36^{a}	80.55±0.4^{a}	13.44±0.05^{a}	423.00±0.58^{a}	46.8±0.06^{b}
ZARIYA	76.23±5.81^{cdef}	40.48±0.38^{d}	5.25±0.05^{k}	278.00±0.58^{r}	30.76±0.29^{m}

Values are mean± standard errors. Values with different letter(s) in the same column are significantly different at P<0.05

4.1.5 Sugar content

The results of sugar contents (glucose, fructose and sucrose) are presented in Table 4.4. The glucose content differed significantly among the accessions ($P<0.05$), with accession R5P8 having the highest mean value of 320.11 mg/g andaccessionR24P9 having the lowest mean value of 30.36 mg/g. The fructose content was highest in accession R15P6, with the mean value of 98.38mg/g, which was significantly different from all the other accessions. The lowest fructose content was obtained in accession R4P29 with a mean value of 32.20 mg/g, which was significantly different from all the other accessions at $p<0.05$. Accession R15P6 had the highest content of sucrose with the mean value of 92.56 mg/g,which was significantly different from the other accessions. The sucrose content was highest in accession R15P6 with the mean value of 92.56 mg/g, which was significantly different from all the other accessions at $p\geq0.05$.

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Table 4.4: Sugar Content of Twenty-One Accessions of Date Palm.

Accession	Glucose(mg/g)	Fructose (mg/g)	Sucrose (mg/g)
R1P10	63.81^{bc}	67.68^{g}	61.19^{gh}
R1P18	39.42^{cd}	40.59^{m}	37.60^{m}
R2P4	56.19^{c}	59.59^{j}	53.88^{j}
R3P22	67.23^{bc}	71.30^{f}	64.46^{f}
R4P12	64.91^{bc}	68.84^{g}	62.24^{g}
R4P29	30.36^{d}	32.20	29.11^{o}
R5P8	320.11^{a}	84.96^{d}	76.81^{d}
R5P20	94.13^{b}	95.83^{b}	90.00^{b}
R5P24	33.92^{cd}	35.97^{n}	32.52^{n}
R6P20	80.11^{b}	85.96^{d}	76.81^{d}
R7P1	41.83^{cd}	44.37^{l}	40.11^{l}
R9P2	68.11^{bc}	73.81^{e}	67.24^{e}
R9P12	55.37^{c}	58.72^{j}	53.09^{j}
R13P1	58.83^{c}	62.39^{i}	56.41^{i}
R13P5	50.88^{c}	53.96^{k}	48.78^{k}
R13P9	89.64^{b}	92.07^{c}	85.95^{c}
R14P21	64.91^{bc}	68.84^{g}	62.24^{g}
R16P31	52.29^{c}	55.46k	50.14k
R15P6	96.53^{b}	98.38^{a}	92.56^{a}
R24P9	62.75^{bc}	66.55^{h}	60.17^{h}
ZARIYA	56.19^{c}	59.59^{j}	53.88^{j}

Values are means ± standard errors. Means with different letter(s) in the same column are significantly different at $p<0.05$

4.1.6 Phytochemical composition

The results of the phytochemical analysis showed that the accession R5P8 had the highest tannin content with a mean value of 5.54 mg/g, which was significantly different from all the other accessions. The lowest value was recorded in the accession ZARIA with the mean value of 0.0005 mg/g. Tannin content in accessions R16P13, R13P9, R6P20 was similar (2.140 mg/g), but differed significantly from the other accessions.

The flavonoid content was highest in accession R6P20 with the mean value of 12.18 mg/g, followed by accessions R1P10 and R4P29 with the mean value of 12.10 mg/g. The lowest value of 2.77 mg/g was observed in R13P5, which was similar to accessions R2P4 and R3P22 with the mean values of 2.88 and 2.85 mg/g, respectively.

The accession R1P18 had the highest alkaloid content of 023 mg/g, which was significantly different from all the other accessions. The lowest alkaloid content of 0.23 mg/g was observed in accession R16P31. The accession R5P8 had the highest phenolic value was observed in accession R13P1 with the mean value of 15.10 mg/g. The saponin content was highest in accession R16P31 with a mean value of 888 mg/g, which was significantly different from all the other accessions. The lowest saponin content of 203.0 mg/g was observed in accession R1P18.

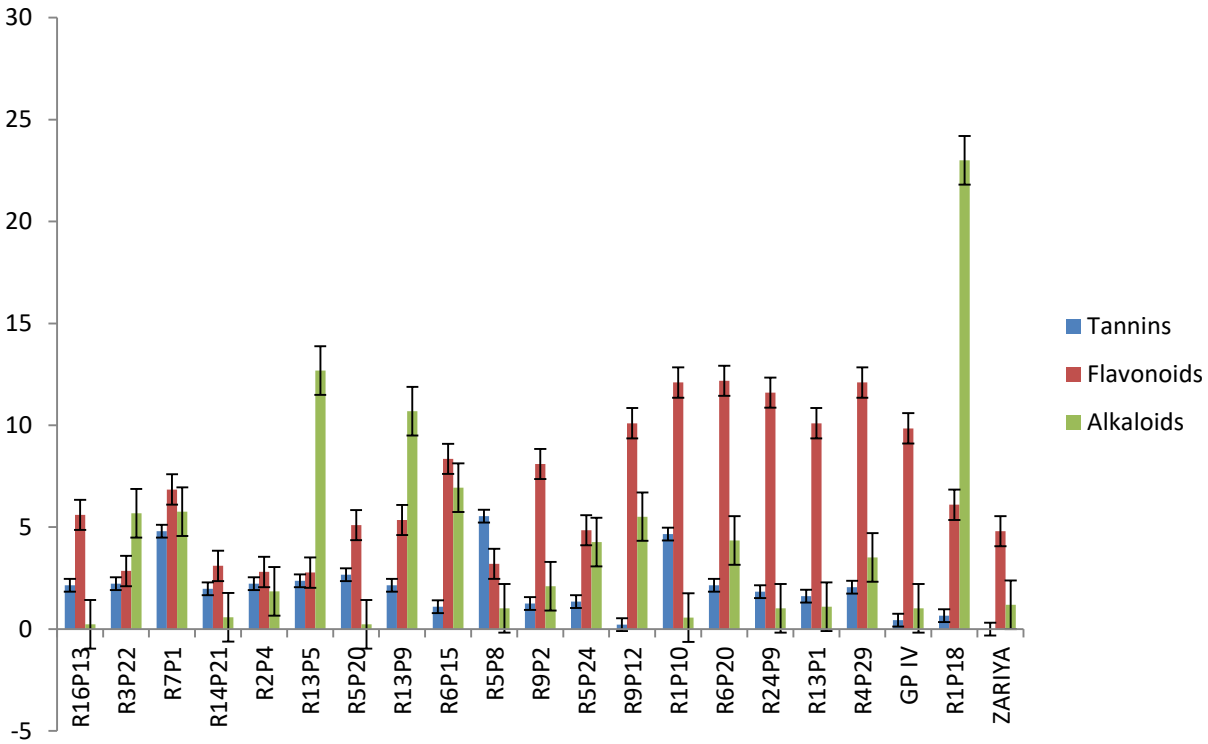


Figure 4.1: Phytochemical composition of the date palm accessions

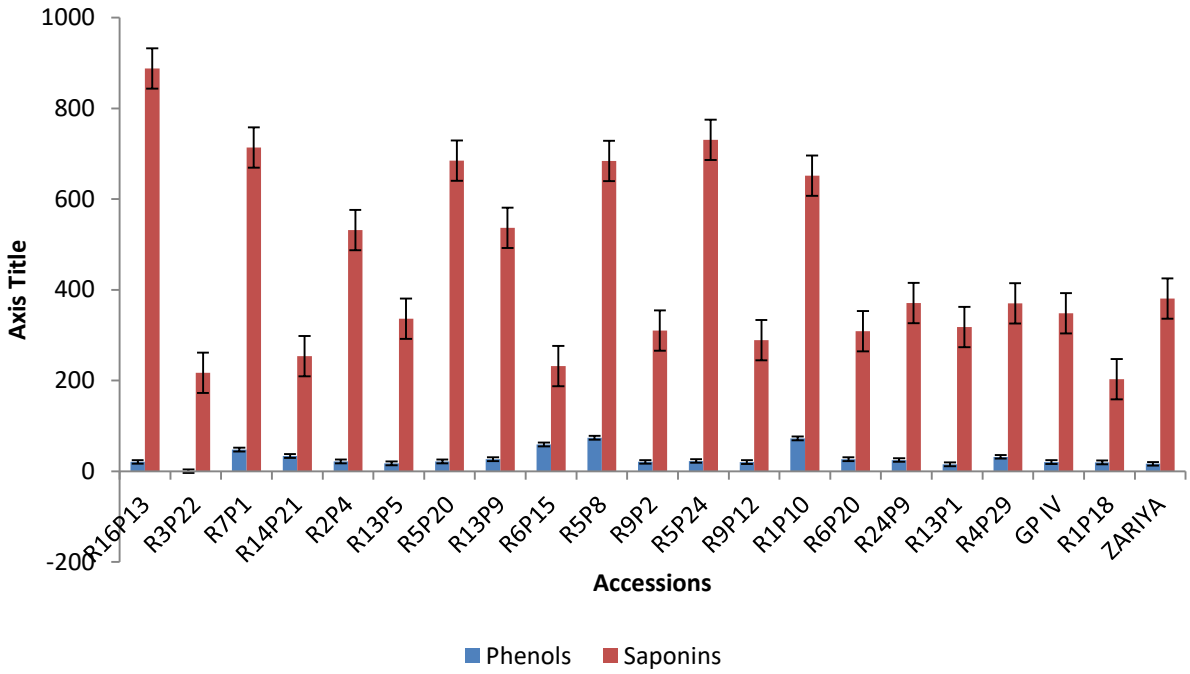


Figure 4.2: Phytochemical composition of the date palm accessions

4.1.6.1 Correlation analysis

The results of correlation matrix of phytochemical composition are shown in Table 4.5. The phenol content was positively correlated with tannin, flavonoid and saponin with r-values of 0.32, 0.30 and 0.05. It was negatively correlated with alkaloid having the value (-0.01). A very strong positive and highly significant ($P<0.001$) correlation was observed between tannin and saponin with the value of 0.42. In addition, flavonoid and saponin had a very weak correlation, though alkaloid was also observed to have a weak correlation with all the phytochemical studied.

Table 4.5: Correlation matrix of phytochemical composition of date palm

	Phenols	Tannins	Flavonoid	Saponin	Alkaloids
Phenols	1.00				
Tannins	0.32**	1.00			
Flavonoid	0.30**	0.07*	1.00		
Saponin	0.05*	0.42***	-0.09	1.00	
Alkaloids	-0.01	-0.12	-0.24	-0.26	1.00

***=strong correlation** = Moderately Correlated *= Weak correlation

4.1.7 Morphological Parameters

4.1.7.1 Plant height at different stages of growth

Plant height at four weeks after planting (4 WAP) was highest in R1P18 which was significantly different from the other accessions. The lowest plant height was recorded in accession R4P29. At 8 WAP, the accession R1P10 had the highest mean of height of 27.33 cm, which was significantly different from the other accessions. The lowest height was recorded in accession R16P31.The accession R1P10 had the highest height of 30.67 cm at 12 WAP, which was closely followed by accession R13P5 (29.93 cm). At 16 WAP, the highest mean height of 35.00 cm was observed in accession ZARIYA, followed by R1P10 (32.17 cm), Plant height was similar in R7P1, R2P4 and R9P12.

Plant height was highest in the accession ZARIYA at 20 WAP with the mean value of 35.00 cm. This was closely followed by the accession R24P9. The lowest height was observed in accession R14P21. At 24 WAP, the highest plant height was observed in accession R13P5 with a mean value of 39.00 cm, this was followed by accession R1P10 (38.67 cm), and the accession ZARIYA (38.33 cm). The lowest plant height was observed in accession R13P9.

4.1.7.2 Length of petiole (LOP)

The highest length of petiole was observed in accession R15P6 with a mean value of (4.27 cm), which was similar to accession R9P2 (4.10 cm), but significantly different from the other accessions (Table 4.6). The lowest length of petiole was observed in the accession R24P9 (Table 4.6).

4.1.7.3 Width of leaf

The accession R6P20 had the highest width of leaf (3.50 cm), which was followed by accession R13P5. The lowest width of leaf was observed in accession R3P22.

4.1.7.4 Girth of stem

The lowest girth of plant (5.10 cm) was observed in accession R13P9, while the highest value of 10.00 cm was observed in accession R5P20 and the difference ($p<0.05$) was significant.

4.1.7.5 Length of leaflets

The highest length of leaflets was observed in the accession R5P8 (25.90 cm), while the lowest was observed in accession R7P1 (13.48 cm). Length of leaflet did not differ significantly in the accessions R5P24, R14P21, R4P29, R5P20 and R13P1 with the mean values of (16.20,15.96, 16.06,16.42, 15.48) cm, respectively.

4.1.7.6 Length of Internodes

There were significant differences among the accessions in the internode length. The highest value was observed in accession R1P10 with a mean value of 3.80 cm, and this was significantly different from all the other accessions. The lowest value was observed in the accession R13P1 with a mean value of 1.60 cm.

Table 4.6: Some Morphological Characteristics of Date Palm Accessions

Parameter	Plant height (4 WAP)	Plant height (8 WAP)	Plant height (12 WAP)	Plant height (16 WAP)	Plant height (20 WAP)	Plant height (24 WAP)	Length of Petiole (cm)	Width of leaf (cm)	Girth of stem (cm)
R13P9	18.00±0.58^{e}	18.00±3.21^{de}	18.83±1.36^{e}	20.00±1.00^{g}	22.67±1.20^{def}	23.33±1.76^{fg}	2.47±0.07^{de}	2.63±0.59^{c}	8.71±0.06^{c}
R16P31	16.13±0.88^{f}	16.33±5.17^{e}	22.00±2.65^{de}	24.67±2.60^{ef}	28.00±0.58^{cd}	29.33±0.67^{de}	2.77±0.07^{cd}	3.10±0.59^{b}	8.01±0.06^{c}
R6P20	13.33±0.29^{h}	22.00±1.53^{cd}	22.33±2.19^{de}	23.00±2.52^{efg}	25.20±1.11^{d}	29.00±0.58^{de}	2.43±0.07^{de}	3.50±0.36^{a}	6.00±0.06^{j}
R5P8	13.00±0.50^{h}	17.67±1.45^{de}	18.67±2.33^{e}	20.33±0.33^{g}	23.002.751^{de}	25.67±3.38^{bcd}	2.33±0.09^{ef}	2.37±0.34^{cd}	10.00±0.06^{a}
R3P22	12.50±0.06^{hi}	18.00±5.29^{d}	19.33±3.93^{e}	21.67±2.91^{fg}	27.50±1.61^{cde}	28.17±0.17^{cde}	2.30±0.06^{ef}	1.10±0.55^{d}	7.10±0.06^{fg}
R5P24	13.67±0.54^{fg}	26.50±1.04^{abc}	26.50±1.26^{bc}	28.17±0.73^{d}	28.83±0.44^{cd}	31.33±2.85^{d}	2.43±0.30^{de}	3.17±0.67^{b}	6.79±0.06^{h}
R1P18	22.83±3.77^{a}	24.17±2.46^{bcd}	29.67±3.28^{ab}	31.00±0.58^{bc}	33.67±2.85^{ab}	34.17±4.62^{cd}	3.87±0.32^{ab}	3.07±0.30^{b}	7.50±0.06^{e}
R1P10	18.87±2.00^{e}	27.33±1.76^{abc}	30.67±1.67^{a}	32.17±1.96^{b}	32.83±3.35^{b}	38.67±1.33^{b}	3.00±0.76^{c}	2.67±0.60^{bc}	6.00±0.06^{j}
R7P1	17.37±0.58^{ef}	25.67±5.17^{bcd}	25.67±3.18^{bcd}	25.83±0.44^{def}	28.00±0.58^{cd}	28.33±0.33^{def}	2.53±0.03^{d}	2.53±0.26^{cd}	6.80±0.06^{h}
R14P21	16.50±0.32^{f}	22.67±2.19^{cd}	24.33±2.03^{cd}	21.33±4.70^{fg}	16.67±5.70^{g}	21.33±3.84^{gh}	2.03±0.09^{ef}	2.67±0.33^{bc}	7.80±0.06^{d}
R15P6	21.00±0.58^{b}	26.00±2.65^{abc}	26.17±4.68^{bc}	27.33±4.10^{de}	27.67±3.53^{cde}	33.53±3.50^{d}	4.27±0.82^{a}	1.93±0.18^{cd}	6.99±0.06^{gh}
R4P29	12.00±0.58^{i}	20.67±1.56^{cd}	20.97±1.45^{de}	21.17±2.09^{fg}	21.50±2.02^{efg}	27.37±4.94^{ef}	2.20±0.38^{ef}	2.07±0.54^{cd}	8.00±0.06^{c}
R4P12	22.00±0.58^{a}	22.67±5.70^{cd}	25.67±3.84^{bcd}	28.03±0.98^{d}	28.50±1.04^{cd}	29.67±0.88^{de}	2.57±0.09^{d}	2.53±0.49^{cd}	7.00±0.06^{fg}
R24P9	20.27±0.37^{c}	23.00±2.65^{cd}	26.00±4.51^{bc}	29.33±3.38^{cd}	34.00±4.04^{ab}	36.67±3.53^{c}	1.87±0.27^{a}	2.57±0.07^{cd}	6.50±0.06^{i}
ZARIYA	18.60±0.78^{e}	27.33±0.93^{abc}	28.33±1.45^{b}	35.00±3.21^{a}	35.00±3.25^{a}	38.33±2.19^{b}	2.17±0.20^{ef}	2.53±0.49^{cd}	7.50±0.06^{e}
R9P2	18.73±0.56^{de}	24.40±4.58^{bcd}	24.67±2.60^{cd}	26.33±0.67^{de}	31.67±1.67^{b}	34.33±3.18^{cd}	4.10±0.46^{f}	2.47±0.32^{cd}	5.20±0.06^{k}
R5P20	20.13±0.20^{c}	20.77±4.30^{cd}	28.67±0.88^{b}	30.67±1.20^{c}	30.83±2.20^{bc}	31.67±0.88^{d}	2.33±0.44^{ef}	3.17±0.43^{b}	5.20±0.06^{k}
R13P1	20.50±3.55^{c}	21.47±0.38^{cd}	28.67±0.67^{b}	28.67±1.20^{d}	30.67±3.53^{bc}	32.50±2.84^{d}	3.33±0.47^{bc}	3.23±0.54^{ab}	8.00±0.06^{c}
R13P5	15.47±0.32^{g}	21.77±1.07^{cd}	29.93±2.54^{ab}	29.33±1.76^{cd}	30.67±4.91^{bc}	39.00±0.58^{a}	3.33±0.60^{bc}	3.40±0.32^{a}	8.10±0.06^{b}
R2P4	17.67±4.81^{ef}	19.33±1.20^{d}	24.67±2.40^{cd}	25.00±4.04^{def}	28.17±2.20^{cd}	35.17±3.88^{c}	2.27±0.15^{ef}	2.87±0.47^{bc}	5.10±0.06^{k}
R9P12	19.33±1.20^{d}	21.83±2.52^{cd}	24.67±2.40^{cd}	25.33±1.45^{def}	26.50±1.32^{cde}	30.17±0.73^{de}	3.77±0.90^{ab}	2.43±0.46^{cd}	5.10±0.06^{k}
Total	17.85±0.780	24.62±0.90	26.13±0.81	27.80±0.78	28.67±0.83	25.48±0.66	2.78±0.12	2.67±0.11	7.19±0.14

Values are mean± standard errors. Values with different letter(s) in the same column are significantly different at $p \leq 0.05$.
WAP= Weeks after planting.

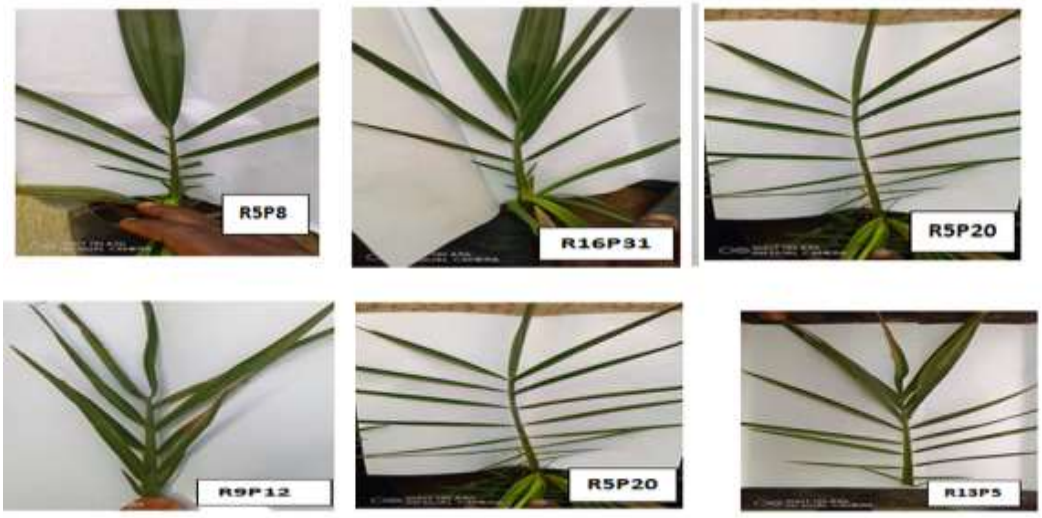


Plate V: Leaf morphology of accessions showing opposite arrangement of leaflets

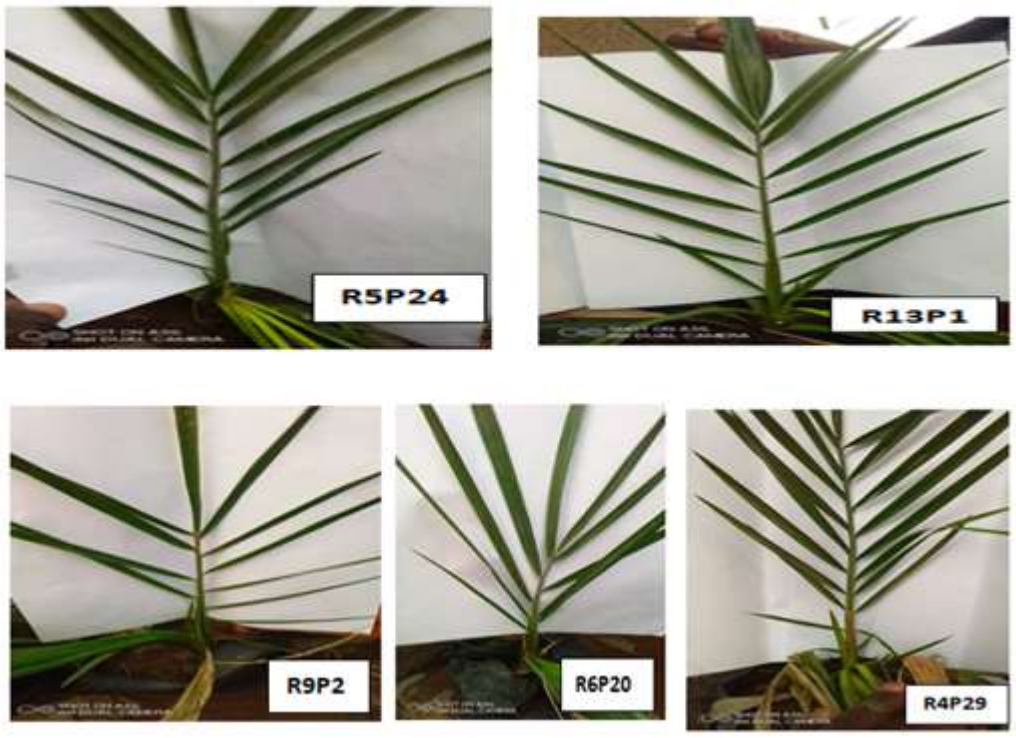


Plate VI: Leaf morphology of accessions showing alternate arrangement of leaflets

4.1.8 Fruit characteristics

4.1.8.1 Fruit length

Table 4.7 shows that the highest fruit length of 4.87 cm was observed in the accession R9P2, followed by R5P24 (4.73 cm), both of which differed significantly from the other accession. The lowest fruit length of 2.70 cm was observed in accession R13P1.

4.1.8.2 Fruit weight

The highest fruit weight of 11.85 cm was observed in accession R7P1, while the lowest was observed in accession R5P8 with a mean value of 2.12 g. Fruit weight was statistically similar in accessions R5P20, R13P1 and R2P4 ($p<0.05$)..

4.1.8.3 Fruit diameter

The accession R7P1 had a significantly higher fruit diameter (2.10 mm), which was significantly different from all the other accessions. The lowest value was observed in R24P9 (1.03 mm). Fruit diameter was similar in accession R13P9 and R9P12.

4.1.8.4 Fruit thickness

The highest value of fruit thickness was observed in accession R7P1 and R15P6, with a mean values of 4.00 mm each. Fruit thickness was similar in accessions R6P20, R14P21, and R5P20, with a mean values of 3.33 mm.

4.1.8.5 Seed weight

The highest mean of seed weight was observed in the accessions R16P31 and R24P9 with the mean values of 1.53 and 1.51 g, respectively. The lowest seed weight of 0.85 g was observed in the accession R4P29.

4.1.8.6 Seed length

The highest seed length of 3.00 g was observed in accession R9P2, while the lowest (1.00 g) was observed in accession R6P20. Differences in seed length were significant at $p<0.05$.

4.1.8.7 Seed diameter

The highest seed diameter of 8.67 mm was observed in R7P1 and this was followed by accession R16P31 with 8.00 mm. The lowest value of 5.67 mm was observed in R4P29.

Table 4.7: Fruit Characteristics of Twenty-One Accessions of Date Palm

Accession	Fruit length (cm)	Fruit weight (g)	Fruit diameter (mm)	Fruit thickness (mm)	Seed weight (g)	Seed length (cm)	Seed diameter (mm)
R13P9	3.40±0.06^{defg}	2.71± 0.08^{hi}	1.10±0.10^{ij}	1.67±0.33^{e}	0.84±0.08^{f}	2.20±0.12^{d}	7.33±0.33^{bc}
R16P31	3.77±0.23^{cd}	4.18±0.26^{g}	1.40±0.06^{cdef}	1.67±0.33^{e}	1.53±0.24^{a}	2.63±0.09^{b}	8.00±0.58^{ab}
R6P20	3.07±0.09^{gh}	3.22±0.27^{h}	1.27±0.03^{fghi}	3.33±0.33^{b}	1.12±0.14^{cde}	1.90±0.06^{g}	6.67±0.33^{cd}
R5P8	3.10±0.15^{gh}	2.12±0.13^{i}	1.23±0.09^{ghi}	2.00±0.00^{de}	1.17±0.17^{cde}	2.27±0.09^{e}	6.33±0.33^{cd}
R3P22	4.13±0.19^{bc}	6.67±0.46^{cd}	1.47±0.03^{cdef}	2.33±0.33^{cde}	1.30±0.07^{c}	2.43±0.12^{d}	7.00±0.00^{c}
R5P24	4.73±0.15^{a}	9.10±0.22^{b}	1.50±0.06^{cde}	3.67±0.33^{ab}	1.31±0.12^{c}	2.60±0.26^{bc}	6.67±0.33^{cd}
R1P18	3.23±0.12^{efg}	2.73±0.03^{hi}	1.37±0.09^{defg}	2.00±0.58^{de}	1.48±0.16^{b}	2.63±0.09^{b}	7.00±0.00^{c}
R1P10	3.80±0.12^{cd}	5.63±0.29^{ef}	1.27±0.09^{fghi}	3.00±0.00^{bc}	1.35±0.11^{bc}	2.50±0.06^{c}	6.67±0.33^{cd}
R7P1	4.50±0.25^{ab}	11.85±0.29^{a}	2.10±0.12^{a}	4.00±0.58^{a}	1.42±0.12^{b}	2.57±0.22^{bc}	8.67±0.33^{a}
R14P21	3.87±0.09^{cd}	4.11±0.13^{g}	1.30±0.0^{6efgh}	3.33±0.33^{b}	1.35±0.06^{bc}	2.87±0.07^{ab}	6.33±0.33^{cd}
R15P6	3.10±0.35^{gh}	4.93±0.58^{fg}	1.73±0.09^{b}	4.00±0.00^{a}	1.20±0.10^{cde}	2.27±0.12^{e}	7.33±0.67^{bc}
R4P29	3.20±0.0^{fg}	2.04±0.30^{i}	1.17±0.03^{hij}	2.00±0.00^{de}	0.85±0.05^{ef}	2.10±0.06^{f}	5.67±0.33^{d}
GPIV	3.27±0.09^{efg}	4.12±0.18^{g}	1.30±0.00^{efgh}	3.00±0.00^{bc}	1.05±0.10^{cdef}	2.60±0.6^{ab}	6.33±0.33^{cd}
R24P9	3.80±0.15^{cd}	4.06±0.37^{g}	1.03±0.09^{j}	2.67±0.33^{bcd}	1.51±0.02^{a}	2.50±0.06^{c}	6.33±0.33^{cd}
ZARIYA	3.70±0.15^{cde}	6.08±0.24^{de}	1.37±0.03^{defg}	2.67±0.33^{bcd}	1.02±0.15^{def}	2.57±0.12^{bc}	0.67±5.67^{cd}
R9P2	4.87±0.09^{a}	7.10±0.20^{c}	1.23±0.03^{ghi}	3.67±0.33^{ab}	1.10±0.05^{cde}	3.00±0.06^{a}	5.67±0.33^{d}
R5P20	3.90±0.06^{cd}	5.19±0.27^{f}	1.57±0.07^{bcd}	3.33±0.33^{b}	0.99±0.03^{abc}	2.43±0.18^{d}	6.67±0.33^{cd}
R13P1	2.70±0.12^{h}	5.11±0.26^{f}	1.57±0.03^{bcd}	3.67±0.33^{da}	1.27±0.06^{cd}	2.03±0.03^{f}	7.33±0.33^{bc}
R13P5	3.60±0.06^{def}	4.89±0.32^{fg}	1.50±0.06^{cde}	2.67±0.33^{bcd}	1.15±0.14^{cde}	2.60±0.06^{bc}	6.33±0.33^{cd}
R2P4	3.47±0.15^{defg}	5.08±0.14^{f}	1.60±0.06^{bc}	3.00±0.58^{bc}	1.29±0.27^{cd}	2.40±0.06^{d}	7.33±0.33^{bc}
R9P12	4.50±0.06^{abd}	6.44±0.06^{cde}	1.13±0.07^{ij}	2.00±0.58^{de}	1.12±0.10^{cde}	2.80±0.06^{ab}	6.00±0.58^{cd}

Values are mean±standard errors. Means with different letter(s) in the same column are significantly different at $P\leq0.05$.

4.1.9 Primer selection and DNA amplification

The result shows that the six (6) primers showed at least one (1) consistent polymorphic band. A total of 125 amplified fragments were distinguished across the selected primers and the statistical analysis showed 106 polymorphic bands among the 21 genotypes with an average of 84.3 polymorphic bands per primer. The highest numbers of fragment bands was produced by the primer OPH-06 with 100 % polymorphism while the lowest number of fragments was produced by the primer OPH-04 with 71.00 % polymorphism. Polymorphic Information Content (PIC) values ranged from 0.616 to 0.920 (Table 4.8). The size of the amplified fragments varied with different primers, ranging from 100 to 1500 base pairs. The polymorphisms revealed by the six (6) primers indicate that they are good and reliable for genetic diversity assessment in date palm and that there is a high degree of diversity in the accessions studied.

Table 4.8: Polymorphism Observed from Six RAPD Primers in Date Palm Accessions

Primer Name	Primer Sequence	Monomorphic Band	Polymorphic Bands	Polymorphism (%)	PIC
OPT-10	CCTTCGGAAG	6	15	72	0.757
OPT-05	GGGTTTGGCA	4	17	81	0.911
OPT-01	GGGCCACTCA	2	18	86	0.920
OPH-04	GGAAGTCGCC	5	16	76	0.891
OPH-06	ACGCATCGCA	0	21	100	0.689
OPH-10	CCTACGTCAG	2	19	91	0.616
Total		19	106	506	
Average		3.17	17.6	84.3	

PIC = Polymorphic Information Content

4.1.9.1 Genetic diversity as revealed by RAPD marker

The genetic distances among the twenty-one date palm accessions obtained using UPGMA are shown in Figure 3.The dendrogram separated the date palm accessions into 6 main clusters groups. Group I consists of four sub clusters, representing 19 % of the accessions which include R24P9, R4P12, R5P8 and R5P20, Group II had four sub clusters representing 19 % of the accessions which include accessions R13P9, R3P22, R2P4 and R13P1, Group III, Group IV, and Group V had two-sub clusters each representing 10 % of the total accessions; these accessions include ZARIYA, R4P29; R5P24, R16P31 and (R13P5 and R7P1). Group VI had the highest number of subclusters with a total number of seven accessions, representing 33 % of the total accessions. These accessions include R1P10, R6P20, R9P12, R15P6, R9P2, R14P21 and R1P18.

The jacquard similarity index for the calculation of distances among the date palm accessions revealed an interesting phenomenon. A distinct relationship and differences among the accessions were observed. The highest closeness (least dissimilar coefficient, 0.07) were observed between accessions R5P24 and R16P31 in group III, indicating low genetic distance and high phylogenetic relationship between the accessions. The least diversity (highest similarity coefficient, 4.04) were observed between accessions ZARIYA and R4P12.

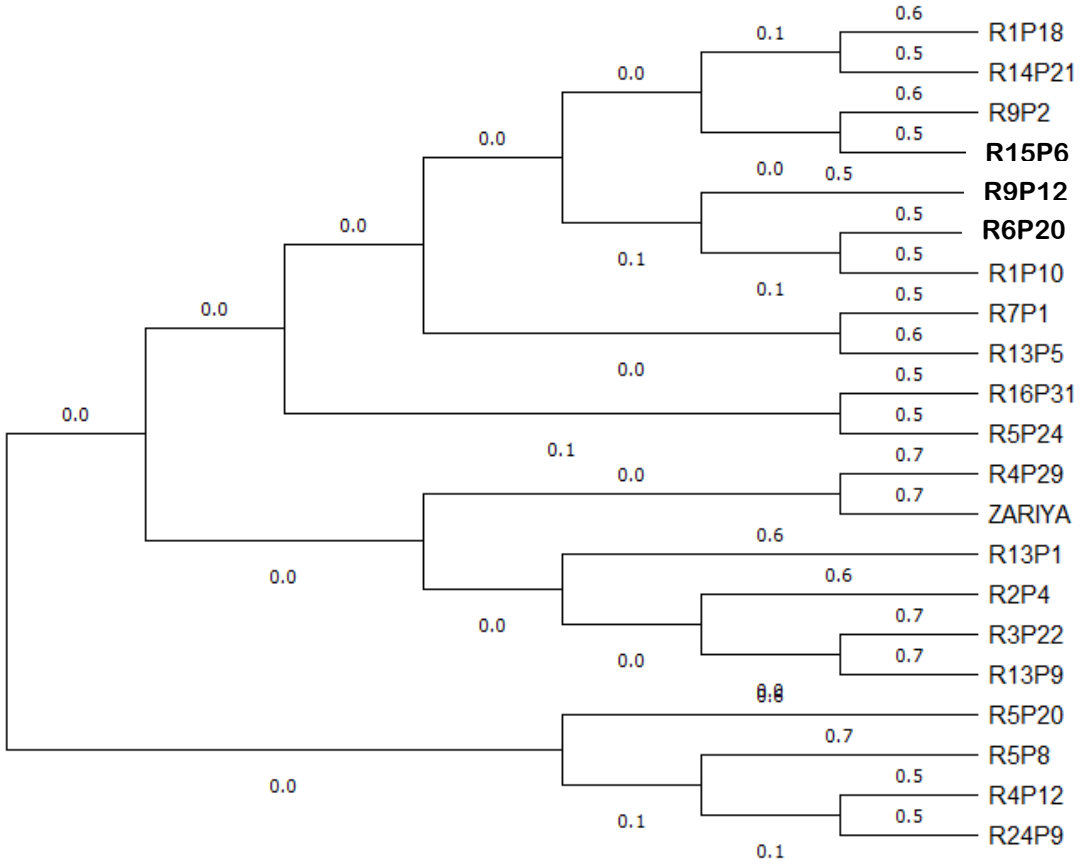


Figure 4.3: Dendrogram Showing the Phylogenetic Relationship Among Twenty-One Date Palm Accessions Based on RAPD Marker

Table 4.9: Jacqard's Similarity Coefficient of the Date Palm Accessions Based on RAPD Marker

	R13P1	R4P12	R5P8	R24P9	R5P20	R3P22	R1P18	R7P1	R13P5	R13P9	R14P21	R4P29	ZARIYA	R16P31	R2P4	R9P2	R6P21	R9P21	R5P24	R5P6	R1P10
R13P1																					
R4P12	0.891																				
R5P8	3.089	0.391																			
R24P9	0.558	0.159	0.279																		
R5P20	0.331	0.891	0.686	0.391																	
R3P22	0.279	0.686	3.702	1.471	2.853																
R1P18	1.471	3.702	3.089	0.891	1.471	3.702															
R7P1	0.279	0.686	0.891	0.235	0.279	0.686	0.391														
R13P5	0.331	0.891	3.881	0.891	0.686	0.891	3.089	0.195													
R13P9	0.331	0.891	1.471	0.891	1.471	0.279	3.089	0.558	1.471												
R14P21	1.471	3.308	3.089	0.891	0.686	3.308	0.331	0.391	3.089	1.471											
R4P29	0.686	0.891	3.512	0.891	0.686	0.558	3.512	0.391	1.471	1.471	3.089										
ZARIYA	1.471	**4.049**	3.881	3.702	0.465	2.853	3.512	2.853	3.089	3.089	1.471	0.465									
R16P31	0.235	0.391	0.465	0.279	0.235	0.391	0.686	0.127	0.465	0.235	0.465	0.235	1.471								
R2P4	0.465	3.308	3.881	2.853	1.471	0.391	3.881	0.558	1.471	0.465	1.471	0.465	0.686	0.331							
R9P2	0.465	2.853	3.512	0.891	1.471	0.558	1.471	0.391	1.471	0.465	0.686	0.331	3.512	0.235	0.686						
R6P21	0.331	2.853	3.881	2.853	0.465	0.391	3.512	0.558	0.686	0.465	3.089	0.465	1.471	0.331	0.465	0.235					
R9P21	0.465	3.308	3.512	0.558	0.331	2.853	0.465	0.195	0.331	1.471	0.686	1.471	3.089	0.331	1.471	0.465	0.235				
R5P24	0.279	0.465	0.558	0.331	0.279	0.465	0.391	0.098	0.391	0.391	0.391	0.279	2.853	**0.071**	0.391	0.279	0.279	0.195			
R5P6	0.235	0.558	3.089	0.279	0.331	0.558	0.331	0.127	0.331	0.465	0.331	0.465	3.089	0.159	0.686	0.159	0.331	0.159	0.127		
R1P10	0.686	0.891	1.471	0.558	0.465	0.558	1.471	0.391	0.686	1.471	3.089	0.686	3.089	0.331	1.471	0.686	0.159	0.159	0.195	0.465	

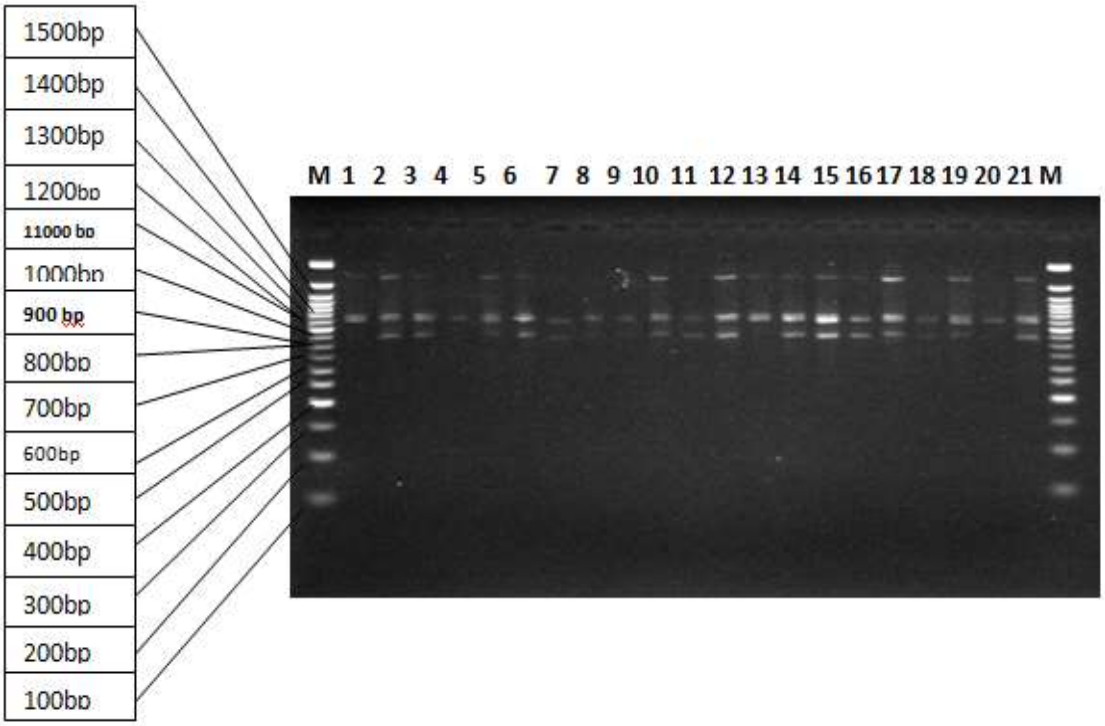


Plate VII: Gel Electrophoresis of Twenty-One Date Palm Accessions

4.1.9.2 RAPD-PCR amplifications and development of marker

In the preliminary study, each of the six primers showed some variations in banding patterns in the amplified fragments of the accessions of unknown male and female genotypes. Almost all of the primers provided patterns that were unrelated with sex. Furthermore, the PCR profile showed that the self designed primer combination of male determinant of Forward (CTCTTCCAATGTTTCTTTCTTGTG) and Reverse (CTACCACTGGCTTCTGCTAAC) fragments, when amplified, was able to distinguish a male-specific fragment of approximately at 200bp (base pairs). All other primers, when amplified with sex-specific fragments, could not provide clear identification of either male or female seedlings. Thus, a second combination of primers was needed to provide co-dominant markers for the identification of the both male and female individuals on a fragment of approximately 400 bp. Hence these markers could generate fragments that consisted of one banding patterns in male DNA extracts and no banding pattern in female DNA extracts.

4.1.9.3 Seedling population

An examination of gender identification for the 21 six months old seedling of date palm genotypes was observed to have a very good differentiation of male and female genotypes (Plate VIII). The analysis of the patterns indicated that there were twelve (12) male and nine (9) female seedlings within the total of 21 (Plate IX)

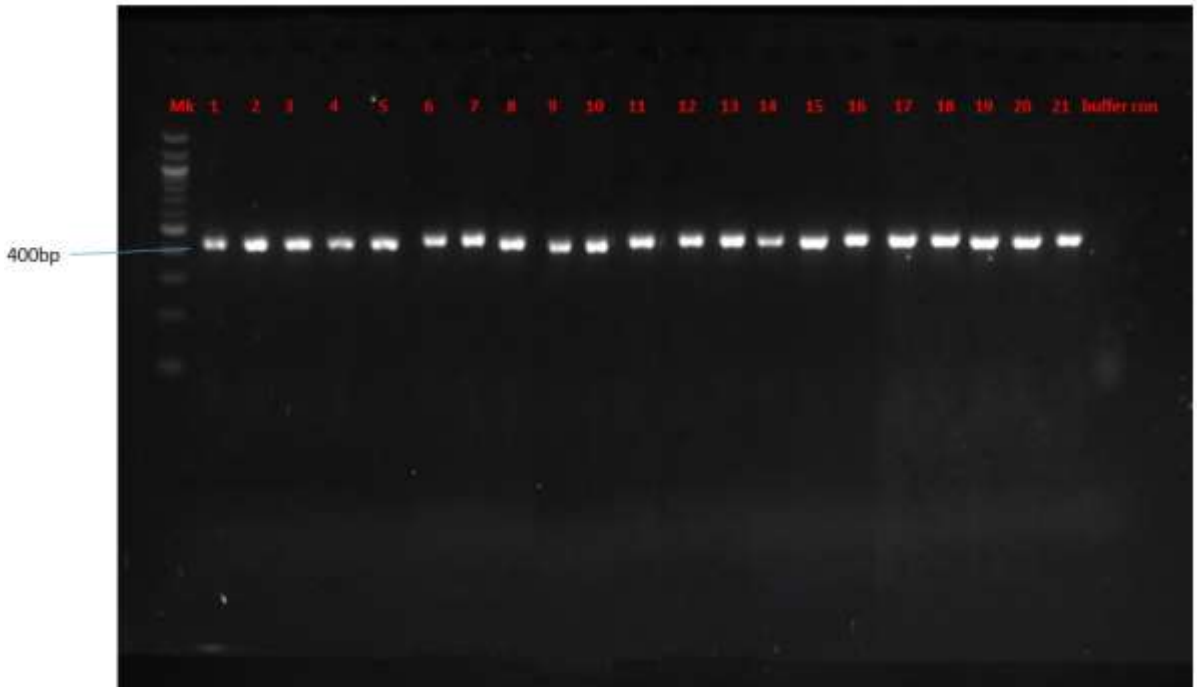


Plate VIII: Agarose gel electrophoresis of the PCR product of both male and female of the date palm accessions. An amplification of 400bp indicates positive amplification. This indicates good and quality extracted DNA. Loading arrangement: (1)R13P1 (2)R4P12 (3)R5P8 (4)R24P9 (5)R5P20 (6)R3P22 (7)R1P18 (8)R7P1 (9)R13P5 (10)R13P9 (11)R14P21 (12)R4P29 (13)ZARIYA (14)R16P31 (15)R2P4 (16)R9P2 (17)R6P21 (18)R9P21 (19)R5P24 (20)R5P6 (21)R1P10.

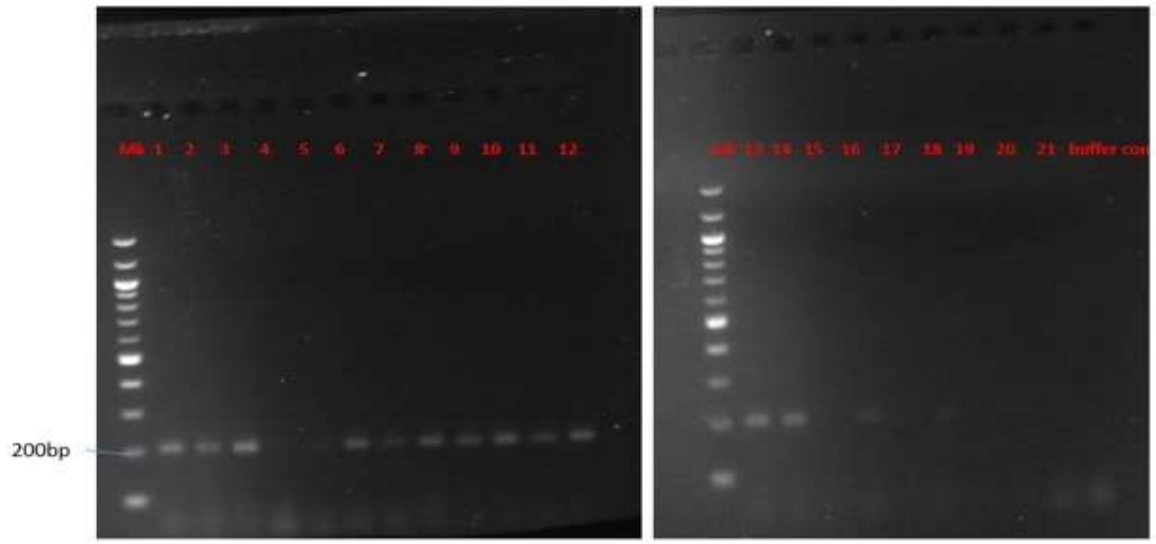


Plate IX: Agarose gel electrophoresis of the PCR product differentiating male date palm seedlings from the female using male specific primers. An amplification of 200bp indicates male loading arrangement: (1)R13P1 (2)R4P12 (3)R5P8 (4)R24P9 (5)R5P20 (6)R3P22 (7)R1P18(8)R7P1 (9)R13P5 (10)R13P9 (11)R14P21 (12)R4P29 (13)ZARIYA (14)R16P31 (15)R2P4 (16)R9P2 (17)R6P21 (18)R9P21 (19)R5P24 (20)R5P6 (21)R1P10. Samples 1 2 3 6 7 8 9 10 11 12 13 and 14 tested positive to the male primers.

Table 4.10: Seedling Population of Twenty-One Date Palm Accessions using Male Specific Primer in the DNA Amplification Procedure

Lane number	Male Lane number	Female lane number
1-----12	1, 2, 3, 6, 7, 8, 9,10 11, 12,	4, 5,
13----21	13, 14.	15, 16, 17, 18, 19, 20, 21

4.1.9.4 RAPD-PCR amplification and validation of male-specific primers

RAPD-PCR of DNA extraction of two commercial varieties namely Ajwah and Tirgal (each with four accessions, totaling eight) and two each of the already determined sexes of the accessions were amplified and determined (Plate X), to further authenticate the efficacy of the male specific primers, the commercial varieties (Ajwah and Tirgal) and the earlier identified male and female accessions were repeated for reproducibility. All the eight commercial varieties indicated male loading while the earlier identified accessions (R5P8 and R13P1 for male while R24P9 and R5P20 for female) gave same sexes of previous results respectively, (Plate XI).

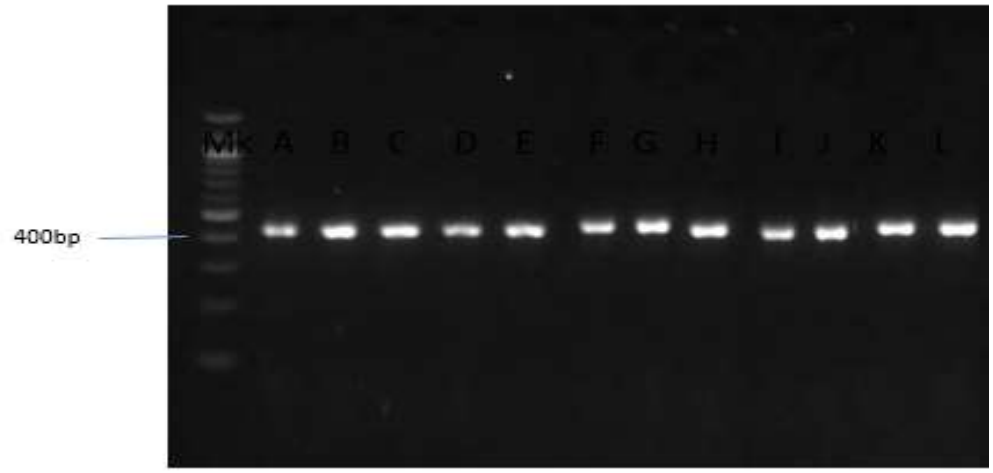


Plate X: Agarose gel electrophoresis of the PCR product of both male and female Date palm. An amplification of 400bp indicates positive amplification: (A) R5P8 (B) R13P1(C) R24P9 (D)R5P20 (E) Ajwah (F) Ajwah (G) Ajwah (H) Ajwah (I) Tirgal (J)Tirgal (K) Tirgal
(L) Tirgal

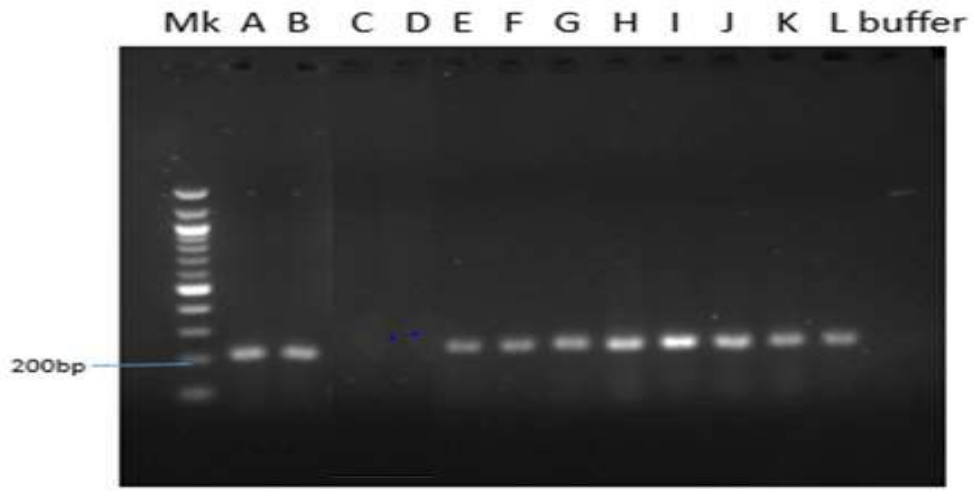


Plate XI: Agarose gel electrophoresis of the PCR product differentiating male date palm seedlings from the female using male specific primers. An amplification of 200bp indicates male loading arrangement: (A) R5P8 (B) R13P1(C) R24P9 (D)R5P20 (E) Ajwah (F) Ajwah (G) Ajwah (H) Ajwah (I) Tirgal (J)Tirgal (K) Tirgal (L) Tirgal.

Table 4.11: Seedling population oftwelve date palm accessions using male specific primer in the DNA amplification procedure.

Lane number	Male Lane number	Female lane number
A-----L	A, B, E,F,G,H,I,J,K,L,M,N,	C, D

4.1.10: Morphological variations in male and female genotypes

Out of the twenty-one accessions studied, eight accessions, that were identified as male samples from the results of the molecular characterisation were morphologically similar. They were all observed to have a larger girth size and possess a higher number of lower leaf spines (Plates XII and XIII). In the female genotypes, however, they were observed to possess a smaller or thinner girth size with little number of lower leaf spines (Plates XIV and XV).

Plate XII: Morphological identification of male accessions. Arrows in accessions R13P1 and R4P12 show large girth; arrows in accessions Zariya, R5P8, R3P22 and R1P18 showed a high number of lower leaf spines.

Plate XIII: Morphological identification of male accessions. Arrows in accessions R13P5 and R4P29 show large girth size; arrows in accessions Zariya, R13P9, R14P21 and R7P1 show high number of lower leaf spines

Plate XIV: Morphological identification of female accessions, arrow in accessions R4P29 and R1P10 show absence of lower leaf spines; arrows in accessions R9P2 and R9P12 show smaller girth size

Plate XV: Morphological identification of female accessions. Arrow in accession R6P20 show smaller girth; arrows in accession R15P6 indicate absence of lower leaf spines

4.11 Cytological Study

In this study, variations in the chromosome number, shape and size were observed among the 21 accessions. Generally, there were marked similarity and differences in the range of size and form among the chromosomes in all the accessions studied. The similarity observed among the accessions showed that the chromosomes were sticky and small in size (Plates XVI and XVII). Results of the mitotic chromosome analysis showed that some accessions were diploid with 2n=36 (accessions R5P8 and R16P31, Plate XVI) while the accession R1P18 was diploid with 2n= 28 (plate XVI). On the basis of the position of the centromere distance from the centre of the chromosome, the accessions R5P8, R3P22 and R4P29 were metacentric or submetacentric. (Plate XVI). Accessions R1P10 and R4P29 were acrocentric (Plate XVI)

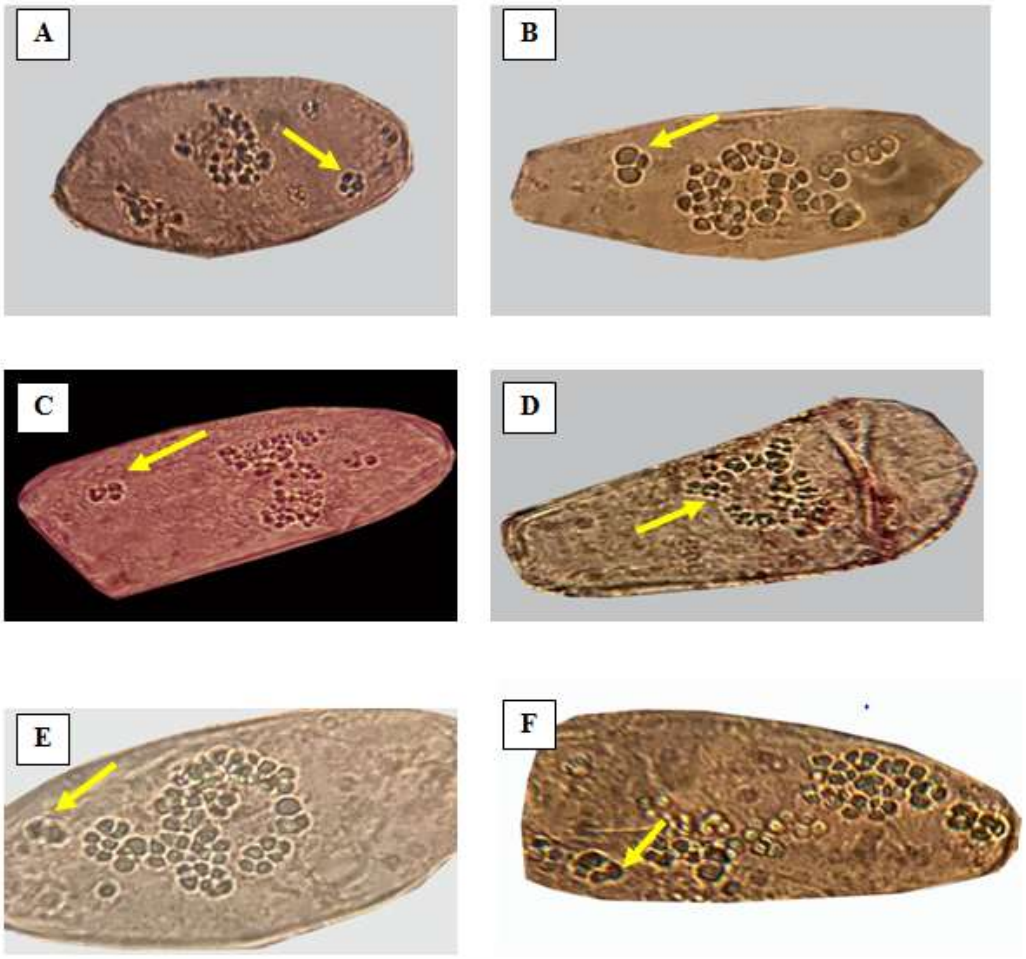


Plate XVI: mitotic metaphase of date palm accessions showing chromosome variation. A=R1P10, B=R5P8, C=R5P20, D=R4P12, E=R4P29, F=R16P31. Yellow arrow show metacentric chromosomes

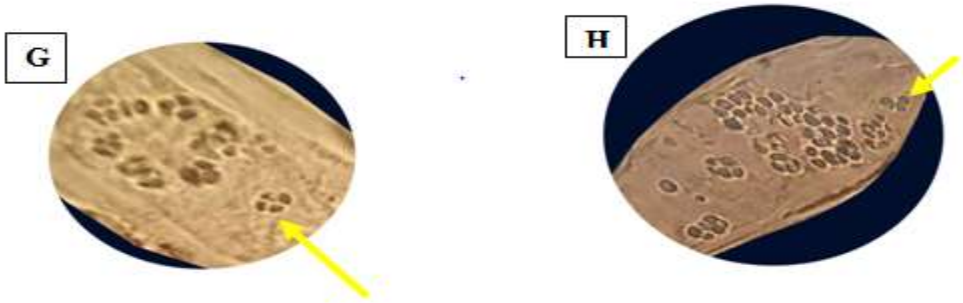
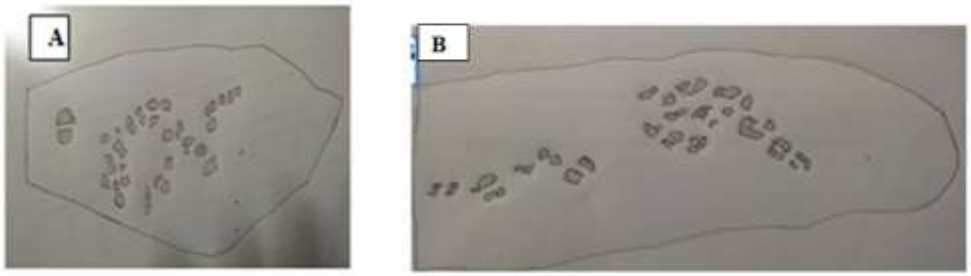


Plate XVII: Mitotic metaphase of date palm accessions showing chromosome variation. G=R1P18, H=R3P22, I=R9P12,J=R6P20. (Yellow arrow: metacentric chromosomes)



A= R5P8 2n=36 chromosomes B=2n=28 chromosomes

Plate XVIII: Idiogram of mitotic metaphase of date palm accessions showing chromosome

4.2 Discussion

4.2.1 Germplasm collection

The high variations in phenotypic characteristics among the date palm accessions collected from the gene pools, which represent the growing states from northern part of Nigeria (Niger, Nassarawa, Kaduna, Katsina, Kano, Kebbi, Sokoto, Jigawa, Bauchi, Yobe, Gombe, Borno, Taraba), is an indication of great diversity in the gene pools among Nigerian Date palms. This is in conformity with the result of Bettencourt *et al.* (1992), who listed ten collections worldwide with Nigeria inclusive and reported that genetic diversity exists in date palm collection. Omamor (2000) reported that date palm cultivation has remained restricted to compounds, homesteads and few orchards in the northern parts of Nigeria. The high diversity among the accessions could be attributed to the adaptation of all the accessions to different environments, lack of transborder sharing of seeds, and wide distance of separation of cultivated species which prevents cross-fertilization.

A high degree of diversity in the fruit characteristics in the gene pools confirms with the report of Ataga *et al*. (2012), who noted that the considerable diversity in fruit characteristics which exist in Nigerian landraces could be broadly classified into large, rfmedium and small fruits; this appears to compare favourably with leading commercial varieties elsewhere. This finding agrees with Markhand *et al*. (2010) in the characterised Pakistani date palm fruits and concluded that the fruits were significantly diverse from one cultivar to another

The assemblage and variability of the studied date palm fruit accessions in the gene pools will serve as a valuable source of parents for hybridisation and subsequent development of the fruit crop.

4.2.2 Proximate composition of the date fruit accessions

Moisture content of date palm depends on their harvesting time, maturation period, environmental conditions such as humidity and temperature during the growing period as well as varietal differences (Nadia *et al*., 2018). The moisture content (2.25 % -7.65 %) obtained was similar to the report of Sadiq *et al.* (2013) on date fruit varieties, but lower than the 8.33 % reported for coconut seeds (Ojobor *et al.*, 2018). The low moisture content indicates that date fruits can be stored for a long period of time without spoilage as it will not be susceptible to microbial growth and enzyme activities.

The ash content which is an index of mineral in most edible fruits was between 1.56 %-2.70 %. The variations observed in the ash content might be due to differences in the nutritional attributes of the fruits. This findings agrees with Ghnimi *et al*. (2017), who reported that the ash content of date fruits ranged from 1.40 % - 2.30 %. However, earlier studies reported ash content of various date fruit varieties ranging from 0.90 % - 2.00 % (Al-Harrasi *et al.,* 2014). The high ash content recorded in this accessions indicates the high percentage of inorganic mineral elements present in date fruits.

The variations in protein content (3.36 %-7.13 %) observed in this study is contrary to the2.85% protein content earlier reported by Borchani *et al.* (2010), in eleven Tunisian date fruits. The protein content recorded in this study was higher than the average protein content of 1.50 % -2.14 % for fresh and dried dates as reported by Kazi *et al*. (2015). The high percentage of protein suggest that Nigerian date fruits could be potentially good for nutritional benefits. The fruits also had a high molecular weight which indicates genetic variability. This finding agrees with El-Sohaimy and Hafez (2010) who reported that most

of the proteins in their study had a high molecular weight of between 80 and 135 KD of 3.00 %.

Date fruit has been reported as a good source of dietary fiber such as cellulose, hemicellulose, lignin and pectin (Al-Shahib and Marshal, 2003; Mrabet *et al.,* 2012). The variations in fibre contents observed among the accessions used in this study might be due to varietal differences as well as duration of fruit collection. The crude fibre content (2.10 % - 12.20 %) observed in this study was within the range (6.63 %-12.21 %) reported for two varieties of date palm in india by Thilagavathi and Gayathri (2019), but its higher (2.50 %) than reported by Al-Harrasi (2014) in Omanian date fruits. The moderately high crude fibre value of date palm fruits recorded in this study indicates its potential in aiding digestion and absorption processes.

The low level of lipids (fatty acid and cholesterol) content of 2.69 % - 5.46 % among the accessions studied is an indication that consumption of date palm fruits is safe for the heart and high blood pressure patients. The date palm fruit is rich in carbohydrate contents such as galactose, glucose and fructose which serve as a source of high energy. Date fruit has been reported to contain carbohydrates that are vital in nutrition and are a good source of energy (Akoma *et al.,* 2018). The carbohydrate value of 64.55 % - 82.89 % observed in this study is within the range of carbohydrate content reported by Mikki *et al.* (2003) in some Saudi date fruits. Variations in the carbohydrate content could be attributed to differences in the cultivar, harvest/post harvest factors and the growing environment. This result shows that some Nigerian date fruits are an excellent source of energy for metabolic processes and can be utilised for confectionary products.

The high level of calcium content (68.83 % - 119.39 %) recorded among the accessions is similar to the value of 105.2 % reported in Nigeria by Sadiq *et al.* (2013), but higher than the 60, 55 and 51 % earlier reported in Bangladesh by Sultana *et al*. (2015). Thilagavathi and Gayathri (2019) reported calcium as the most abundant mineral in the body because it regulates many cellular processes and has important structural role in living organisms. Calcium has been found to help keep muscles working correctly (El-Sohaimy *et al*., 2010). The high amount of calcium observed among the accessions indicates that date fruits are a good source of bone strength and growth.

Magnesium is the best supporting factor needed every day in human health as it forms an important role in bone-formation by keeping muscles and nerves healthy. The magnesium value of 39.16 %-78.41 % observed in this study falls within the recommended daily intake. This result agrees with the findings of Bouhlali *et al*. (2017), on some Moroccan date fruit varieties and Ricardo *et al.* (2019) on medjool date cultivar. The sodium content (6.20 %-13.44 %) was low when compared with 38.55 % of sodium observed on some Nigerian date fruits as reported by Omowunmi and Adejumo (2013), this could be due to differences in cultivar, environment and soil conditions. The result obtained in this study disagrees with the 91.03 % or 89.58 % reported by Thilagavathi and Gayathri, (2019) on two different varieties of unripe date palm.

The concentration of potassium in this study was between 275 % - 424 %. Thilagavathi and Gayathri (2019), reported that a high potassium and low sodium level was beneficial for the people suffering from hypertension, which confirms with the findings in this study, where low sodium content and high potassium concentrations were observed. Thus this supports date fruits as an excellent food for hypertensive patients.

4.2.3 Sugar contents of the date fruit accessions

The glucose content (30 % - 96 %) in the date palm accessions used in this study is higher than the value of 37.21 % reported by Ricardo *et al.* (2018) on medjool variety in Mexico, and 37.79 % glucose content observed in some Moroccan date fruits as reported by Bouhlali *et al.* (2017). The variation in glucose content might be due to differences in the genetic make-up of the different accessions, climate, soil and other environmental conditions. This corroborates the findings of Khaled *et al.* (2019), who in their study on Genome-wide association mapping of date palm fruit and concluded that the cell wall invertase genes show large expression differences between varieties with different fruit sugar compositions. Glucose is an important energy source that is needed by all the cells and organs in human bodies. The high level of glucose recorded in this study is an indication that Nigerian date palm fruits are excellent source of energy in the human body.

The high amount of fructose (32 mg/100 g – 102 mg/100 g), observed in the date palm accessions used in this study is in conformity with the findings of Biglari *et al.* (2009) who reported that fructose was about twice as sweet as glucose which is considered less diabetogenic. Miller *et al.* (2003) also reported that low glycaemic index (GI) in dates is due to the high amount of fructose present. This implies that consumption of date fruits may be very useful in the management of patients with diabetes.

It was observed that the sucrose content was lower than the glucose and fructose content in all the accessions. This agrees with the findings of Amira *et al.* (2011) on Tunisian dates that as the fruit ripens there is reduction in sucrose and increase in glucose and fructose, which might be due to increasing activity of the invertase enzyme that converts sucrose into

reducing sugars. Rastegar *et al.* (2012) reported that sucrose undergoes a complete hydrolysis, especially at tamar stage which is also the fruiting stage of the date palm accessions studied. Maria *et al.* (2013) reported that most of the date palm varieties belong to the inverted sugar type where sucrose is totally converted into glucose and fructose at the mature edible tamar stage.

4.2.4 Phytochemical composition

Date fruits are considered as staple fruits and they are widely cultivated in semi-arid regions. The phytochemical analysis of the date fruit studied showed the presence of alkaloids, tannins, flavonoids, phenols and saponins, indicating the presence of secondary metabolites. The tannin content (0.005 mg/g - 5.540 mg/g) observed in the accessions studied is in line with the findings of Sadiq *et al*. (2013) and Saha *et al*. (2017) on date palm as well as Ojobor *et al.* (2018) on coconut. The variations in the tannin content might be due to the genetic variations in the astringency content in date fruits. The presence of tannin shows that date fruits could quicken the healing of wounds and inflamed mucus membrane. Tannins also play an important role in the prevention of cancer and are also used for the treatment of inflamed and ulcerated tissue (Aiyegoro and Okoh, 2010). The presence of tannin in date fruits makes it serve as raw material for pharmaceutical industries and orthodox medicine in the treatment of many health challenges.

The flavonoid content ranged from 2.77 mg/g – 12.18 mg/g among the accessions studied. This is in line with the findings of Tapas *et al.* (2008) on *Phoenix sylvestris,* Saha *et al.* (2017) on date palm and Ojobor *et al.* (2018) on coconut. This shows that date fruits can serve as antioxidants and metal chelators. The presence of flavonoids in fruits has long

been recognised to perform anti-inflammatory, anti-allergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities.

Alkaloids are in great demand for pharmaceutical formulations especially for terminal diseases such as cancer and inflammatory disorders. Alkaloids are a heterogeneous group of naturally occurring compounds found in the leaves, bark, roots or seeds of plants. (Sarah *et al.,* 2015). They are the most effective plant substances used therapeutically as analgesic, antimicrobial and antibacterial agents. The presence of alkaloids in this study indicates that date fruit could play a wide range of physiological actions in human healthcare such as antibiotics, anticancer and degenerative disorders. The variations in alkaloids contents (0.23 mg/g - 10.684 mg/g) among the accessions studied are in contrast with the value (1591.00 mg/g) reported in some date palms cultivated in Niger.

The phenol content(15.00 mg/g and 73.92 mg/g) recorded in this study is in line with the reports of Mohammed *et al.* (2014) on Sudanese date fruits (35.82-199.34 mg/g), but lower than the findings reported by Al-Turki *et al.* (2010) on the Tunisian date fruits and Krishnamoorthy *et al.* (2018) on Palmyra palm. The variations in phenol content among the accessions studied might be due to varietal differences as well as geographical locations, as has been reported studies by Kchaou *et al.* (2013) on some Tunisian date palm varieties grown in different geographical locations. Phenols are very important plant constituents because of their free radical-scavenging ability. Dietary phenols from date consumption may supply substantial antioxidants which, in turn, may prevent (Tohidi *et al.,* 2017). This implies that the fruits of date palm are a rich source of anti-oxidants because studies have shown that antioxidant capacities of plants are highly correlated with phenol compounds.

Variations observed in saponin contents of the date palm fruits are in line with the report of Saha *et al.* (2017), who observed significant differences in the saponin contents in all the fruits of the accessions studied. Similar findings have been reported by Hassan *et al.* (2012) on some medicinal plants in Nigeria and Tiwari *et al.* (2014) on *Gymnema sylvestre*. The results of saponin content in observed fruits in this study (217 mg/g – 888 mg/g), shows that date palm fruits could be used as a traditional medicine.

4.2.5 Correlation analysis

In this study, a correlation coefficient was performed to understand how the five phytochemical components namely flavonoid, alkaloid, saponin, phenol and tannins contribute to the overall phytochemical profile of the fruit of date palm. The correlation matrix describes how intricately the correlation amongst the phytochemical constituents exists. From the result obtained in this study, the positive correlation between phenol and saponin (0.05) and phenol with flavonoid (0.30) is in agreement with the findings of Chaudhuri *et al.* (2013) between phenol and saponin (0.96) and phenol and flavonoid (0.96) on the leaf extract of some species of croton plants in India. The positive correlation observed in some of the parameters is an indication of high quantities of these bioactive phytochemicals in date fruits, which may be responsible for its medicinal value. The negative correlation (-0.09262) observed between flavonoid and saponin contrasts the positive correlation reported by Chaudhuri *et al*. (2013).

4.2.6 Morphological parameters

These differences might be due to varietal differences, geographical location as well as the ripening stage of the date fruits.

The pronounced variation observed in the vegetative characters revealed the genotypic differences among the accessions which were evident in the phenotypic expression. Hanane and Halima (2020) noted that traits related to vegetative and reproductive organs could be a useful tool to assess phenotypic diversity and are therefore, used as tools for characterisation of plant species. Salem *et al.* (2008), Eissa *et al.* (2009) and Hammadi *et al.* 2009) reported that morphological traits such as plant height, length of petiole, girth of plant , length and grouping of spines, spathe, fruit and spideces contain quantitative markers used for identification, description, differentiation and characterisation of date palm cultivars.

Differences were observed among the accessions in all the morphological parameters observed in this study. This is in line with the report of Djerouni *et al.* (2015), who noted that vegetative characteristics could be used to differentiate date palm varieties. Mohamed *et al.* (2014) and Haider *et al.* (2015) in studies using 18 Mauritanian date palms and 16 Pakistani date palms, respectively, reported that pinna number, length and width and the palm leaf could be used to differentiate date palms.

Leaf width which ranged from 1.10cm-3.40cmand differed among the accessions studied. This is in contrast to the findings of El-Merghany and Al-Daen (2014), who observed no significant difference in leaf base width of date palm cultivars under Toshki conditions in Egypt. The result of this study is similar to the findings of Saleem *et al*. (2008) who reported width of leaf as an important discriminant parameter among date palm cultivars. Length of leaf has also been reported as an important characteristic that can be used to discriminate among date palm varieties (Faqir *et al.*, 2016, 2018).

The girth size in date is used to determine maturity (Marie *et al.*, 2007). Variations in girth size as seen in this study might be due to division and enlargement of parenchymatous cells in the ground tissue (secondary growth), which is genetically controlled. Marie *et al.* (2007) noted that repeated divisions cause increase in girth of stem, resulting in what is referred to as diffused secondary growth. The accession R5P20 which had the highest girth size could be considered in the development of breeding programmes for growth selection among the accessions. In the study of Barhee cultivar and two strains of Barhee palm seedlings in Egypt, El-Kosary (2009) found slight variation in the girth.

The leaflets observed in this study were of the short type, which is similar to the findings of Ahmed *et al*. (2016) who reported short leaves of less than 325 cm. The variations observed in the length of leaflets might be due to varietal differences among the accessions.

The length of internode plays an important role in the growth and health of plants. Pearson *et al.* (1995) reported that the final stem length is determined both by number of internodes or length of internode. The variations in the length of internode observed among the accessions might be due to varietal differences or environmental conditions. Carvalo *et al.* (2002) suggested that the length of internode could be used as an indicator of a plant's health and productivity. The length of internode is affected by several factors including fertilizer application, sunlight and other environmental conditions.

This study highlighted some strong relationship among some accessions with regard to some quantitative vegetative characteristics. For example, the arrangement of leaflets in the accessions was either alternate or opposite. In some accessions, both opposite and alternate arrangements of leaflets with a single leaflet in-between were observed.

4.2.7 Fruit characteristics

The extensive variations fruit size among the accessions studied revealed that there is diversity in fruit characters which can be used for phenotypic characterisation and development of breeding programmes. The variations observed in the fruit characteristics among the accessions might be due to genotypic differences and genetic variability of pollens on fruit characters (a process known as metaxenia effect). This finding is in line with the report of Merwad *et al.* (2015), who reported that the type of pollen used in pollination affected physical quality of the fruit in date palm.

The fruit weight (2.04 g-9.10 g) observed in this study was similar with the range (5-12 g) reported by Muhammad *et al.* (2016) on some yield components of major date palm cultivars in Pakistan. Abdoulhadi *et al.* (2011) reported mean fruit weight of 11.60- 7.05 g in the study on the assessment of fruits in date cultivars of Saudi Arabia. However, Mansour (2005) reported the fruit weight of 23.80 g in the study on morphological and genetic characterisation of some common date palm cultivars in Ismailia region in Egypt. Jaradat and Zaid (2004) noted that characters such as fruit length are unique with a high polymorphic index. The significant differences in fruit length (2.70 cm-4.87 cm) among the accessions studied is in conformity with the range (2.4–3.9 cm) reported by Ghulam *et al.* (2010).

Farag *et al.* (2012) noted that date palm fruits are influenced by the type of pollinator used for pollination of the mother tree. Significant differences in the fruit length could be due to varietal differences among the accessions studied, similar findings reported by Ghulam *et al.* (2010) on some Pakistani date palms. Al-Hooti *et al.* (1997) reported that fruit size

varied from one cultivar to another. The accessions R5P24, R7P1, R9P2, R9P12 and R3P22 could be utilised for mass propagation.

The range of fruit diameter (1.03-2.10cm) observed in this study is similar to the range (1.5–2.4 cm) reported by Ghulam *et al.* (2010) on some Pakistani date palm cultivars. The variations in the fruit diameter among the accessions might be due to varietal differences as well as the effect of pollen sources used to pollinate the mother plant. Iqbal *et al.* (2012) reported that the time of pollination at different times significantly affected fruit length and diameter of Dhakki variety. ElMardi *et al.* (2002) noted that variations in diameter of date fruits could be due to varietal differences.

Fruit seed is considered as one of the most important physical characters. Sometimes two varieties are similar in everything except the seed properties. The result on fruit characteristics in this study is in agreement with the report of Mohammed Elsafy (2000), who reported a wide range of diversity in fruit size, and noted that the large number of accessions in Nigeria might indicate the benefit of screening date palm germplasm for agronomically important traits. In the study on some local Algerian date palm cultivars, Hanane and Halima (2020) reported that farmers use fruits more than the palm in distinguishing date palms. Fruit traits are therefore, used in the morphological characterisation of Nigerian date palm.

4.2.8 Genetic diversity as revealed by RAPD Marker

Results of the RAPD analysis showed genetic diversity among the date palm genotypes in Northern Nigeria. The phylogenetic tree constructed showed divergence among the date

palm accessions studied as evidenced by the cluster groups. This agrees with the findings of Nadia *et al.* (2012) and Hussein *et al.* (2005).

The high level of polymorphism (84.5 %) observed among the accessions is in agreement with Khierallah *et al.* (2011), who reported 83 % polymorphism in some Iraqi date palms. An example of genetic polymorphism across the 21 date palm accessions was recorded in primer OPH-06 and OPH-10. The high polymorphism observed is an indication of genetic diversity among the accessions studied.

Results also showed that some accessions (e.g. R9P12, R6P20 and R1P10) from the same gene pools (e.g gene pool II) were grouped together, an indication that genetic divergence followed geographical isolation. Ataga *et al.* (2012) reported that date palm had a common evolutionary relationship and that some accessions have adapted to their local environments as land races through gene re-arrangement due to long periods of cultivation.

In groups I and II, the UPGMA showed slight intra-varietal polymorphism among the accessions. This is an indication of close relationships among the accessions and suggests the existence of gene flow in the experimental field from which the fruits were originally collected. Al-Khalifah and Askari (2003) noted that the ex-change of varieties between the different plantation areas, clonal propagation of ecotypes and limited sexual reproduction might have resulted in narrowing the genetic base among them. A similar finding was reported in Tunisia using the RAPD Marker, (Zehdi *et al.,* 2004) and in Saudi using the RAPD and ISSR markers (Munshi *et al.*, 2010).

Genetic diversity can be enlarged by combining desired traits from different local and wild populations of different geographical origins into the breeding lines (Bisht *et al*., 1998).

Nevertheless, the rich genetic diversity available among the date palm accessions from Northern Nigeria can be utilized for current and future breeding programmes in order to select genetically distinct parents.

The use of RAPD for assessment of genetic diversity is more reliable than the morphological and biochemical markers since they are completely devoid of the effects of environment and the stage of the experimental material. Xuemei *et al*. (2012), Khierallah *et al*. (2011) and Ameer *et al*. (2012) noted that RAPD is simple to use for the evaluation of genetic diversity and phylogenetic studies in many plant species.

The present study also confirmed the suitability of RAPD as a reliable, simple, easy to handle and elegant tool in molecular diagnosis of different accessions available in the germplasm collection. The results showed that RAPD markers can be efficiently used to evaluate genetic variation in date palm germplasm. Molecular markers like RAPD are effective tools to discriminate various date palm genotypes, and commonly used in modern-day breeding to achieve genetic improvement in date palm.

4.2.9 Sex determination studies

In Nigeria, sex determination in date palm has been a major challenge in the development of breeding programme as it has been difficult to differentiate morphologically between male and female date palm at the seedling stage (i.e. prior to first flowering). In this study, the use of sex-determinant primers were developed which has been proved as a reliable technique for sex determination in date palm. Out of a total of 21 seedlings, one from each accession, twelve (12) were observed to be male and nine (9) were female. The sex determinant primers produced positive results when tested on commercial varieties and pre-

determined genotypes. The ability to identify gender by DNA methods provides a distinct advantage as it is difficult to distinguish sex in the early seedling stage from morphological differences. Sex-specific DNA markers have been used to differentiate sexes in date palm cultivars (Al-mahmoud *et al.*, 2012). It was reported that the use of sex specific DNA markers such as RAPD primers has been successful in different varieties of date palm at seedling stage to identify sex-linked markers in the crop. A number of sex-specific DNA markers have been identified in several dioecious plants using RAPD primers (Shibu *et al*., 2001, Dhawan *et al.,* 2013).

In this study, morphological differences observed among the male and female genotypes using molecular characterisation is an indication that morphological parameters are under genetic control. Jingshan *et al.* (2020) reported genetic and morphological variations in the leaf of *Populus simonii* and *P. nigra* plants in China. The male-specific RAPD primer developed in this study was used to differentiate between male and female plants at the seedling stage.

Molecular marker-based techniques are reliable in determining the sex of dioecious plants as they are stable and independent of age and environment. In addition, DNA identification of male and female genotypes provides a fast and reliable approach to sex differentiation in plants regardless of reproductive age. Gao *et al*. (2009) and Dhawan *et al.* (2013), noted that SCAR marker has been used for sex-determination in many dioecious plant species in which male and female plants look similar at the vegetative stage. Qacif *et al.* (2007) reported the successful use of biochemical studies for gender identification of date palm plants. Al-Qurainy *et al.* (2011) reported the use of male-specific SCAR markers which

were developed to differentiate male and female plants such as *Humulus scandens, Rumex nivalis* and *Phoenix dactylifera* using molecular marker profiling.

Findings of this study provide a baseline information needed for genetic and morphological studies of on date palm. Dioecism and the long time taken to attain sexual maturity have led to selection programmes based on clonal propagation of females from good date palm cultivars. This procedure promotes genetic uniformity, accelerates the process of genetic erosion and makes crops vulnerable to environmental stresses. Plant breeders may adopt sex determining markers as a potential tool for gender identification of date palm seedlings before their plantation in field.

4.2.10 Cytological studies

The common feature in the mitotic metaphases among the date palm accessions studied is that all the chromosomes were sticky and variable in sizes. Tahira *et al.* (2019) studied karyotype variations among date palm cultivars in Pakistan and concluded that date palm chromosomes are recalcitrant in nature, very sticky and exceptionally small in size. This, perhaps, accounts for the interspecific hybridization which has been demonstrated through crosses made between several species. Structural differences in chromosomes such as the chromosome length and centromere deviation frommedian to telocentric were observed in these accessions. According to Haroun (2010), the variation in length between chromosomes and centromere position indicates the degree of heterogeneity in the genome structure of the species. This suggests that the species is widely distributed and cross-pollinated, with high chances of intraspecific and interspecific hybridization to produce new cultivars. Kuterekar and Wanjari (1983) noted that varietal differences results from

changes in heterochromatic part of chromosomes as well as repetitive sequences in the genome. Tayyar *et al.* (1996) reported that variation among chromosomes could be as a result of re-arrangement in the chromatin part of the chromosomes.

The 2n=36 number of chromosomes obtained in this study confirms earlier reports (Aly and Bacha, 2000; Mohamed *et al.,* 2010; Alzahrani 2016; Tahira *et al.,* 2019). Using tissue culture-derived date palm plants, Mohammed *et al.* (2010) observed that there were about 36 chromosomes arranged in 18 pairs according to their lengths in each of the varieties studied. The somatic chromosome number, 2n=36,observed in some date palm accessions could be due to stable genome which did not show fusion or duplication of whole chromosome pair. On the contrary, Al-Salih and Al-Rawi (1987) reported different numbers of chromosome pairs such as 14, 16, 17, 18, and 32 in cv. Lilwi. The variation in somatic chromosome number could be due to differences in origin of date palm varieties. Variations in the number and morphology of chromosomes may also arise from mutations in the natural populations, chromosomal re-arrangements such as translocation, duplications, deletion and inversion (Mukherjee and Roy, 2012; Awe and Akpan, 2017).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Genetic diversity is of great importance in the development of breeding programmes. The variations observed among the parameters studied showed that a degree of variability exists among date palm germplasm ibbbn Nigeria for the characters studied.

The present study revealed that the most promising accessions in terms of nutritional and morphological compositions were R1P18, R2P4, R4P29, R5P8, R5P20, R13P5, R16P31 and ZARIYA.

The use of RAPD-PCR analysis is very useful in determining the relationships that exist among the accessions and for selection of genetically distinct genotypes for the improvement of the crop.

The developed Sex determining RAPD marker used in this study could be used for gender identification at the seedling stage of date palms in order to save time, as the plant takes 5–7 years to reach the reproductive stage

The cytological examination of some of the accessions confirmed 2n=36 chromosomes for the date palm, with variant type of chromosomes based on the position of the centromere. This shows heterozygosity of the plant genetic composition and high chances of hybridisation

5.2 Recommendations

i. The accessions with nutritional and medicinal values should be recommended to the farmers as well as marketers.

Ii Further research should be carried out on the morphological and fruit characteristics that showed greater genetic diversity for selection and improvement of the crop in the germplasm gene pools.

iii Further study is required to evaluate genetic diversity of the crop using other molecular markers like Amplified fragment length polymorphism, Simple sequence repeats, Restriction fragment length polymorphism and Inter-Simple sequence repeats e.t.c.

iv Larger populations should be used in screening for morphological differences that are related to sex differentiation using the sex-determining primers.

V Inheritance studies such as metaxenia effect should be carried out for the improvement of the crop

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