الجمهورية الجزائرية الديمقراطية الشعبية
République Algérienne Démocratique et Populaire
وزارة التعليم العالي والبحث العلمي
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
جامعة مولاي الطاهر، سعيدة
Université MOULAY Tahar, Saida

كلية العلوم الطبيعية و الحياة
Faculté des S.N.V
قسم الفلاحة و علوم التغذية
Département d'agronomie et sciences de nutrition

N° d'Ordre

Mémoire pour l'obtention du diplôme de master

En Biotechnologies vegetales

Spécialité : biotechnologie et génomique végétale

Thème

Molecular sex determination in the date palm (*Phoenix dactylifera L*)

Presentè par :

- Mr : NADRI Yasser Nidal Nizar

Soutenu le : 23/0602024

Devant le jury composé de :

Présidant	Mme. CHALANE Fatiha	MCA Université Moulay Taher de Saida
Examinateur	Mme. BENDAOUD Amina	MCB Université Moulay Taher de Saida
Rapporteur	Mme. BERBER Naima	MCA Université Moulay Taher de Saida

Année universitaire 2023/2024

Dedications

I dedicate this modest work to:

My mother A. Bakhta, NASRI Asmaa Messaouda for their help and support throughout my academic journey.

Acknowledgements

I would like to thank:

My supervisor, Mme. BERBER Naima, members of the jury, Mr. ANTEUR Djamel and Mme. CHALANE Fatiha, all the professors and teachers who have helped me from primary school to my master's degree and the Saida university establishment.

List of abbreviations

SSR: Simple Sequence Repeats

SNP: Single Nucleotide Polymorphisms

AFLP: Amplified Fragment Length Polymorphism

RFLP: Restriction Fragment Length Polymorphism

RAPD: Random Amplified Polymorphic DNA

ISSR: Inter-Simple Sequence Repeat

EST: Expressed Sequence Tag

CAPS: Cleaved Amplified Polymorphic Sequence

DArT: Diversity Array Technology

SCAR: Sequence Characterized Amplified Region

BCE : Before Common Era

CAPS : Cleaved Amplified Polymorphic Sequence

cm : Centimeters

DNA : Deoxyribonucleic Acid

FAO : Food and Agriculture Organization

°C : Degrees Celsius

ha : Hectares

kg : Kilograms

m : Meters

m3 : Cubic Meters

mm : Millimeters

NGS : Next-generation Sequencing

PCR : Polymerase Chain Reaction

CYP703: Cytochrome P450 family 703.

GPAT3: Glycerol-3-phosphate acyltransferase 3.

LOG: LONELY GUY gene.

2n: Diploid chromosome number.

PAGE: Polyacrylamide Gel Electrophoresis.

TPC : Total Phenolic Content.

ISH: In Situ Hybridization.

FISH: Fluorescent In Situ Hybridization.

CODFISH: Concomitant Oncoprotein Detection Fluorescence In Situ Hybridization.

SKY: Spectral Karyotyping.

PCR: Polymerase Chain Reaction.

dNTPs: Deoxynucleotide Triphosphates.

XX: Female chromosomal arrangement.

XY: Male chromosomal arrangement.

List of tables

List of figures

Abstract

The date palm (*Phoenix dactylifera L.*) is a diecious Angiospermes-monocotyledones, plant, it is very economically important plant primarily for it's fruit, which is produced by the female plant, however it's sex is phenotypically identifiable near it's first inflorescence at the 4-5 year mark of it's life, which makes it very difficult for farmers to manage planting habits for optimal fruit production in Algeria and around the world.

However, there is a molecular solution to this issue, in the form of sex linked molecular markers developed from Sequence Characterized Amplified Region (SCAR), Amplified Fragment Length Polymorphism (AFLP), and Simple Sequence Repeats (SSRs) …etc. markers, which are used as PCR primers to identify the Presence of the Date-SRY gene which is responsible for the control of sex linked gene expression, later through electrophoresis the results are viewed and if the band is present the plant is male, if not it's a female, this newly developed technique is not yet widely used and still under research, to make it concrete and accessible.

Keywords: Date palm, Algeria, Molecular markers, Gender determination, Genetic engineering.

Résumé

Le palmier dattier (*Phoenix dactylifera L.*) est une plante angiosperme-monocotylédone dioïque, économiquement très importante principalement pour son fruit, produit par la plante femelle. Cependant, son sexe n'est phénotypiquement identifiable qu'à partir de sa première inflorescence, vers l'âge de 4 à 5 ans, ce qui rend la gestion des habitudes de plantation très difficile pour les agriculteurs afin d'optimiser la production de fruits en algerie et mondialement

Il existe toutefois une solution moléculaire à ce problème sous forme de marqueurs moléculaires liés au sexe, développés à partir de la région amplifiée caractérisée par séquence (SCAR), du polymorphisme de longueur de fragment amplifié (AFLP) et de séquences répétées simples (SSR), etc. Ces marqueurs sont utilisés comme amorces de PCR pour identifier la présence du gène Date-SRY, responsable du contrôle de l'expression des gènes liés au sexe. Ensuite, grâce à l'électrophorèse, les résultats sont visualisés : si la bande est présente, la plante est mâle, sinon elle est femelle. Cette technique nouvellement développée n'est pas encore largement utilisée et est encore en cours de recherche pour la rendre concrète et accessible.

Mots-clés: Palmier dattier, Algerie, Marqueurs moléculaires, Détermination du sexe, Génie génétique.

ملخص

نخيل التمر (*Phoenix dactylifera L*) هو نبات مغطى البذور-أحادي الفلقة، وهو نبات ذو أهمية اقتصادية كبيرة بشكل أساسي لثماره التي تنتجها النبتة الأنثى. ومع ذلك، فإن جنسها لا يمكن تحديده ظاهرياً إلا عند ظهور أول نورة لها بعد مرور 4-5 سنوات من عمرها، مما يجعل من الصعب على المزارعين إدارة عادات الزراعة لتحقيق إنتاج مثالي للثمار في الجزائر و عالميا

ومع ذلك، توجد حلول جزيئية لهذه المشكلة على شكل مؤشرات جزيئية مرتبطة بالجنس، تم تطويرها من مناطق محددة مكبرة بواسطة التسلسل (SCAR)، تعدد الأشكال لطول الشظايا المضخمة (AFLP)، وتكرارات التسلسل البسيطة (SSRs)، وغيرها. تُستخدم هذه المؤشرات كبادئات لتفاعل البوليميرات المتسلسل (PCR) لتحديد وجود جين Date-SRY المسؤول عن التحكم في التعبير الجيني المرتبط بالجنس. بعد ذلك، من خلال عملية الرحلان الكهربائي، يتم عرض النتائج: إذا كانت الفرقة موجودة، فإن النبات ذكر، وإن لم تكن موجودة فهو أنثى. هذه التقنية المطورة حديثاً لم تُستخدم على نطاق واسع بعد وما زالت تحت البحث لجعلها ملموسة ومتاحة.

الكلمات المفتاحية: نخيل التمر الجزائر مؤشرات جزيئية، تحديد الجنس، الهندسة الوراثية.

Table of contents

INTRODUCTION

Phoenix dactylifera, commonly referred to as the date palm, belongs to the flowering plant species within the palm family, Arecaceae. It is primarily cultivated for its delectable, sweet fruit known as dates. This species finds extensive cultivation across northern Africa, the Middle East, and South Asia, and has naturalized in numerous tropical and subtropical regions globally. Notably, P. dactylifera serves as the type species of the genus Phoenix, which encompasses a range of 12 to 19 species of wild date palms.

In the first chapter of this work we will go over the date palm general information, an introduction to the date palm world from the botanical description to the general methods used to determine date palm sex, along the way we will go over the karyotype of the date palm tree and the production and cultivation varieties all over the world and delve into the Algerian date palm market and cultivars, while also citing the worldwide market and cultivars from the middle east all the way to California.

The second chapter is focused on general information about the genetic techniques and markers interpreted in date palm sex determination, from Polymerase chain reaction to genetic mapping and sequencing, involving different markers such as Sequence-characterized amplified region (SCAR), and Simple Sequence Repeats (SSRs) or Microsatellites.

Later, we will essentially mix up both chapters as we will talk about the use of the genetic techniques and markers mentioned in the second chapter in the determination of sex in the date palm through some recently tuned markers and techniques developed by researchers.

CHAPTER I: THE DATE PALM

Date palms (Phoenix dactylifera L) hold immense cultural, historical, and economic significance, particularly in arid and semi-arid regions (Zaid, de Wet, 2002). Their importance spans millennia, with references found in ancient texts and archaeological evidence indicating their cultivation dating back to 4000 BCE (Al-Shahib, Marshall, 2003). Today, date palms are cultivated worldwide, contributing significantly to the livelihoods of millions of people and serving as a vital food and income source in many countries (FAO, 2021).

Figure 1: Picture of a date palm tree (https://starnursery.com)

II.1. Taxonomy and botanical description

Belonging to the Angiosperms-Monocotyledones, Palmaceae is a family of about 200 genera and 1, 500 species (Dowson, 1982). Phoenix (Coryphoideae Phoeniceae) is one of the genera which contains a dozen species, all native to the tropical or subtropical regions of Africa or Southern Asia, including Phoenix dactylifera L. (Munier, 1973). According to Dransfield and Uhl, (1986) date palm is classified as follows

- Group: Spadiciflora
- Order: Palmea
- Family: Palmaceae
- Sub-family: Coryphyoideae
- Tribe: Phoeniceae
- Genus: Phoenix
- Species: Dactylifera L

The date palm is an evergreen palm tree that can reach 15-40 m in height. Its fasciculated root system can grow to a depth of 6 m (Zaid et al., 2002). The stem, or stipe, is cylindrical, straight, up to 1-1.1 m in diameter. The date palm bears 100-120 large fronds, 4-7 m long. Phoenix dactylifera is a dioecious species with male and female plants. A female tree bears about 12 inflorescences per year. These spikelike clusters of up to 10,000 flowers have a central rachis and 50-100 spikelets (Zaid et al., 2002; Ecocrop, 2011). Date palm trees begin fruiting within 2 to 4 years and reach full production at 5-8 years (Ecocrop, 2011).

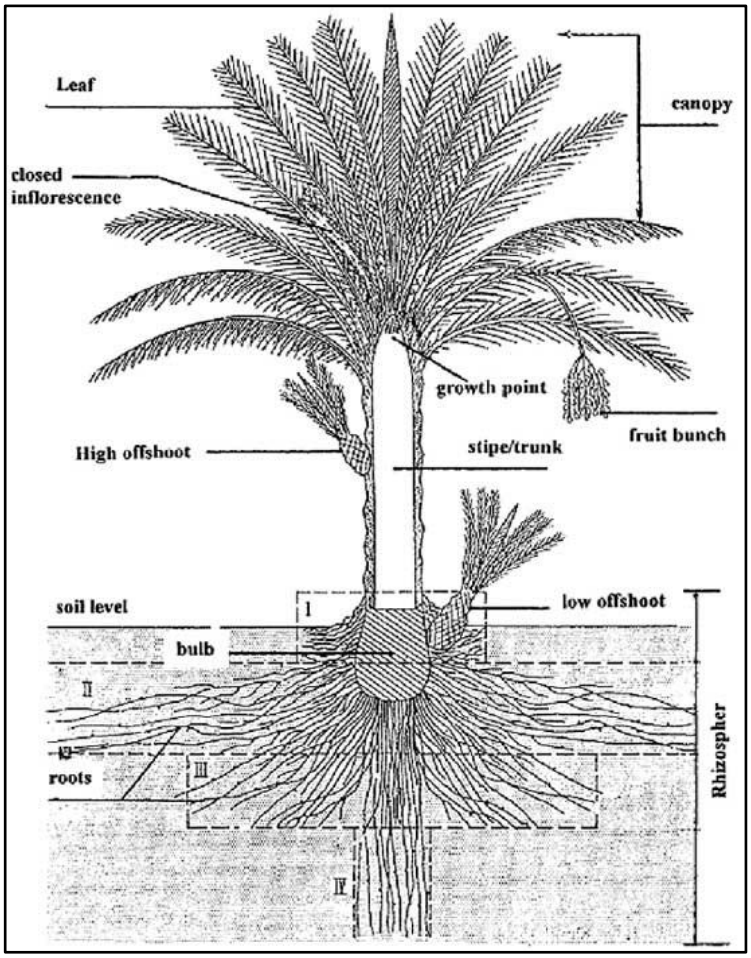

Figure 2: Diagrammic construction of a date palm with it's root system (Zaid, de Wet, 2002).

II.2. Date palm karyotype

The date palm (Phoenix dactylifera) has a diploid karyotype consisting of 36 chromosomes (2n = 36). These chromosomes include a pair of sex chromosomes that determine the sexual characteristics of the plant. In date palms, males typically exhibit an XY chromosomal arrangement, whereas females display an XX configuration. The chromosomes are relatively small and similar in appearance (homomorphic), making it challenging to distinguish between them through traditional cytogenetic techniques (Mathew et al., 2015; Hazzouri et al., 2019). Studies on the date palm karyotype have focused on understanding the structure, function, and evolutionary dynamics of these

chromosomes, particularly in relation to sex determination and plant breeding efforts (Mohamoud et al., 2019).

The sex determination system in date palms involves specific genes located on the Y chromosome, such as CYP703 and GPAT3, which are linked to male sterility, and the LOG gene, which suppresses female development to promote male characteristics (Hazzouri et al., 2019). The evolution of the sex chromosomes has involved structural changes like inversions and translocations, leading to recombination suppression in the sex-determining region and ensuring the stability of sex-specific genes (Westergaard, 1958; Charlesworth, Charlesworth, 1978). High-throughput genomic studies have utilized kmer analysis to differentiate male-specific regions, offering insights into the genetic basis of sex determination (Torres et al., 2018).

Understanding the karyotype and genetic mechanisms of sex determination in date palms is crucial for breeding programs, as early and accurate sex identification can enhance the selection of desirable traits, thereby optimizing orchard productivity and resource management (Mathew et al., 2015).

II.3. Life cycle and growth patterns

The life cycle of a date palm begins with the germination of a seed, which can take several months to complete (Barreveld, 1993). Once germinated, the palm progresses through various growth stages, including the juvenile phase, vegetative growth, and reproductive maturity (Zaid, de Wet, 2002). Germination typically occurs within 4 to 8 weeks under optimal conditions, with subsequent growth stages varying in duration depending on environmental factors such as temperature and water availability (Hijmans et al., 2003).

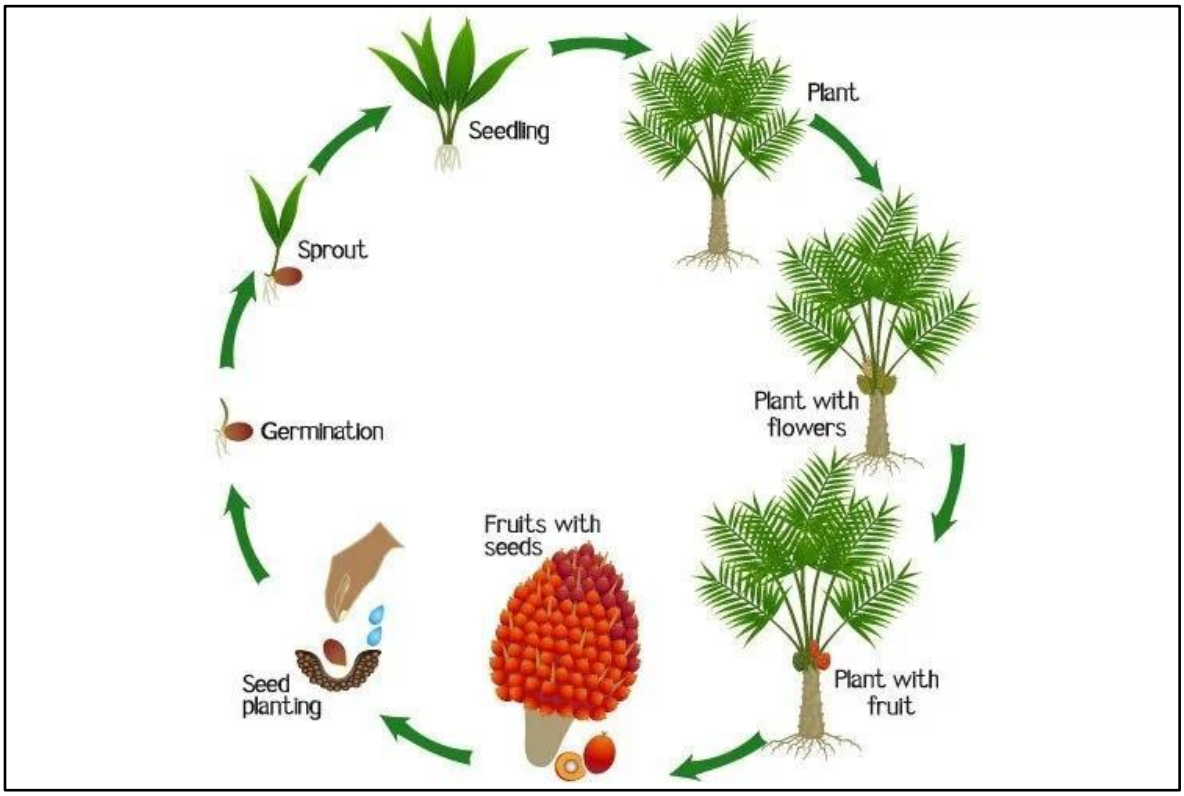

Figure 3: Date palm growth cycle (https://Designbundles.com)

II.4. Distribution by chevalier, 1952

Table 1: Distribution of date palm species (Chevalier, 1952)

Species	Common name	Distribution
Phoenix dactylifera L	Date palm	Mediterranean countries, Africa and part of Asia; introduced in North America and Australia
P. atlantica A. Chev.		Occidental Africa and Canary Islands
P. canariensis chabeaud	Canary palm	Canary Islands and Cape Verde
P. *reclinata* Jacq.	Dwarf palm	Tropical Africa (Senegal and Uganda) and Yemen (Asia)
P. sylvestris Roxb.	Wild Date Palm or Sugar Palm	India and Pakistan
P. humilis Royle.		India, Burma, and China
P. hanceana Naudin.		Meridional China and Thailand
P. robelinic O'Brein.		Sri Lanka, Toukin, Annam, Laos and Thailand
P. farinifera Roxb.	Pigmy palm	India, Ceylon and Annam
P. rupicola T. Anders	Rocky date palm	India
P. acaulis Roxb.	Dwarf palm	Bengaladesh and India
P. paludosa Roxb.	Hental or juliana palm	Bengaladesh, Tenasherim, Andaman, Nikobaren and Thailand

Besides date palm, five of the above species bear edible fruit (P. Atlantica chev., P. Reclinata Jacq., P. Farinifera Roxb., P. Humilis Royle., and P. Acaulis Roxb.).

Most of the 12 Phoenix species are well known as ornamentals, the most highly valued is P. Canariensis Chabeaud, commonly called the Canary Island Palm. P. Sylvestris Roxb. Is widely used in India as a source of sugar. P. Dactylifera L. Is distinguished from the above two species by several characteristics which could be summarised as follows:

- Production of offshoots;
- Tall, columnar and relatively thick trunk. If the crown of fronds is included, the date palm could reach a height of over 20 m (Blatter, 1926); and
- Dark green leaves, (instead of the shiny green colour of the two other species).

II.4.1. In Algeria

Date palm is grown in numerous oases spread over the southern part of the country, where the climate is hot and dry. The oases are living spaces which have been artificially established iduringa large arid area where water is present. In these locations, a ksar (a village made of clay) was built and date palms were planted around it. These oases systems of complex intensive production are maintained with a very fragile balance. Given the geography of Algeria, it is possible to describe several regions of date palm cultivation (Figure 4)

- In the Atlas Mountains foothills (Ksour Ouled Naïl, Zibans, and Aures), there is an oasis chain that marks the gateway of the Sahara.
- In the east, Zibans (Biskra), Oued Ghir, Oued Souf (El Oued), and the basin of Ouargla especially with the Deglet Noor cultivar of high commercial value.

- In the west, Saoura (Beni Abbes), the Touat (Adrar), the Gourara (Timimoun), and the Tidikelt (Reggane) where palm groves include cultivars of relatively low commercial quality.
- At the center. El Golea, the M'zab (Ghardaïa), and Laghouat.

There are different types of oases depending on the nature and operation of water resources, the type of the soil, and topography. Four types have been distinguished (Zella, Smadhi 2006):

- Oases in erg (dune fi eld) depressions, where irrigation water is sourced from groundwater by wells and drilling (Ouargla oasis)
- Oases in Ghouts where irrigation water is drawn up by capillary action (Souf oasis).
- River oases, supplied with water from rivers (Oued of Ghoufi , Oued M'zab, Oued Saoura).
- Oases of depressions, supplied with water by foggaras (Touat, Gourara, and Tidikelt).

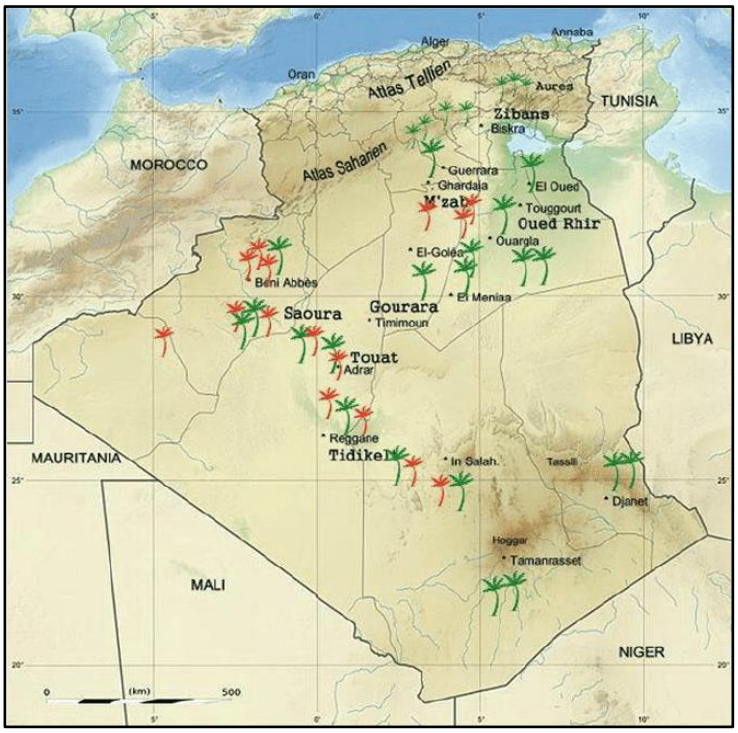

Figure 4: Map of Algeria indicating the different areas with date palms (Bouguedoura et al., 2015)

In Algeria, nearly 1,000 cultivars have been inventoried, and their distribution shows a very marked breakdown into eastern, center, and western portions of the country. Fifty cultivars are found in two or three regions, but most cultivars are endemic to the region and their area of origin. Brac de la Perriere and Benkhalifa (1989) found a very high rate of endemism of 70 % for the dates of the southwestern and more than 60 % on average in those of the southeastern parts of Algeria (Bouguedoura et al., 2015).

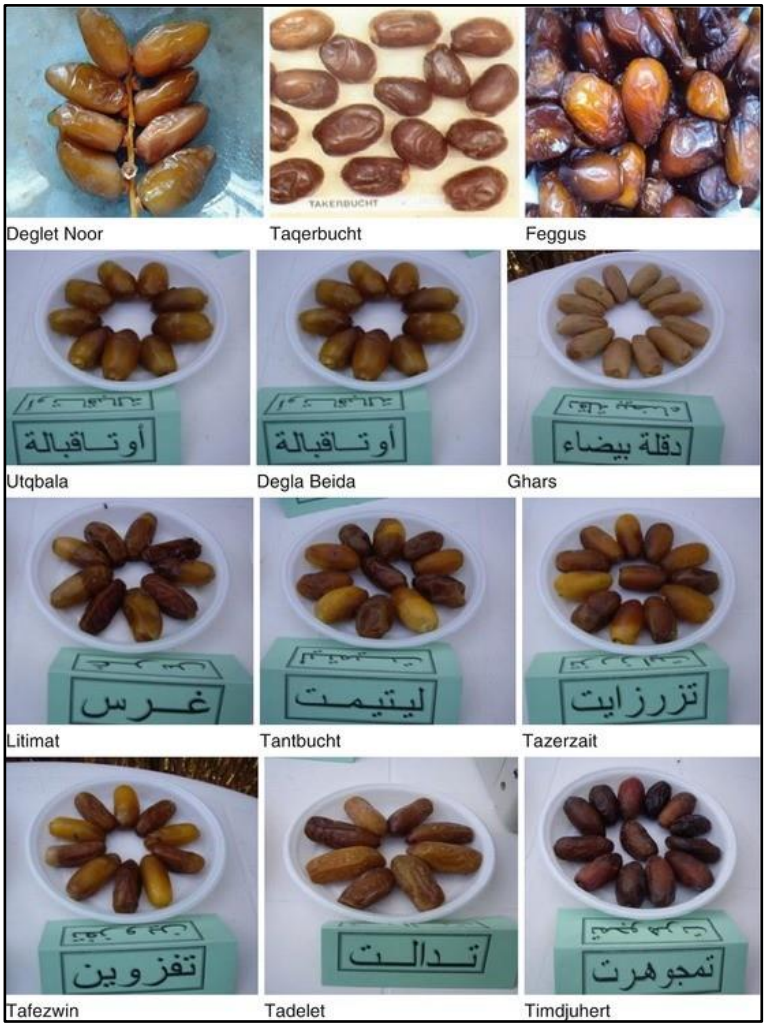

Figure 5: Some date palm cultivars in algeria (Bouguedoura et al., 2015)

II.4.1.1. Date palm production in algeria

Date production in Algeria varies annually which correlated with the alternate bearing of the date palm, cultural practices, climatic hazards, and region of cultivation.

This production has surged from 205,907 mt in 1990 to 755,000 mt in 2011, an increase of 266 %. The average production during the period of analysis is estimated at 420,290 mt (Figure 6).

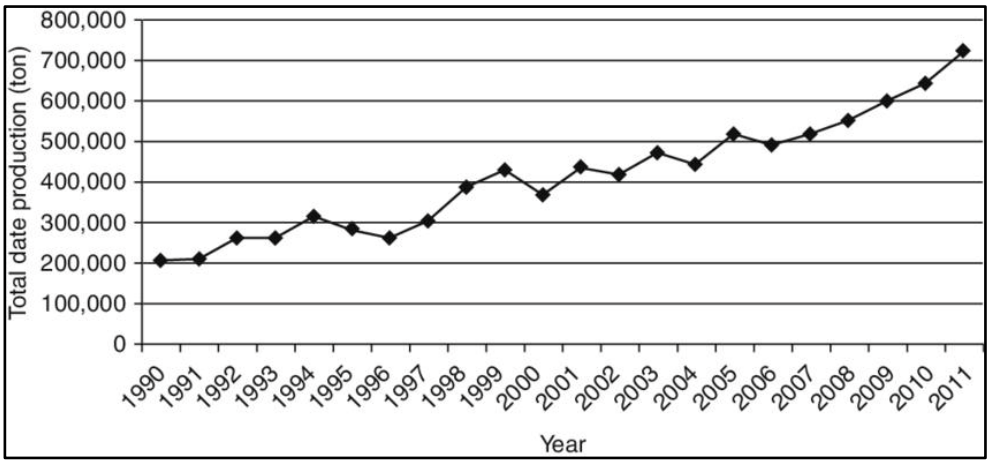

Figure 6: date palm production in Algeria from 1990 to 2011 (benziouche, 2012)

Over 92 % of the increase in date production is due to new plantations established within the framework of the Accession to the Agricultural Land Ownership (APFA) and the National Program of Agriculture Development (PNDA) (Benziouche, 2010), as well as to the strong recent interest given to this crop.

Statistics show that date production in Algeria is mainly concentrated in the southeastern part of the country, which is responsible of 76 % of national production. The province of Biskra ranks fi rst with nearly 31 % followed by El Oued (27 %) and Ouargla with 18 %. The edaphic and pedoclimatic specifi cities, as well as the crop management and the market value of cultivars, justify the importance of the production in these regions. In date groves elsewhere, production is less important, contributing 24 % of total national date production allocated as follows: southwest (15 %) and south center (9 %).

II.5. Research in Genetics, Breeding, and Conservation in algeria

Because date palm is heterozygous and dioecious, it is very diffi cult to study, especially from the genetic and improvement perspective. Therefore, these domains have resulted in few scientifi c works. Initially, there was the need to understand the biology of the development of new combinations, which was very poorly known. Studies by Bouguedoura (1979 , 1980) helped to understand the structure and evolution of different axillary productions of date palm and undertook multiplication and improvement using biotechnology, namely, tissue culture and haplomethods (Bouguedoura 1989).

In Algeria, this is the issue of bayoud that has dominated the orientation of research. It also is why research into the recognition of Fusarium , its culture, and its genetics has developed along with investigations of soil resistance to this fungus (Amir et al. 1996).

Recently, signifi cant results have been achieved in the production of date palm protoplasts; however, plant regeneration from protoplast-induced callus remains limited and requires further research (Assani et al. 2011 ; Chabane et al. 2007 , 2010).

Protoplast fusion made between the sensitive variety Deglet Noor and resistant variety Taqerbucht has not yet resulted in mass production of somatic hybrids. Some plantlets obtained shown 4 n level of ploidy (Chabane 2007). Moreover, induced mutagenesis is another potentially powerful biotechnological tool for date palm improvement (Jain 2012). Currently, selected mutants induced from cvs. Deglet Noor and Teggaza are being evaluated for disease resistance in bayoud-infested fields as a part of a joint project (No. AlG/5/023) realized by INRAA and sponsored by the International Atomic Energy Agency (IAEA) (unpublished results).

II.5.1. Current Status and Prospect of Genetic Resources in algeria

A census by researchers from Research Laboratory of Arid Lands (LRZA), INRAA, and the Commission for the Agricultural Development of Saharan Regions (CDARS) shows the rich genetic heritage of Algerian date palms (Acourene et al.2007 ; Belguedj 2002 ; Belguedj and Tirichine 2011 ; Brac de la Perrière and Benkhalifa 1989 ; Brac de la Perrière and Bounaga 1990 ; Hannachi et al. 1998).

However, Algeria's date palm heritage is yet to be fully recorded.

II.6. Cultural and traditional uses

Date palms have deep-rooted cultural and traditional significance, particularly in regions where they are endemic (Barreveld, 1993). Throughout history, date palms have played multifaceted roles in the daily lives and ceremonies of various cultures.

In the Middle East, dates hold symbolic importance in religious ceremonies and rituals. For example, during Ramadan, the Islamic holy month of fasting, dates are traditionally consumed to break the fast at sunset, followed by a communal meal (Al-Shahib, Marshall, 2003). This practice dates back centuries and is believed to have originated from the traditions of Prophet Muhammad.

In addition to religious significance, date palms are integral to cultural celebrations and social gatherings. In many Middle Eastern and North African cultures, dates are served as a gesture of hospitality to guests, symbolizing generosity and abundance (Zohary, Hopf, 2000). Furthermore, date palms feature prominently in traditional folklore, poetry, and art, serving as a source of inspiration and cultural identity for communities across the region (Barreveld, 1993).

Beyond their cultural significance, date palm products have been used in traditional medicine for centuries due to their purported health benefits (Al-Shahib, Marshall, 2003). Date fruits are rich in vitamins, minerals, and antioxidants, making them valuable in promoting overall health and well-

being. In traditional medicine, date palm products are used to treat various ailments, including digestive disorders, respiratory ailments, and fatigue (Al-Shahib, Marshall, 2003). Date syrup, for example, is believed to possess therapeutic properties and is used as a natural remedy for coughs and sore throats in some cultures

II.7. Cultivation

Date palm grows well in areas with long and hot summers, no or low rainfall, and very low relative humidity levels during the ripening period (Zaid et al., 2002). Date palm can be grown from sea level up to an altitude of 1500 m (Ecocrop, 2011). The optimal temperature for growth is about 32°C, but the date palm can withstand up to 56°C under irrigation (Zaid et al., 2002). A date palm tree requires 0.21-0.56 m3 (0.72 m3; Rolland, 1894 cited by Zaid et al., 2002) of water per day. If ground water is inadequate, irrigation is necessary (Ecocrop, 2011; Peyron, 2000). Date palm is tolerant of windy conditions and can bear strong, hot, dusty and dry winds. It is thus used as wind breaks for more sensitive plantations. Date palm has also some tolerance to soil salinity but cannot be considered a true halophyte since it grows better on sweet soils than on salty soils (Zaid et al., 2002).

II.8. Cultivars and varieties

Date palms exhibit considerable diversity in cultivars and varieties, reflecting centuries of selection and breeding efforts by growers (Loutfi, Chakir, 2013). Table 2 provides an overview of selected date palm cultivars, their origins, characteristics, and growing regions.

Table 2: Date palm fruit varieties, growth regions and characteristics(Loutfi, Chakir, 2013)

Cultivar	Origin	Characteristics	Growing region
Medjool	Morocco	Large, soft, and sweet fruit	Middle East, California
Deglet Noor	Algeria	Semi-dry with a caramel-like flavor	North Africa, California
Barhi	Iraq	Small, round, and soft with a honey-like taste	Middle East, California
Zahidi	Iraq	Medium-sized, semi-dry with a nutty flavor	Middle East, California
Khadrawy	Saudi Arabia	Small, soft, and extremely sweet	Middle East
Halawi	iraq	Medium-sized, soft with a caramel flavor	Middle East, California
Dayri	Algeria	Large, elongated, and soft with a rich flavor	North Africa, California

II.9. Economic importance

The economic importance of date palms extends beyond their nutritional value to encompass various industries, including agriculture, food processing, and tourism (Al-Khayri et al., 2015). Date production contributes significantly to the economies of date-growing countries, generating income and employment opportunities for millions of people (FAO, 2021). Furthermore, date palms play a crucial role in sustainable development, providing ecosystem services such as soil stabilization and biodiversity conservation (Hijmans et al., 2003).

The data of date production is testimonial to it's cultivation requirements, in the table presented under is date palm prodcution in the year 2021 according to FAO

Table 3: Date production (FAO, 2021)

Country	Date production (tonnes)
Egypt	1,747,715
Saudi arabia	1,565,830
Iran	1,303,717
Algeria	1,188,803
Iraq	750,225
Pakistan	532,880
Sudan	460,097
Oman	374,200
United arab emirates	351,077
Tunisia	345,000

II.10. Importance of Early Sex Determination

Early sex determination in date palms is crucial for several reasons. Firstly, it allows growers to identify and eliminate male trees, which do not produce fruit and thus do not contribute to commercial date production (Al-Khayri et al., 2015). This selective removal of male trees optimizes orchard space and resources, leading to increased yields and profitability. Secondly, early sex determination enables the selection of superior female cultivars based on desirable traits such as fruit size, flavor, and yield potential (Al-Khayri et al., 2015). By identifying and propagating high-quality female trees at an early stage, growers can establish orchards with consistent and high-value fruit production. Moreover, early sex determination aids in the development of new cultivars through controlled breeding programs, facilitating the creation of improved varieties with enhanced characteristics (Al-Khayri et al., 2015).

Additionally, early sex determination in date palms facilitates efficient orchard management practices, such as irrigation scheduling, fertilization regimes, and pest control strategies (Al-Khayri et al., 2015). By knowing the sex of individual trees early on, growers can tailor management practices to optimize resource allocation and minimize inputs, resulting in improved overall orchard health and productivity. Furthermore, early identification of male and female trees allows for the establishment of balanced pollination ratios within orchards, essential for maximizing fruit set and quality (Al-Khayri et al., 2015). This ensures consistent and reliable yields year after year, essential for commercial date production.

II.11. Methods of sex determination

II.11.1. Morphological basis

Plant identification through morphological features is comparatively the simplest method of selection. In the past, genetic variation in date palm was characterized through morphological descriptions such as plant form, shape, and structure, but results provided by these markers are ambiguous due to environmental and development stage effects (Haider et al. 2015; Naqvi et al. 2015; Raza et al. 2020).

Traditional date farmers in southeastern Niger customarily identify the date palm sex at two stages: seed and seedling. Seed having curved pointed tips and a smooth appearance can germinate a female seedling; straight with a smooth appearance would be male seedling (Figure 7a). Secondly, for sex determination at the seedling stage, the seed is sown under the straw mat, and if the seedling pushes the straw mat and emerges out straight, it would be male (Figure 7b). If a seedling bends under the straw mat, it would be female (Zango et al. 2016). No scientific studies support this means of gender identification.

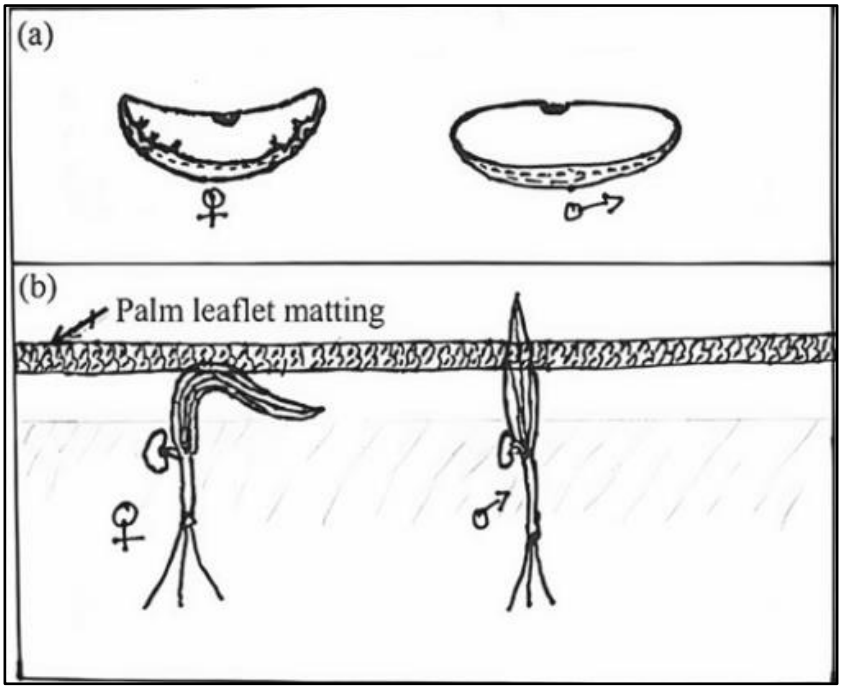

Figure 7: Discrimination of date palm seedling sex. a: Seed morphology, b: Seedling emergence pattern (Zango et al. 2016)

II.11.2. Biochemical basis

Biochemical markers have been used by Bekheet et al. (2008) to differentiate the sex in date palms and reported that female adult plants and female offshoots showed an elevated level of peroxidase activities. The sex estimation of in vitro developed lines was identified by the activity of enzymes (glutamate oxaloacetate and acid phosphatase) that provide the strong differentiation in male and female plants.

II.11.2.1. Proteomics

The biomarkers are also used for gender discrimination in date palm by analyzing leaf proteomics through mass spectrometry and twodimensional polyacrylamide gel electrophoresis (PAGE) (Sonia et al. 2013). Male and female comparison of the proteomic map identified one clear protein spot which is linked to gender in date palm and corresponds to the ABC superfamily ATP-binding cassette transporter. ABC protein is associated with pollen development and male fertility.

II.11.2.2. Sugar content

Sugar is an essential element in the sap of date palms irrespective of sex (Rao et al. 2009). Plant sex may affect the sap quality in Phoenix and male date palms reveal higher levels of sugar and dry matter contents in comparison to female palms. Similarly, higher total phenolic content (TPC) is recorded in male date palm sap compared to female (2.04 vs.1.648 lg gallic acid equivalent mL−1 , respectively) (Makhlouf-Gafsi et al. 2016).

II.11.3. Cytological studies

Techniques like in situ hybridization (ISH), fluorescent in situ hybridization (FISH), concomitant oncoprotein detection (CODFISH), and spectral karyotyping (SKY) are applied to identify chromosomes and chromosomal aberrations; hence identification of sex in seedlings may be worked out using these techniques (Al-Ani et al. 2010). Sex chromosomes were unidentified in date palm until Siljak-Yakovlev et al. (1996) stained root chromosomes with chromomycin A3 and identified clear variations in isolated male and female chromosome heterochromatin (Juarez and Banks 1998).

Sexual discrimination of palm trees based on fluorescent in situ hybridization (FISH) was tested by Atia et al. (2017a), who hybridized complementary probe sequences to visualize the identified DNA sequences of cell preparations. Since then, in situ fluorescence hybridization (FISH) has been established as an effective and important method for specific genome DNA fragment detection.

II.11.4. Molecular markers

This technique starts with the DNA extraction that further divides into four different processes: polymerase chain reaction (PCR)-based, non-PCR-based, sequence analysis, and hybridization. Several DNA-based markers have been tested for genetic variation analysis, cultivar identification, gene mapping, and phylogenetic analysis (Zango et al. 2017; Zehdi-Azouzi et al. 2015).

Gender-specific PCR-based molecular markers have been used effectively for gender discrimination in date palm. Al-Mahmoud et al. (2012) used the polymorphic region in male and female date palms to design the DNA-based assay that differentiates the male and female seedlings at an early growth stages.

CHAPTER II: GENETIC TECHNIQUES AND MARKERS IN DATE PALM SEX DETERMINATION

II.1. Introduction to Genetic Sex Determination

Sex determination in plants is a fundamental biological process that regulates the development of male and female reproductive structures (Diggle et al., 2011). In dioecious plant species like the date palm (Phoenix dactylifera L.), sex determination mechanisms vary widely and can be influenced by genetic, hormonal, and environmental factors (Diggle et al., 2011). Understanding the genetic basis of sex determination is crucial for plant breeding, crop improvement, and conservation efforts (Diggle et al., 2011).

In date palms, sex determination is of particular interest due to their economic importance as a major fruit crop in arid regions worldwide. Date palms exhibit a clear separation of male and female individuals, with female plants bearing the economically valuable fruit. However, the molecular mechanisms underlying sex determination in date palms remain largely unexplored (Gros-Balthazard et al., 2021).

Genetic sex determination in date palms involves the identification of specific genetic markers or genomic regions associated with sex expression. Molecular techniques, such as PCR and DNA sequencing, along with various genetic markers, have been employed to elucidate the genetic basis of sex determination and facilitate the selection of elite breeding lines with desirable sex traits (Gros-Balthazard et al., 2021).

By unraveling the genetic pathways governing sex determination in date palms, researchers aim to enhance our understanding of plant reproductive biology and develop strategies for sustainable date palm cultivation in diverse agro-climatic conditions (Diggle et al., 2011).

II.2. Genetic Techniques

II.2.1. Polymerase Chain Reaction (PCR)

PCR is a cornerstone technique in molecular biology for amplifying specific DNA sequences. It has been extensively utilized in genetic sex determination studies of date palms due to its sensitivity, specificity, and versatility (Aradhya et al., 2002). PCR allows researchers to selectively amplify target DNA regions, such as sex-linked markers, from genomic DNA samples obtained from date palm tissues (Salem et al., 2005).

II.2.1.1. The process of PCR

II.2.1.1.1. Denaturation

Polymerase Chain Reaction (PCR) is a molecular biology technique that enables the amplification of specific DNA sequences (Saiki et al., 1985). The process begins with denaturation, where the double-stranded DNA template is heated to around 95°C to separate the strands, allowing access to the target sequence (Saiki et al., 1985).

II.2.1.1.2. Annealing

Following denaturation, annealing occurs, where two oligonucleotide primers anneal to complementary sequences on each strand of the template DNA. This step typically occurs at a lower temperature (typically 50-65°C) to allow for primer binding (Saiki et al., 1985).

II.2.1.1.3. Extension

Once annealed, the primers serve as starting points for DNA synthesis. The extension step, carried out by a heat-stable DNA polymerase such as Taq polymerase, involves the synthesis of new DNA strands complementary to the template strands, utilizing nucleotide triphosphates (dNTPs) as building blocks (Saiki et al., 1985).

II.2.1.1.4. Amplification

This process is repeated for multiple cycles, with each cycle doubling the amount of DNA, resulting in exponential amplification of the target sequence (Saiki et al., 1985)

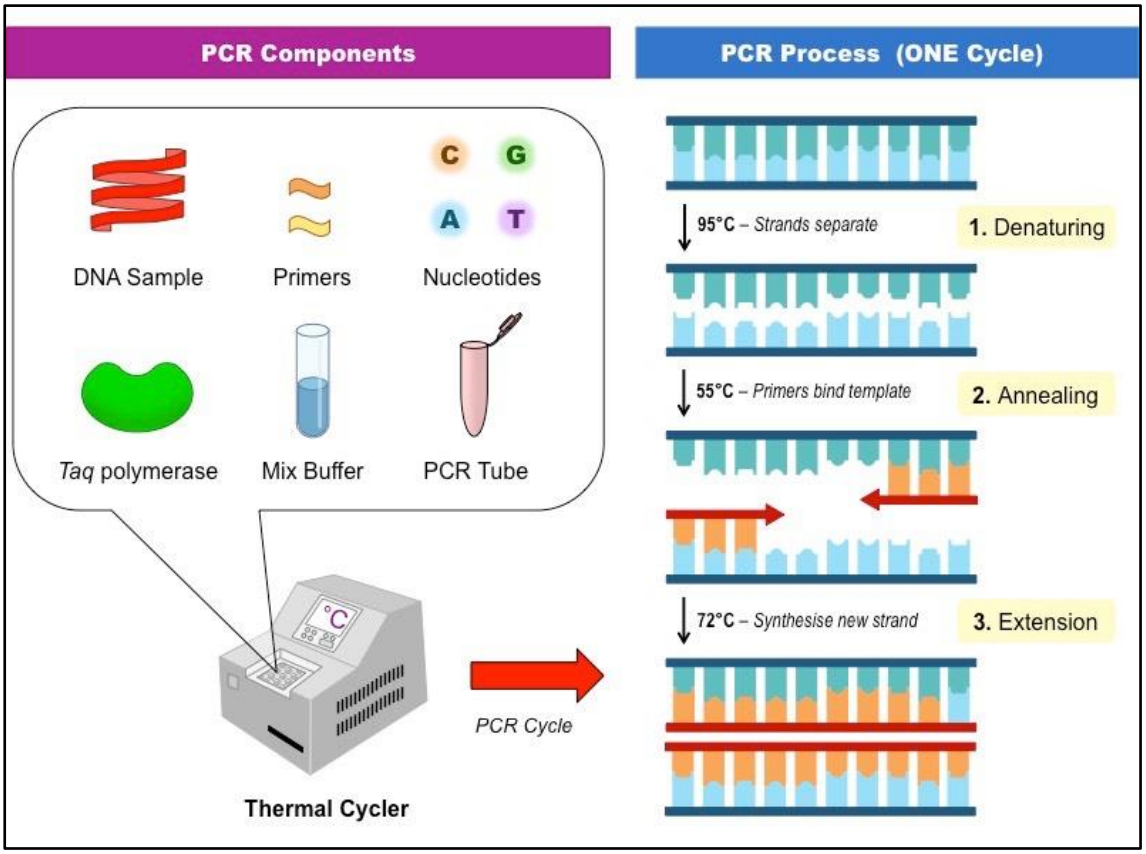

Figure 8: PCR Process (https://Facellitate.com)

II.2.2. DNA Sequencing

DNA sequencing technologies have advanced significantly over the years, enabling comprehensive analysis of genomic DNA sequences. Next-generation sequencing (NGS) platforms, including Illumina and Pacific Biosciences, have been instrumental in deciphering the genetic basis of sex determination in date palms (Al-Mssallem et al., 2013). Whole-genome sequencing approaches have facilitated the identification of sex-linked loci and candidate genes associated with sex determination in date palms (Gros-Balthazard et al., 2021).

II.2.2.1. The process of DNA Sequencing

II.2.2.1.1. Initiation

The process commences with the extraction and purification of DNA from various sources, including cells, tissues, or environmental samples (Sanger et al., 1977).

II.2.2.1.2. Fragmentation

Subsequently, the isolated DNA is fragmented into smaller pieces, either enzymatically using restriction enzymes or physically through sonication or nebulization (Smith et al., 1986).

II.2.2.1.3. Sequencing Reaction

Each fragmented DNA segment undergoes a sequencing reaction, wherein DNA polymerase catalyzes the incorporation of fluorescently labeled nucleotides (dideoxynucleotides) into newly synthesized DNA strands (Sanger et al., 1977).

II.2.2.1.4. Separation

The resulting fragments are then separated based on size using gel electrophoresis or capillary electrophoresis, allowing for the determination of the sequence of nucleotides within each fragment (Maxam, Gilbert, 1977).

II.2.2.1.5. Detection

Finally, the fluorescently labeled fragments are detected using specialized instruments, such as automated DNA sequencers, which detect the emitted fluorescence and convert it into nucleotide sequences (Sanger et al., 1977).

II.2.2.1.6. Assembly

The sequence data obtained from multiple fragments are assembled using bioinformatics software, facilitating the reconstruction of the original DNA sequence (Maxam, Gilbert, 1977).

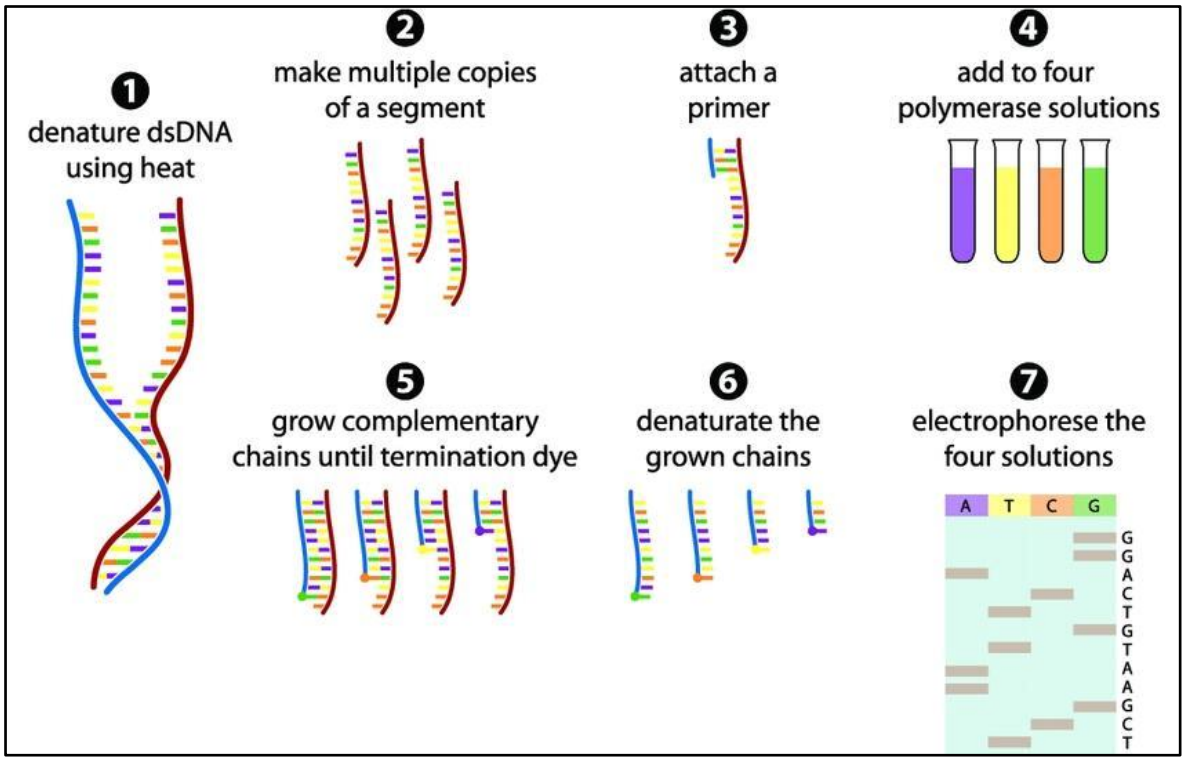

Figure 9: Sagner method of DNA Sequencing (Gauthier, Michel. 2007)

II.2.3. Genetic Mapping

Genetic mapping involves the construction of genetic linkage maps to identify the relative positions of genes or genetic markers on chromosomes. The concept of genetic mapping originated from genetic linkage studies conducted by Thomas Hunt Morgan in the early 20th century (Morgan et al., 1915). Mapping is typically performed using molecular markers, such as restriction fragment length polymorphisms (RFLPs), simple sequence repeats (SSRs), or single nucleotide polymorphisms (SNPs), which can be genotyped across populations (Morgan et al., 1915).

II.2.4. Transcriptomics

Transcriptomics involves the study of the complete set of RNA transcripts (the transcriptome) produced by a cell, tissue, or organism under specific conditions. The field of transcriptomics has its origins in early gene expression studies using techniques such as northern blotting and reverse transcription-polymerase chain reaction (RT-PCR) (Alwine et al., 1977; Saiki et al., 1985). Transcriptomic analyses, including RNA sequencing (RNA-seq), allow for the quantification and characterization of gene expression patterns, providing insights into the regulation of biological processes, including sex determination.

II.2.5. Functional Genomics

Functional genomics aims to understand the function and interactions of genes and gene products on a genome-wide scale. The field emerged from advances in genomic sequencing and high-throughput functional assays (Collins et al., 2003). Functional genomics approaches, such as CRISPR/Cas9-mediated gene editing, RNA interference (RNAi), and gene expression profiling, are used to study gene function, elucidate molecular pathways, and identify potential therapeutic targets.

II.3. Markers Used in Genetic Sex Determination

Although molecular markers have been introduced into breeding programs for date palms, little research effort has been focused on the early determination of sex in the plant (Bekheet and Hanafy, 2011). The most popular markers for sex determination in plants are RAPD (Random Amplified Polymorphic DNA), SCAR (Sequence-characterized Amplified Region), AFLP (Amplified Fragment Length Polymorphism), and microsatellites or SSRs (Simple Sequence Repeats) (Figure 10).

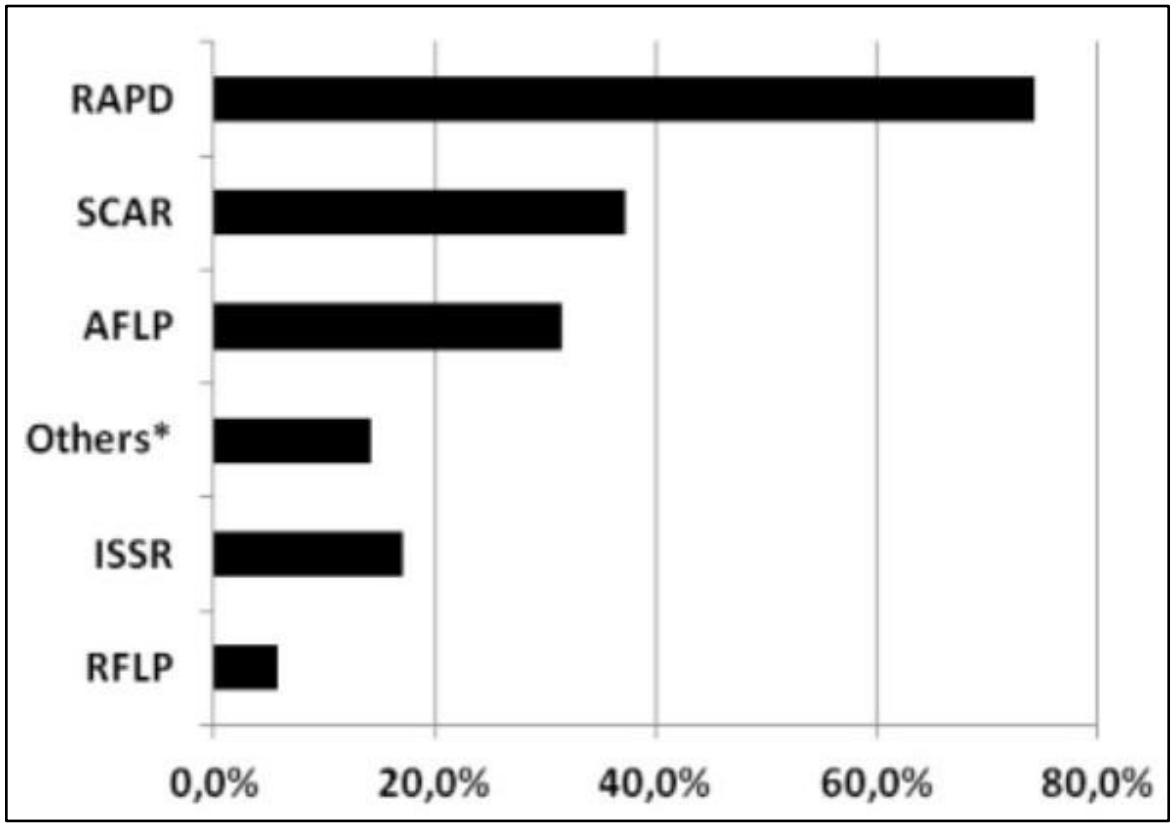

Figure 10: The most popular markers for sex determination in plants, depending on the species studied (frequency of application in percentage) (Milewicz and Sawicki, 2013).

II.3.1. Sequence Characterized Amplified Region (SCAR) Markers

SCAR markers were introduced by Michelmore et al. in 1991 (Michelmore et al., 1991).

SCARs are genomic fragments amplified by PCR with specific primers obtained after sequencing RAPD products. They serve as markers with the advantage of being codominant and providing more robust analysis (Shan et al., 1999), making them more suitable for breeding programs. A potentially interesting band identified in a RAPD gel is excised, then the DNA fragment is cloned into a vector and sequenced. Specific primers (16-24 bp) are designed for this DNA fragment. Re-amplification of the target DNA with these new primers will show a simpler PCR profile (Figure 11).

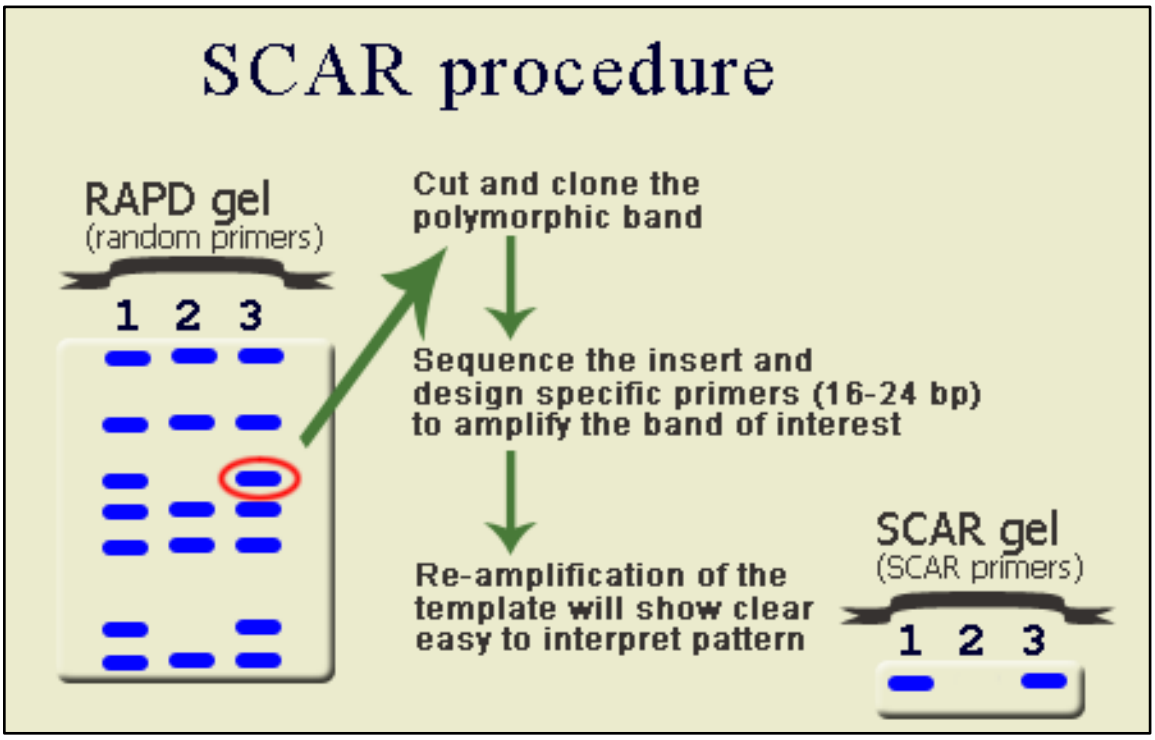

Figure 11: SCAR procedure (https://NCBI.com)

II.3.2. Simple Sequence Repeats (SSRs) or Microsatellites

SSRs were first described by Jeffreys et al. in 1985 (Jeffreys et al., 1985).

SSRs are tandemly repeated DNA sequences consisting of short motifs (1-6 nucleotides) that are highly polymorphic. They are widely used as molecular markers in date palm genetic studies due to their co-dominant inheritance and abundance throughout the genome (Billotte et al., 2004).

II.3.3. Single Nucleotide Polymorphisms (SNPs)

SNPs were first described by Botstein and Risch in 2003 (Botstein, Risch, 2003).

SNPs are single nucleotide variations occurring at specific positions in the genome. High-throughput SNP genotyping assays, such as SNP arrays and sequencing-based methods, have been employed in date palm sex determination studies to identify sex-linked markers and elucidate the genetic basis of sex determination (Khalil et al., 2016).

II.3.4. Amplified Fragment Length Polymorphism (AFLP)

AFLP was developed by Vos et al. in 1995 (Vos et al., 1995).

AFLP is a PCR-based technique that involves the digestion of genomic DNA with restriction enzymes followed by ligation of adaptors and selective amplification of fragments. AFLP markers have been utilized in date palm genetic studies to assess genetic diversity, population structure, and sex determination (Zehdi-Azouzi et al., 2015).

The process

- Genomic DNA is digested with restriction enzymes.
- Adapters are ligated to the ends of the fragments, followed by selective PCR amplification.
- Amplified fragments are separated by size using gel electrophoresis.

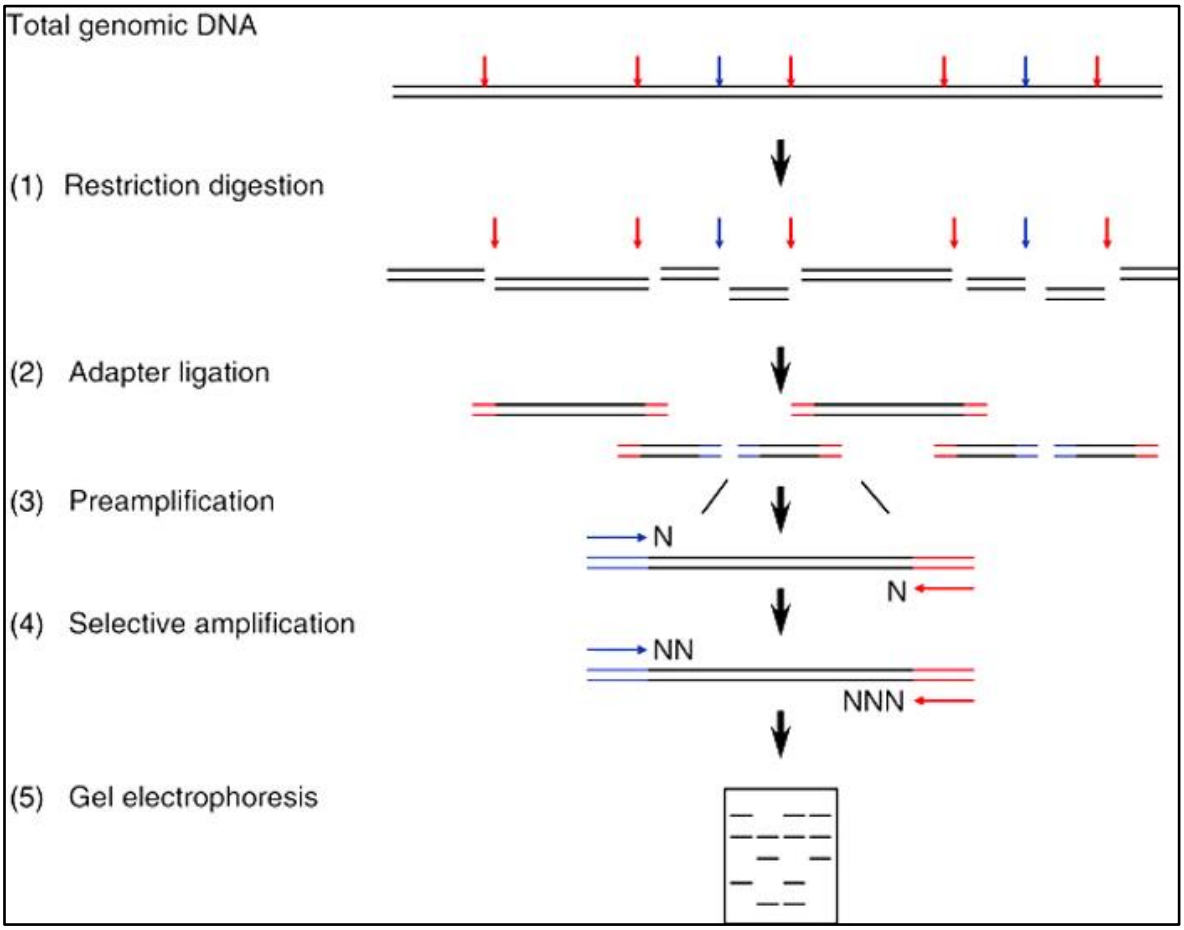

Figure 12: AFLP Procedure outline (https://nature.com)

II.3.5. Restriction Fragment Length Polymorphism (RFLP)

RFLP was pioneered by Botstein et al. in 1980 (Botstein et al., 1980).

RFLP analysis involves the digestion of genomic DNA with restriction enzymes, followed by gel electrophoresis to detect fragment length polymorphisms. Although less commonly used in recent years, RFLP markers have been valuable tools in date palm genetic mapping and linkage analysis (Nadaf et al., 2005).

The process

- DNA is extracted and digested with restriction enzymes.
- The resulting fragments are separated by size using gel electrophoresis.
- Fragments are visualized using molecular probes specific to the target sequence.

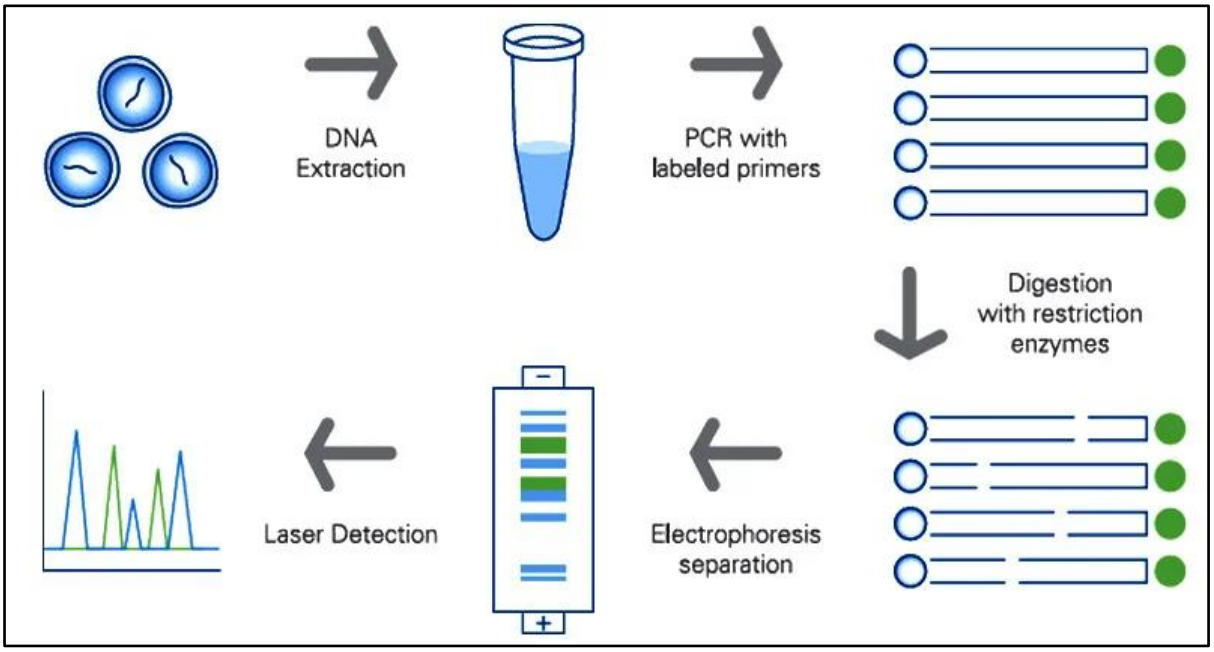

Figure 13: RFLP Procedure (https://Microbenotes.com)

II.3.6. Random Amplified Polymorphic DNA (RAPD)

RAPD was first introduced by Williams et al. in 1990 (Williams et al., 1990).

RAPD (Random Amplified Polymorphic DNA) is a molecular technique based on PCR, which amplifies a set of DNA fragments randomly distributed throughout the genome using short, single oligonucleotide primers (Khosla and Kumari, 2015). Its main variant involves using a single oligonucleotide primer, about ten bases long, to amplify one or more DNA segments from the sample by PCR (Figure 14). This primer randomly hybridizes within the genome. If two hybridization sites are close to each other on opposite DNA strands, amplification will occur. Each band in the amplified product corresponds to the presence of a complementary motif to the primer on each DNA strand. The polymorphism revealed is a polymorphism of primer hybridization sites.

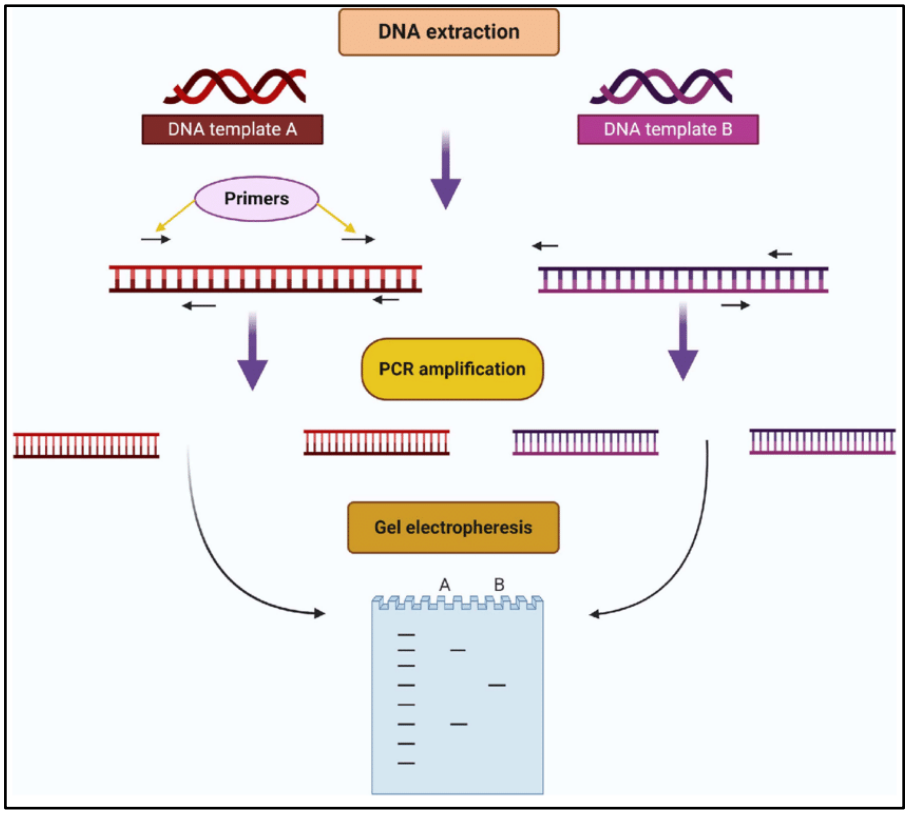

Figure 14: RAPD procedure (https://ResearchGate.com)

II.3.7. Simple Sequence Repeat (SSR)

SSR markers were introduced by Zietkiewicz et al. in 1994 (Zietkiewicz et al., 1994)

Microsatellites, or simple sequence repeats (SSRs), consist of a variable number of tandemly repeated units ranging from 1 to 6 base pairs each and represent a class of repetitive DNA commonly found in eukaryotic genomes (Tautz and Renz, 1984). The number of repeated units varies widely among organisms, ranging up to 50 copies of the repeated unit. They are characterized by their high abundance (Condit and Hubbell, 1991), high variability (Schug et al., 1998), and widespread distribution in different genomes (Liu et al., 1996). Polymorphisms in the repeat region can be detected by performing PCR with primers designed in the flanking region. Variation in the size of PCR products is caused by differences in the number of repeat units of the microsatellite. SSR polymorphisms can be visualized by electrophoresis on agarose or polyacrylamide gel (de Vicente and Fulton, 2003).

II.3.8. Expressed Sequence Tag (EST) Markers

EST markers were first described by Adams et al. in 1991 (Adams et al., 1991).

EST markers are derived from expressed sequence tags, representing transcribed regions of the genome. EST-based markers have been utilized in date palm research to identify candidate genes associated with sex determination and other important traits (Yaish et al., 2018).

II.3.9. Cleaved Amplified Polymorphic Sequence (CAPS) Markers

CAPS markers were introduced by Konieczny and Ausubel in 1993 (Konieczny, Ausubel, 1993).

CAPS markers are PCR-based markers generated by amplification of DNA fragments followed by digestion with restriction enzymes. CAPS analysis

has been used in date palm genetic studies to detect DNA polymorphisms associated with sex-linked loci and genetic diversity (Tranchant et al., 2013).

II.3.10. Diversity Array Technology (DArT)

DArT markers were developed by Jaccoud et al. in 2001 (Jaccoud et al., 2001).

DArT is a high-throughput genotyping platform that detects DNA polymorphisms based on the presence or absence of specific genomic fragments. DArT markers have been applied in date palm genetic studies for genetic mapping, diversity analysis, and marker-assisted breeding (Al-Dous et al., 2011).

II.4. Considerations and Challenges

Genetic sex determination in date palms presents several challenges, including the influence of environmental factors on sex expression, the complexity of the sex determination mechanism, and the limited availability of genomic resources. Overcoming these challenges requires interdisciplinary approaches integrating genetics, genomics, and bioinformatics to unravel the genetic basis of sex determination and develop molecular breeding strategies for date palm improvement (Hazzouri et al., 2020).

CHAPTER III: MOLECULAR SEX DETERMINATION IN THE DATE PALM

For decades of extensive date palm research, there have been numerous attempts to identify the sex of the plant, but most were unsuccessful, resulting to unpredictable number of female plants in the field that leads to uncertainty in the production potential of the crop. The number of female plants must be more than the male plants. Consequently, it is not possible to know the sex of the plant until it flowers and reaches the reproductive age spanning from five to ten years (Bendiab et al., 1993, Juarez and Banks, 1998, El Hadrami and El Hadrami, 2009).

III.1. Sex determining gene

The SRY gene was first identified in 1990 by Peter Goodfellow and colleagues through studies on individuals with disorders of sex development (DSD), such as Swyer syndrome, where individuals with XY chromosomes develop as females due to mutations in SRY or other genes involved in male sex determination (Goodfellow et al., 1990).

III.1.1. In plants

The identification of SRY-like genes in plants began with studies in species such as *Silene latifolia*, *Rumex acetosa*, and *Dioscorea tokoro* (Akagi et al., 2014; Bergero et al., 2019; Harkess et al., 2017). These genes, often termed as "plant SRY" or "plant Y-specific genes," are located on sex chromosomes and govern the development of male reproductive organs.

The SRY gene, also known as the Sex-determining Region Y gene, is located on the Y chromosome. It plays a crucial role in the determination of male sex. Studies on *Silene latifolia* have revealed that the SRY gene acts as a transcription factor, regulating the expression of genes involved in male sex differentiation. This includes genes responsible for the development of male reproductive structures such as stamens and pollen (Bergero et al., 2019).

III.1.2. In the date palm

Due to its dioecious nature, breeding in date palm (*Phoenix dactylifera*) has not been practiced because the sex of the plant cannot be known until it reaches its reproductive stage (Bendiab et al., 1993).

To identify sex-linked markers, Cherifet al. (2013) studied 52 male and 55 female geographically diverged date palm genotypes using three microsatellite (SSR) markers and reported three genetically linked loci that are heterozygous only in males and thereby confirming the existence of an XY chromosomal system with a non-recombining XY-like region in the date palm genome. The existence of an XY chromosomal system in date palm suggests the possibility of discovering Y chromosome-specific DNA marker for identification of male plants.

The SRY gene in the date palm (*Phoenix dactylifera*) was discovered as part of a broader effort in 2011 to sequence and analyze the genome of the date palm (Al-Dous et al., 2011).

III.2. The use of molecular markers in date palm sex determination

DNA or the genetic fingerprinting method to identify individual organisms is based on the organization of an organism's genetic material. The DNA fingerprinting technique has several advantages; the cell's DNA content is not influenced by environmental factors, growth stages, or organ specificity.

In date palm, restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR) markers have been used to genetically differentiate cultivars, analyze genetic diversity and genetic relationships (Khanam et al. 2012; Maryam et al. 2016)

III.2.1. Around the world

III.2.1.1. Amplified fragment length polymorphism (AFLP)

An AFLP analysis conducted by Abdoulkader (2009) on four date palms (two males and two females) revealed the existence of potential male-specific sequences (Figure 15). The result obtained through capillary electrophoresis shows amplified fragments after digestion of the DNA from male and female individuals. Arrows indicate fragments amplified in male individuals and absent in females. These fragments can serve as potential sex markers.

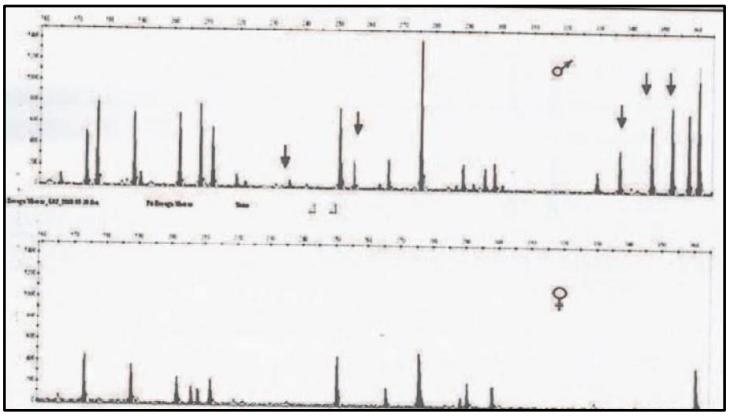

Figure 15: AFLP profile obtained through capillary electrophoresis (Abdoulkader, 2009).

III.2.1.2. PCR based Restriction fragment length polymorphism (PFLP)

Al-Mahmoud et al. (2012) used the polymorphic region in male and female date palms to design the DNA-based assay that differentiates the male and female seedlings at an early growth stage. PCR-RFLP with the enzyme BclI restricts the male allele only and this site is absent in female allele, and HpaII has 3 restriction sites but the site at 180 bp out of 452 bp is only present in female date palm and absent in male date palm. They designed only PCR-based primers for gender-specific polymorphism and up to 90% accuracy these primers differentiated the gender in date palm, where the male samples amplified two bands while the female a single band.

III.2.1.3. Randomly amplified polymorphic DNA (RAPD)

Adawy et al. (2014) tested a set of 122 random primers on ten date palm samples to identify sex-specific markers. Four RAPD primers (OP-A11, OP-M11, OP-O07, and OP-S07) showed differential fragments/bands between male and female date palms (two markers associated with male plants and five markers associated with female plants) (Figure 16). To determine the genetic difference between male and female date palms, genomic DNA was extracted from the leaves of four female cultivars (Deglet Noor, Allig, Kentichi, Menakher), a male pollinator genotype T23, and an F1 hybrid. The RAPD results produced reproducible polymorphic bands with 11 out of 53 primers used (Ben-Abdallah et al., 2000).

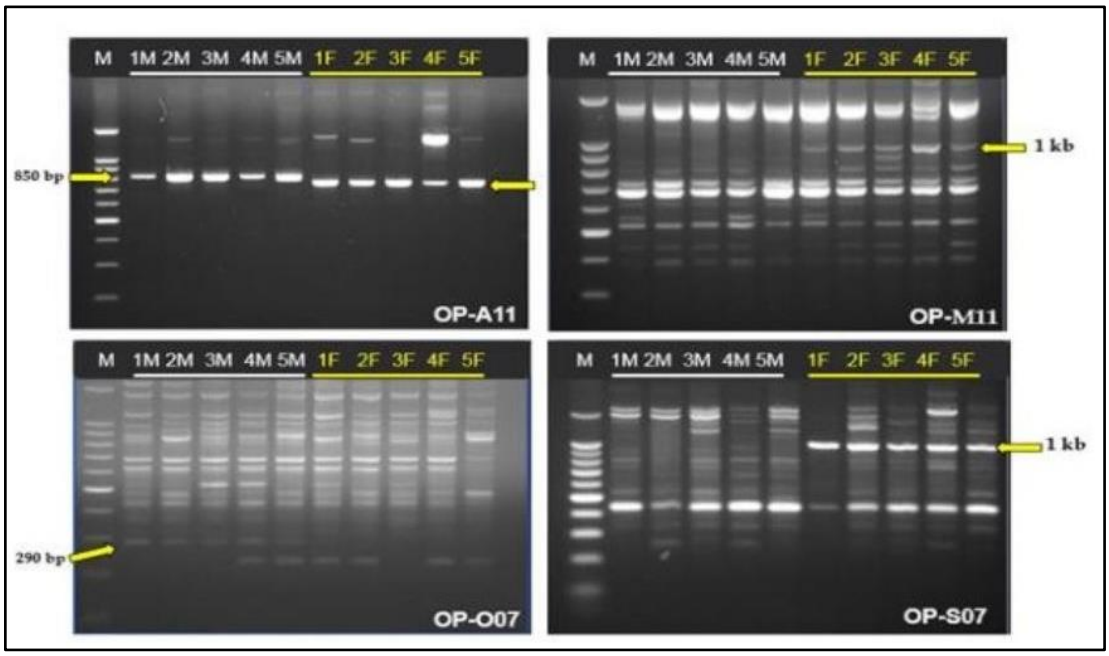

Figure 16: RAPD profiles of date palms (Male: M and Female: F), revealed by primers OP-A11, OP-M11, OP-O07, and OP-S07 (Adawy et al., 2014).

III.2.1.4. Sequence-characterized amplified region (SCAR)

SCAR markers have codominance and are found to be more reliable, reproducible, and less sensitive to reaction conditions than RAPD markers. SCAR primers are longer (20–30 bp) from the RAPD decamer and sequence-specific primers are used in many dioecious plants for gender discrimination. These primers can be designed from cloning and then sequencing of required polymorphic band amplified from the RAPD (PCR) amplification.

Al-Qurainy and his collaborators (2018) developed a specific SCAR marker for male date palms (Figure 17). The marker clearly differentiated all male plants from female plants based on the presence or absence of the 186 bp band.

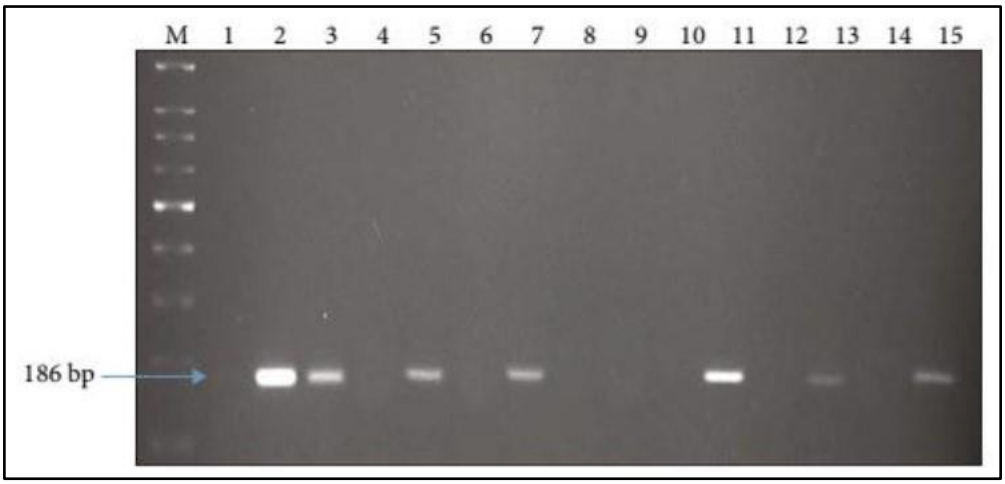

Figure 17: Screening of male and female date palm plants with a developed SCAR marker. The presence of a band indicates male seedlings, and the absence of a band indicates female seedlings (AlQurainy et al., 2018).

III.2.1.5. Simple sequence repeats (SSR)

The study conducted by Awan et al. (2017) on date palms identified a male specific SSR marker of 300 bp. This marker is absent in female palms and present in 4 samples of their descendants (Figure 18).

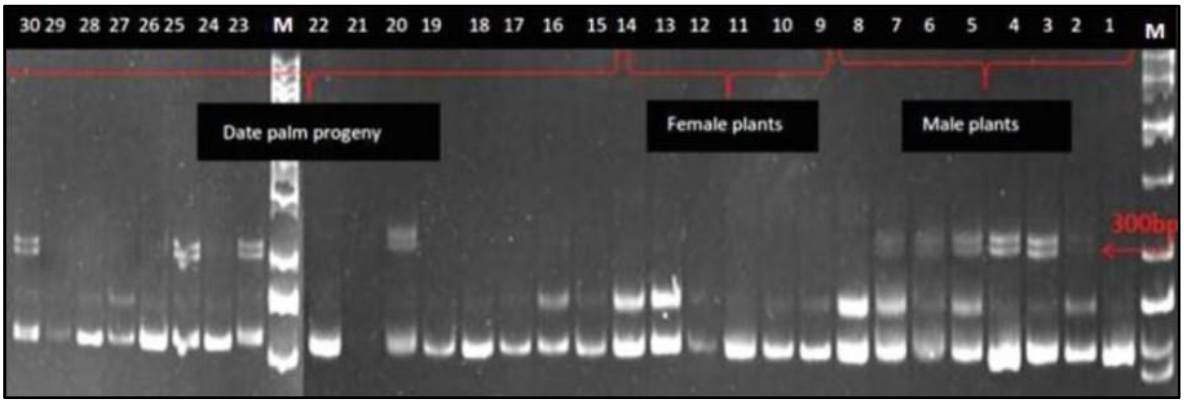

Figure 18: SSR analysis of male and female parent date palms and their seedlings (Awan et al., 2017).

III.2.2. Genetic mapping

Cherif et al. (2013) added that malespecific DNA markers can provide genetic evidence of an XY chromosome system. In the postgenomic era, identification of Date-SRY Gene and GWAS mapping of sex determination locus (Hazzouri et al. 2019) are some of the novel tools for sex identification in date palm (Mohi et al. 2019). Recently, Wang et al. (2020) developed the sex-linked SSR markers and validated them in date palm. Markers mPdIRDP52 and DPM4 proved to be sex-linked with 100% accuracy. Mathew et al. (2014) constructed the first genetic map for date palm and identified the putative sex chromosome. They placed *4000 markers on the map using nearly 1200 framework markers spanning a total of 1293 cM. Approximately *1.9 cM/Mb were revealed on the map by this analysis. Date palm sex determination region analysis reported telomere repeats on linkage group 12 and displayed recombination in the full chromosome.

III.2.2.1. Combination of multiple markers

Atia et al. (2017b) identified and developed PCR-based molecular markers against gender discrimination in date palm genotypes. These markers were AFLP, conserved DNA-derived polymorphism (CDDP), start codon targeted polymorphism (SCoT), intron-targeted amplified polymorphism (ITAP), and RAPD (Figure 19).

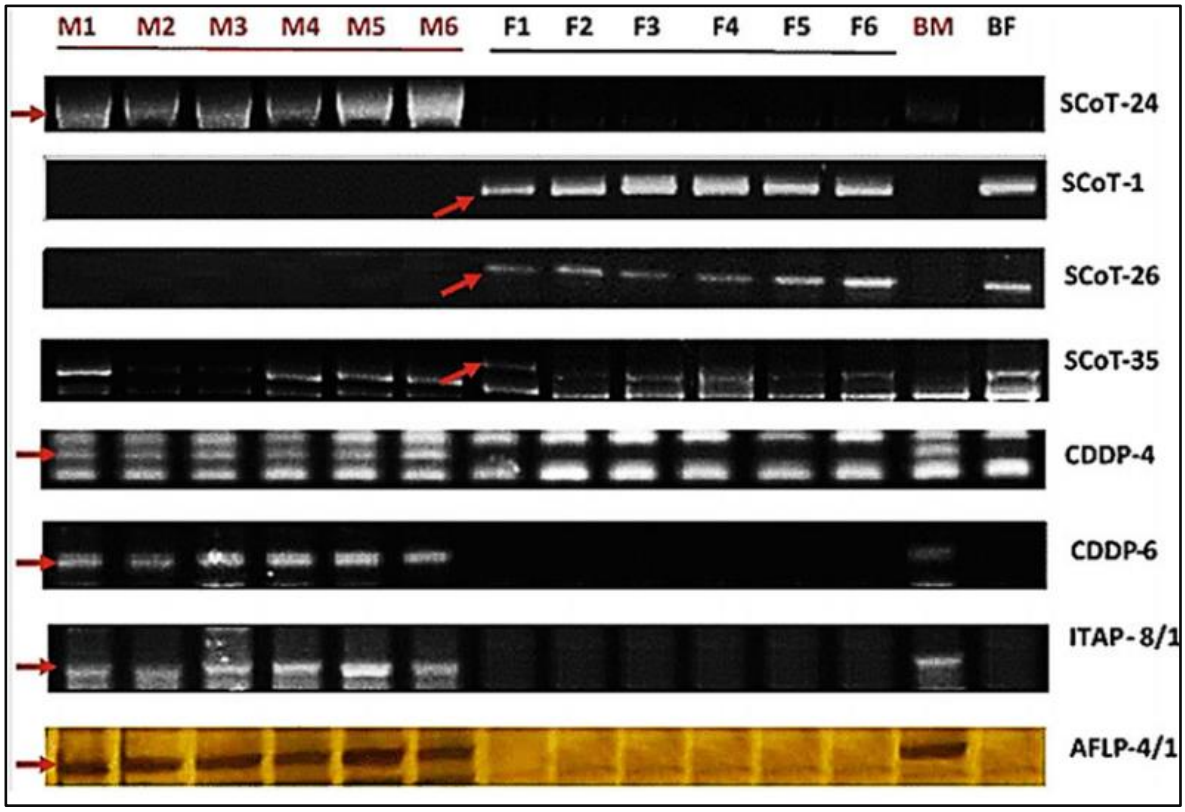

Figure 19: The amplicon pattern established from SCoT, CDDP, ITAP, and AFLP marker types discriminating male and female date palm varieties. (Atia et al. 2017b)

They developed the procedure based on PCRbased sex-specific markers starting from genomic DNA purification to sequencing up to BLAST analysis (Figure 20).

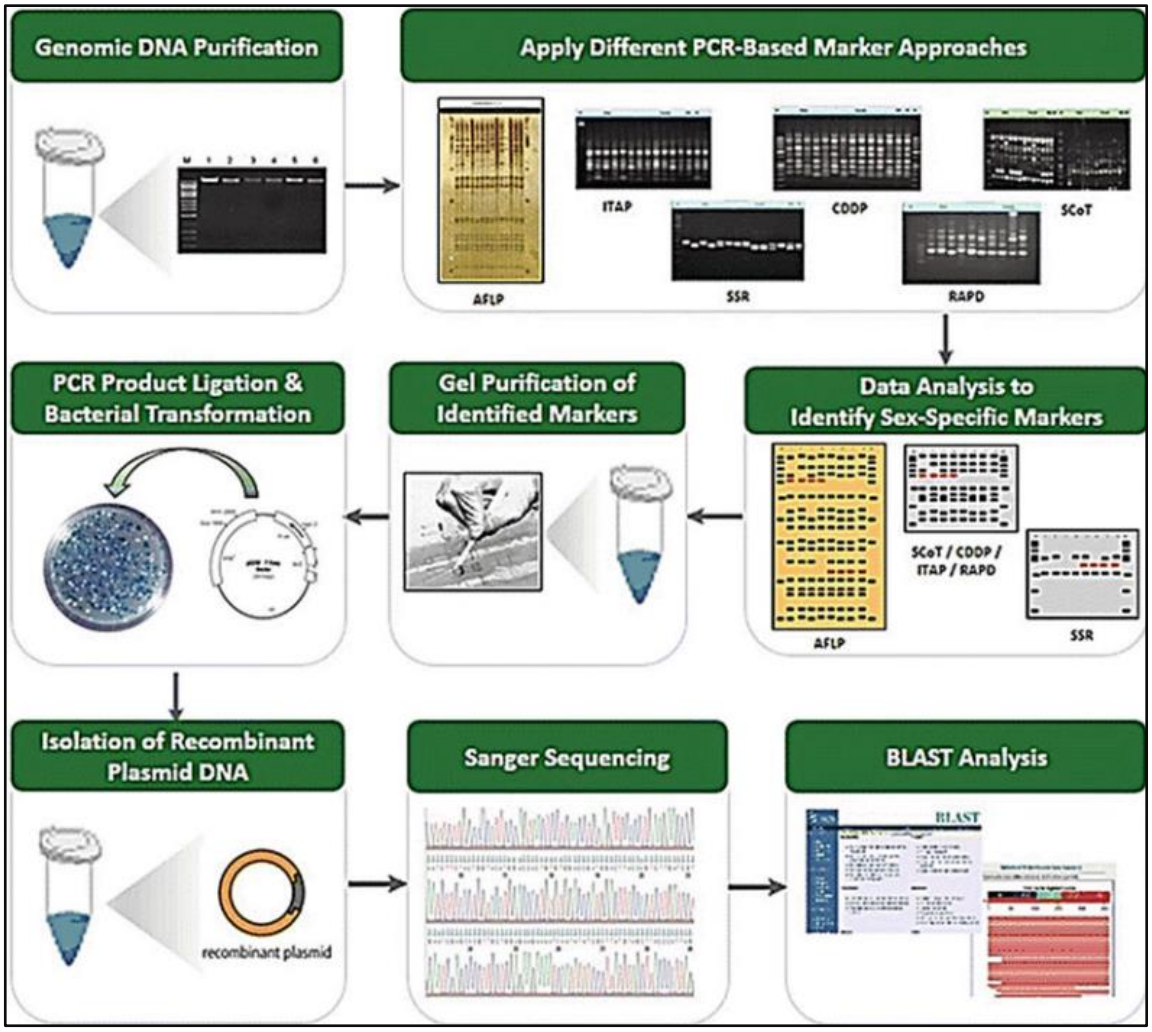

Figure 20: The procedures used to develop sex-specific PCR-based marker in date palm (Atia et al. 2017b)

III.2.3. In Algeria

The use of molecular markers in date palm sex determination is relatively new, in 2022 Benhamada and bougheddad under the supervision of Dr.Bouchemal in the Mentouri brothers university of Constantine used SSR markers (mPdCIR010, mPdCIR052, mPdCIR016, mPdCIR32 and mPdCIR70) linked to sex to identify male and female date palms, following this protocole

- Genomic DNA isolation from date palm leaves through the classic CTAB method with a little modification, or Al-Qurainy et al., (2018) CTAB method
- Control of DNA quality through migration electrophoresis using agarose at 0.8% and ethidium bromide.
- Quantative DNA analysis using an Nanodrop spectrophotometer to separate pure DNA for use in this procedure.
- Dilution of DNA
- Choice of markers, they landed on SSR markers (specifically mPdCIR010, mPdCIR016, mPdCIR032, mPdCIR070 and mPdCIR052)
- PCR amplification
- SSR separation through electrophoresis

But as they stated in their research there weren't any distinctive differences between the 15 male and female genotypes analyze, forcing them to change the focus of their study towards genetic diversity between cultivars.

PERSPECTIVE AND CONCLUSION

This subject is very new and exciting and even more challenging, however it being so new and relatively untapped successfully, it makes it very hard for non-scientifically developed countries to delve into.

which complicates things even further considering that the non-scientifically developed countries are most in need of research in this subject due to the distribution of the date palm around the world, with it's majority present in Africa and the middle east.

So the scientifically advanced countries (Europe and the us) essentially have no incentive to further research this, because this field doesn't yield them much economically, and if there isn't an economic value to a field, it won't be researched properly and with a sense of urgency, it is of the utmost necessity for countries of interest to invest in the scientific material and personnel needed to research this field, or sponsor research abroad, to cut losses through early molecular sex determination in the date palm.

BIBLIOGRAPHIC REFERENCES

Bibliographic references

Ahmed, M. M., Al-Qurainy, F., Alatar, A. A., & Khan, S. (2013). ISSR markers: Genetic diversity and population structure of date palm (Phoenix dactylifera L.) from Hofuf, Al-Ahsa, Saudi Arabia. African Journal of Biotechnology, 12(35), 5446-5455.

Al-Dous, E. K., Al-Abdallat, A. M., Al-Mahmoud, M. E., Almajali, D. A., & Al-Ghazi, Y. M. (2009). RAPD markers reveal polymorphism among some Jordanian date palm (Phoenix dactylifera L.) cultivars. Australian Journal of Crop Science, 3(1), 38-43.

Al-Dous, E. K., Al-Azwani, E. K., George, B., Al-Mahmoud, M. E., Al-Jaber, M. Y., Wang, H., ... & Malek, J. A. (2011). The genome of the date palm Phoenix dactylifera L. Nature Communications, 2(1), 1-9.

Al-Dous, E. K., George, B., Al-Mahmoud, M. E., Al-Jaber, M. Y., Wang, H., Salameh, Y. M., ... & Al-Azwani, E. K. (2011). De novo genome sequencing and comparative genomics of date palm (Phoenix dactylifera). Nature Biotechnology, 29(6), 521-527.

Al-Khayri, J. M., Jain, S. M., & Johnson, D. V. (Eds.). (2015). Date Palm Genetic Resources and Utilization: Volume 2: Asia and Europe. Springer.

Alwine, J. C., Kemp, D. J., & Stark, G. R. (1977). Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. Proceedings of the National Academy of Sciences, 74(12), 5350-5354.

Aradhya, M. K., Zee, F. T., Manshardt, R. M., & Goldman, I. L. (2002). Molecular characterization and genetic relatedness among 50 date palm (Phoenix dactylifera L.) cultivars grown in United Arab Emirates (UAE), using AFLP markers. Emirates Journal of Agricultural Sciences, 14(1), 36-44.

Billotte, N., Marseillac, N., Brottier, P., Noyer, J.

Blatter, E. (1926). The palms of British India and Ceylon. Milford, London.

Charlesworth, D., & Charlesworth, B. (1978). A model for the evolution of dioecy and gynodioecy. The American Naturalist, 112(988), 975-997.

Chao, C. C. T., & Krueger, R. R. (2007). The date palm (Phoenix dactylifera L.): overview of biology, uses, and cultivation. HortScience, 42(5), 1077-1082.

Collins, F. S., Green, E. D., Guttmacher, A. E., & Guyer, M. S. (2003). A vision for the future of genomics research. Nature, 422(6934), 835-847.

Diggle, P. K., Di Stilio, V. S., Gschwend, A. R., Golenberg, E. M., Moore, R. C., Russell, J. R., ... & Stevenson, D. W. (2011). Multiple developmental processes underlie sex differentiation in angiosperms. Trends in Genetics, 27(8), 368-376.

Ecocrop. (2011). Ecocrop database. FAO.

El-Juhany, L. (2010). Degradation of date palm trees and date production in Arab countries: causes and potential rehabilitation. Australian Journal of Basic and Applied Sciences, 4(8), 3998-4010.

FAO. (2011). FAOSTAT. Food and Agriculture Organization of the United Nations.

FAO. (2021). Date Palm. Food and Agriculture Organization of the United Nations. Retrieved from http://www.fao.org/faostat/en/#data/QC

Gauthier, M. (2007). Simulation of polymer translocation through small channels: A molecular dynamics study and a new Monte Carlo approach. Retrieved from https://facellitate.com/advantages-and-disadvantages-of-pcr-technology/

Gros-Balthazard, M., Lesur, I., Grover, C. E., & Aberlenc-Bertossi, F. (2021). A review on sex determination in Phoenix dactylifera L.: from discovery to application. Tree Genetics & Genomes, 17(4), 1-20.

Hazzouri, K. M., Flowers, J. M., Visser, H. J., Khierallah, H. S. M., Rosas, U., Pham, G. M., ... & Purugganan, M. D. (2019). Whole genome re-sequencing

of date palms yields insights into diversification of a fruit tree crop. Nature Communications, 10(1), 1-10.

Hijmans, R. J., Guarino, L., & Mathur, P. (2003). DIVA-GIS Version 5. A geographic information system for the analysis of biodiversity data. Manual available at http://www.diva-gis.org

Janick, J., & Paull, R. E. (2008). The encyclopedia of fruit and nuts. CABI Publishing Series.

Loutfi, K., & Chakir, A. (2013). Date palm: Traditional uses and recent applications. Journal of Medicinal Plants Research, 7(38), 2780-2787.

Mathew, S., Spannagl, M., Al-Malki, A., George, B., Abdel Rahman, M. E., Murali Mohan, C., ... & Al-Mssallem, I. S. (2015). A first genetic map of date palm (Phoenix dactylifera) reveals long-range genome structure conservation in the palms. BMC Genomics, 16(1), 1-13.

Maxam, A. M., & Gilbert, W. (1977). A new method for sequencing DNA. Proceedings of the National Academy of Sciences, 74(2), 560-564.

Mohamoud, Y. A., Bester, A. C., Mathew, S., Flowers, J. M., Filatov, D. A., & Purugganan, M. D. (2019). Genomic insights into the evolution of date palms: Evidence for a complex history of domestication. bioRxiv, 753996.

Morgan, T. H., Sturtevant, A. H., Muller, H. J., & Bridges, C. B. (1915). The mechanism of Mendelian heredity.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G., & Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor symposia on quantitative biology, 51(1), 263-273.

Munier, P. (1973). Le Palmier-dattier-Techniques agricoles et productions tropicales. Maison Neuve et Larose.

Ramawat, K. G. (2010). Desert Plants: Biology and Biotechnology. Springer-Verlag.

Risch, N., & Merikangas, K. (1996). The future of genetic studies of complex human diseases. Science, 273(5281), 1516-1517.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., ... & Mullis, K. B. (1985). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science, 239(4839), 487-491.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A., & Arnheim, N. (1985). Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science, 230(4732), 1350-1354.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences, 74(12), 5463-5467.

Shendure, J. et al. (2017). DNA sequencing at 40: past, present and future. Nature, 550(7676), 345-353.

Smith, L. M., Sanders, J. Z., Kaiser, R. J., Hughes, P., Dodd, C., Connell, C. R., ... & Hood, L. E. (1986). Fluorescence detection in automated DNA sequence analysis. Nature, 321(6071), 674-679.

Torres, M. F., Mathew, S., Ahmed, I., Al-Azw